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Research Article

THE POTENTIAL IMMUNOMODULATORY EFFECT OF ALLICIN ADMINISTRATION IN AUTOMMUNE DISEASE PROCESS OF TYPE 1 DIABETES MELLITUS

MUHAMED T OSMAN[1*], ARIZA ADNAN[1], NOR SALMAH BAKAR[1], FATMA ALASHKHAM[1]

[1]Centre of Pathology, Diagnostic and Research Laboratories (CPDRL), Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sg. Buloh Campus, 47000 Sg Buloh, Selangor, Malaysia. Email: mtosman2004@gmail.com

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ABSTRACT

Many studies have examined the hypoglycaemic effect of allicin in both types of diabetes mellitus, but till now the mechanism has not been discussed regarding type 1 diabetes (IDDM). To the best of our knowledge the effect of this plant on autoimmunity process of IDDM has never been examined.This study was carried out in UiTM Malaysia to investigate the potential immunomodulatory effect of administration of allicin in the autoimmune disease process of IDDM. We have evaluated with the help of ELISA kits the levels of anti- islet cell antibodies, Pan T lymphocytes marker (CD90), Pan B lymphocytes markers (CD19), and Pan innate cells marker (CD11b) in male Sprague-Dawley rats with Streptozocin-induced IDDM. The four groups (6 rats each) under study received or not different doses of allicin. The results have been compared to the ones obtained from healthy and non treated diabetic rats. The administration of allicin especially in high doses to the type1 diabetic rats leads to a significant decreases in the levels of all immunological parameters and stop the disease process ($p<0.001$). These experimental results suggest that allicin treatment has a therapeutic protective effect against autoimmune reactions occurs in IDDM.

Keywords: Allicin, Garlic, Type1 diabetes mellitus, Autoimmune disease, CD markers, Anti islet cell antibody

INTRODUCTION

Type I diabetes mellitus or insulin-dependent diabetes mellitus (IDDM), is an autoimmune disease process in which pancreatic islet β-cells are targeted for destruction by an aberrant host immune system [1]. This process involves both cellular and humoral branches of the immune system, with the generation of islet-specific T-cell reactivity, as well as autoantibodies directed against islet cell antigens [1].

The disease process in IDDM is primarily caused by the destruction of pancreatic beta cells that is thought to result mainly from the action of T-lymphocytes; the key players in autoimmune disease development [2]. Meanwhile, in subjects newly diagnosed with IDDM, up to 90% have autoantibodies to islet cell antibodies (ICA) [2]. These autoantibodies appear during the preclinical period of β-cell destruction before the clinical manifestation of diabetes [2]. The hallmark of the autoimmunity of IDDM is the presence of circulating ICA autoantibodies, they thought to signal a T-cell mediated immune response which sets the stage for beta cell destruction [3].

Experimental studies on animals especially rats showed that these animals develop a form of autoimmune diabetes that resembles human IDDM [4]. Studies with animal model have established that islet-infiltrating cell– reactive T-cells are the major effectors of ß-cell damage. However, other immune system cells are also crucial in the disease development. Among these cells, B-cells are essential in the onset and progression of type 1diabetes [4-5] and although it is not fully understood when and how these cells participate in IDDM, it is known that they produce ICA autoantibodies against many ß-cell autoantigens and act as antigen-presenting cells [6]. On the other hand, the production of specific ICA autoantibodies directly correlates with the progression of IDDM in both humans and laboratory animals [5-6].

Natural immunomodulators are becoming a viable adjunct to established modalities offering a novel approach for the treatment of immunological diseases in the coming decades of 21st century [7-8]. Among these natural immunomodulators is garlic and its components. Allicin is the principle active ingredient of garlic oil (*Allium sativum*), which is present in the form of a thermostable precursor visualizing alliin in the whole garlic bulb [9]. On crushing a garlic clove, allicin and allinase combine to form allicin which on storage rearranges to form diallyl sulphides and other sulphides[9]. The antimicrobial [10], antitumor[11-13], antifungal [14], and antigenotoxic [15] activities of allicin have been reported. Meanwhile, various researches have indicated that garlic and allicin have inhibitory immunumodulatory action. It modulates immune responses, enhance humoral immunity and minimize immunological stress will affect growth performance most positively [16].Studies demonstrated that garlic enhances natural killer (NK) activity and T-lymphocyte proliferation [16-17].

Many studies have examined the hypoglycemic effect of allicin in both types of DM [18-21] , but till now the mechanism has not been discussed regarding IDDM while the probable mechanism underlying garlic and allicin hypoglycemic effects in type 2 diabetes most likely is increased insulin secretion and sensitivity [22-23].

