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Eeffects of Garlic Oil Administration on Some Immunological Parameters in Induced Type 1 Diabetes Mellitus

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ABSTRACT

Garlic has hypoglycaemic effects in both types of diabetes mellitus, but the mechanism has not been clarified regarding type 1 diabetes (T1DM). This study was carried out in UiTM Malaysia to investigate the effects of garlic oil administration on some immunological parameters in the disease process of T1DM. We have evaluated by using 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 T1DM. The four groups (6 rats each) under study received different doses of garlic oil. The results have been compared to the ones obtained from healthy and non treated diabetic rats. Type1 diabetic rats