To date, there are no successful treatment interventions that have been found to delay the onset of type 1 diabetes. Thus, our present study was carried out in Faculty of Medicine, UiTM Malaysia to investigate the potential immunomodulatory effect of administration of major component of garlic;(allicin) in the autoimmune disease process of type 1 diabetes mellitus as by determination its effect on the levels of main immunological cell markers in IDDM: autoimmune anti- islet cell antibodies (ICA), pan T- lymphocytes marker (CD90), pan B-lymphocytes marker (CD19), and pan innate cells marker (CD11b).

MATERIALS & METHODS

Experimental animals

Twenty four male Sprague-Dawley rats with an average weight of 150-250g and an average age of 12-16 weeks were used throughout the experiment, obtained from Nano Life Quest Company. The rats were acclimatized for a period of 21 days. A standard environmental condition such as temperature (20-22 ^{0}C), relative humidity (45-55%) and 12 hrs dark/light cycles was maintained. The animals were fed daily with rodent pellet diet and tap water *ad-libitum* under strict hygienic conditions.

Ethical clearance for performing the experiment on animals was approved by Animal Care and Use Committee (ACUC), Faculty of Medicine, Universiti Teknologi MARA (UiTM) Malaysia that conforms to the Guide for the Care and Use of Laboratory Animals [24], and all efforts were made to minimize animal suffering and the number of animals used.

Chemicals

Streptozotocin (STZ) used in the present study was purchased from Nano Life Quest Company (Sigma); Allicin (2-propene-1-sulfinothioc acid S-2-propenyl ester; thio-2-propene-1-sulfinic acid S-allyl ester) was purchased from Nano Life Quest Company. The allicin was administered once a day by intraperitoneal injection (i.p) at a dose of (8 mg/kg and 16mg/kg) for 30 days.

Induction of type 1 diabetes mellitus and treatment of rats

A single injection of STZ is widely used to generate a rat model of type I diabetes, which results from the selective toxicity of STZ towards the insulin-producing β-cells in pancreatic islets [25-26]. IDDM was induced in overnight fasted animal group by intra-peritoneal injection with a single dose of STZ (65 mg/kg body weight). This dose of STZ lies within the range used in most of studies to produce IDDM, in which blood glucose levels are 3–4 times normal, by causing substantial depletion of pancreatic insulin [26]. STZ was dissolved in sodium citrate buffer solution (PH 4.5) immediately before use. The development of IDDM was confirmed by the presence of hyperglycaemia with blood glucose above 13.9mmol/L (250 mg/dL), which last for at least three days. The rats were divided into four groups comprising 6 rats each. Group A (GA; control group), rats were injected with an equal volume of vehicle (citrate buffer, 65 mg/ Kg body weight) ; Group B (GB; untreated STZ-diabetic rats) ; Group C (GC; STZ-diabetic rats treated with 8 mg/ kg, i.p., allicin) ; Group D (GD; STZ-diabetic rats treated with16 mg/ kg, i.p., allicin).

The treatment by allicin was started for a period of 30 days. During this period, all animals had free access to standard diet and water until 6pm. None of the rats was treated with insulin at any time during the experiment. Animals were sacrificed at 30th day of experiment immediately after measuring blood glucose [25-26]. Blood glucose levels were tested every morning (at 8 am). Blood was collected from the tail of fasting (14 h) animals. A drop of blood was used for the blood glucose test with the help of a One Touch Glucometer (Roche, USA).

Laboratory tests

On the last day (30th day) and after completion of the experimental protocols, blood samples were collected from overnight fasting rats by sacrificing each diabetic and control rats. The animals were anesthetized in a chamber containing diethyl ether. Cardiac puncture was done using a heparin syringe and blood was collected into a heparin containing container. Immediately after collection, 2.0 ml of blood was transferred into fresh tube and centrifuged at 3000 rpm for 10 minutes. The serum was collected and stored at – 80°C until serological analysis.

Serum was assayed for autoimmune anti- Islet cell antibodies (ICA), pan T- lymphocytes marker (CD90), pan B-lymphocytes marker (CD19), and pan innate cells marker (CD11b) in addition to serum insulin using enzyme-linked immunosorbent assay (ELISA) by using a commercially available kits (USCNK, CHINA).

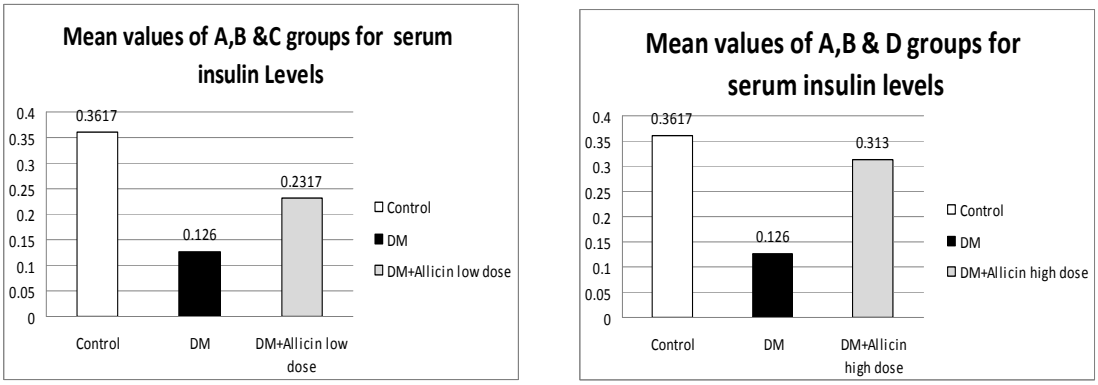
Statistical analysis

The data are expressed as mean ± SE. with 'n' referring to the number of rats used. Two way analysis of variance (ANOVA) was carried out using SPSS 16 software to assess the overall effects and interaction of treatment and time on parameters and followed by repeat one way analysis of variance (ANOVA) with post hoc least significant difference (LSD) test to determine the effect of treatments on differences among means when the analysis of variance indicated a significant result. $P < 0.05$ was taken to indicate significance.

RESULTS

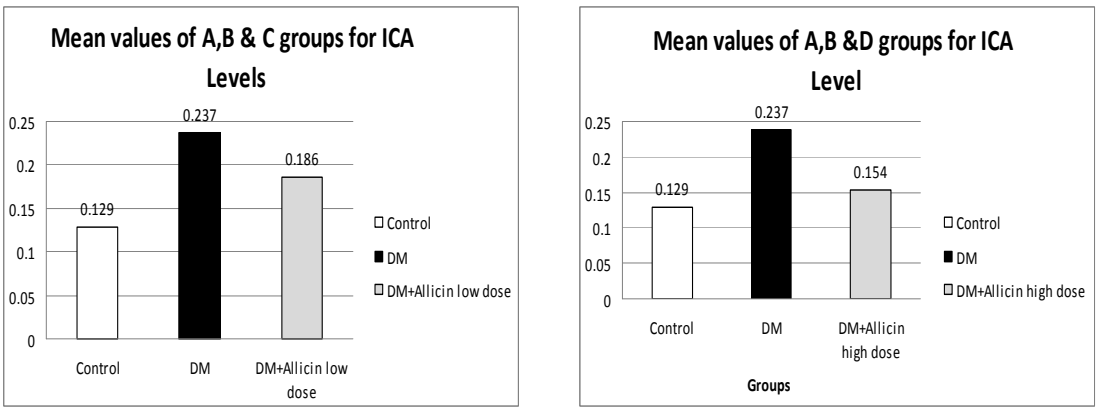
The diabetic animals exhibited consistent hyperglycemia. Meanwhile allicin treatment caused a decrease in the elevated serum glucose, and an increase (P=0.001) in the lowered serum insulin concentrations in STZ induced diabetic rats by the end of the experiment. (Figure 1)

After induction of IDDM, the diabetic animals showed increase the levels of anti islet cell auto antibodies ICA, however, by the end of the experiment the allicin treatment specially in high doses diabetic group significantly (P= 0.001) caused decreased in elevated ICA levels (Figure 2). The same effect was noticed with other immunological markers; CD19, CD90, and CD11b which increased after diabetic induction but by the end of the experiment, allicin treatment significantly caused decrease in all elevated markers (Figures 3, 4, and 5 respectively).



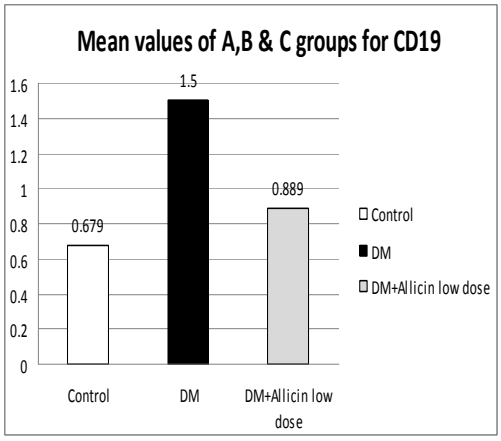
Low dose **High dose**

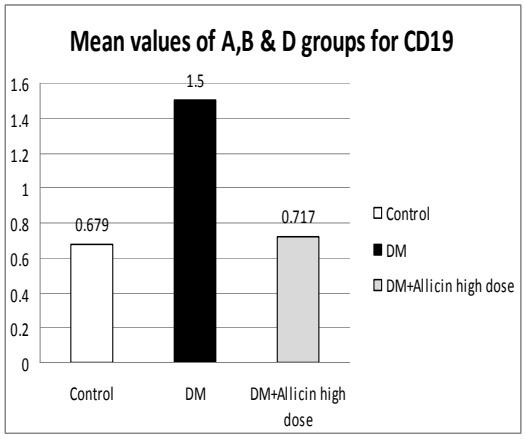
Fig. 1: Effects of allicin administration on insulin level production.



Low dose **High dose**

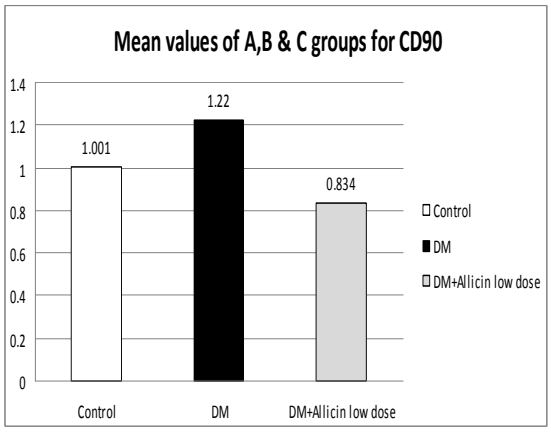
Fig. 2: Effects of allicin administration on the ICA levels

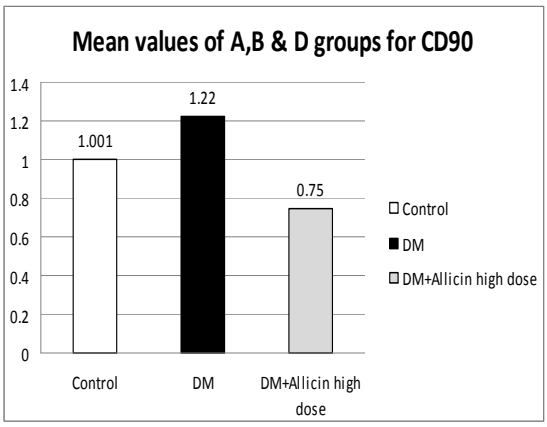




Low dose **High dose**

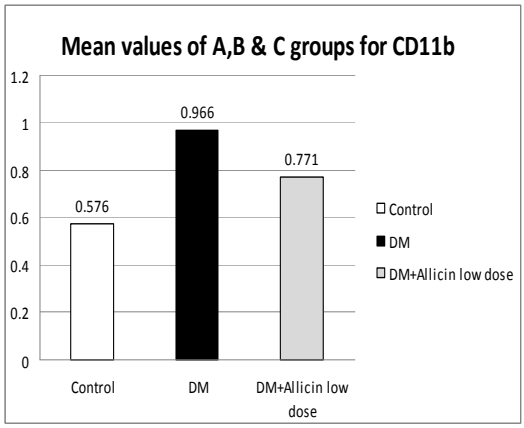
Fig. 3: Effects of allicin administration on the levels of CD19

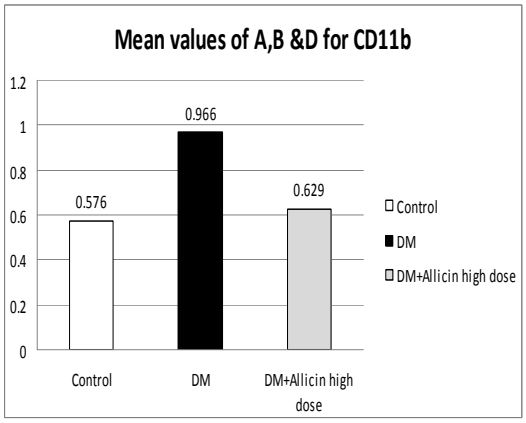




Low dose **High dose**

Fig. 4: Effects of allicin administration on the levels of CD90





Low dose **High dose**

Fig. 5: Effects of allicin administration on the levels of CD11b.

DISCUSSION

This study demonstrates for the first time, to the best of our knowledge, the effect of allicin (the principle active ingredient of garlic) on the autoimmune disease process in IDDM.

Our principal findings are: (1) daily intra-peritoneal administration of allicin (both low dose 8 mg/kg or high dose 16 mg/kg but more with high dose) for up to 30 days to type1 diabetic rats effectively reduces levels of anti islet cell antibodies ICA which are the main antibodies produced in autoimmune process of the disease; and (2) the elevated pan B cell marker (CD19), elevated pan T cell marker (CD90) and elevated pan innate cells marker (CD11b) that increased due to autoimmunity process were significantly reduced after the administration of allicin; (3) reduced level of insulin due to damaged Langerghans islet cell was significantly increased in the serum due to repairing tissue process after allicin treatment.

While the therapeutic anti diabetic effects of allicin on type 2 diabetes mellitus are numerous and well-documented, evidence presented in this study actually shows a novel immunomodulatory effect of allicin in IDDM of STZ induced diabetic rats. This finding is made more interesting by the fact that many studies [18-21] have shown that allicin is hypoglycaemic in both types of diabetes through biochemical evidences while in this study we have proved that allicin has significant effect on the production of main autoimmune antibodies in IDDM in addition to its effects on the levels of other immunological markers as well, that lead to affect and stop the disease autoimmune process of IDDM.

Researchers have determined that during the first stage of IDDM, ICA antibodies are synthesized that act against the insulin-producing cells of the pancreas[4]. The consequence of these autoantibodies is a destruction of the insulin-producing beta cells of the islets of Langerhans' cells and an absence or deficiency of circulating insulin [3]. Indirect immunofluorescence stains of human pancreas sections demonstrate that majority of recently diagnosed diabetics have ICA [5]. The autoimmune attack of these antibodies appears to destroy ß cells selectively [2, 4]. Moreover, many studies have considered that ICA serum autoantibodies are an important hallmark of this disease, and assays for these islet cell antibodies have facilitated the investigation and understanding of several facets in the pathogenesis of autoimmune diabetes. Their applications have begun to extend into clinical practice and have opened new avenues for early preclinical prediction and preventive prophylaxis in IDDM [2-5].

The immunomodulatory and immunostimulatory effects of allicin alone or within garlic were reported in many studies. It potentially induces the lymphocytes proliferation and macrophage phagocytosis, stimulates the infiltration of macrophages and lymphocytes in transplanted tumours, induces splenic hypertrophy, stimulates release of interleukin-2, tumour necrosis factor-alpha, interferon-gamma and enhances natural killer cell and lymphokine-activated killer cell activity. These activities reflex effective stimulation of the immune response [27-28]. Meanwhile, because certain diseases can be caused by immune dysfunction, modification of immune functions by garlic may contribute to the treatment and prevention of diseases. Thus, some pharmacologic effects of garlic might be mediated through immunomodification [27, 29].

The present study showed additional biochemical evidence to that of immunological effect of allicin and our results indicate that allicin affects blood glucose and insulin level. However, most of the studies showed that garlic can reduce blood glucose levels in diabetic mice, rats and rabbits [30-33]. It is not clear how garlic and allicin actually works in alleviating hyperglycaemia but the hypoglycaemic action of allicin could possibly be due to an increase in pancreatic secretion of insulin from β-cells, release of bound insulin or enhancement of insulin sensitivity also has been previously suggested that allicin can enhance serum insulin by effectively combining with compounds like cysteine [33].

The goals of treatment of autoimmune diseases are to reduce symptoms, control the autoimmune process and maintain the body's ability to fight disease [34]. Some patients may need supplements to replace a hormone (like insulin injections in IDDM) [35] or immunosuppressive medicines include corticosteroids to control or reduce the immune system's response, but these medicines are often cause many side effects [36]. Meanwhile, there are no data were recorded regarding possible garlic harmful effects in animals and humans except that uncooked garlic has been reported to have an irritant effect on human stomach [37].

CONCLUSION

In conclusion, these experimental results suggest the immunomodulatory effect of allicin "the principle active ingredient of garlic" against autoimmune reactions occurs in IDDM, since the immune defense in this disease was significantly improved by the administration of this plant. According to the best of our knowledge; the preliminary data of our study provide for the first time a new strategy for using allicin as a natural product to be recommended in the clinical management, control, and prevention of IDDM. Moreover, these data may open a wide field to study the effect of allicin on autoimmune diseases in general; thus, further studies need to be done to conceptualize the exact role of allicin alone or within garlic on autoimmunity not in IDDM but other autoimmune diseases as well.

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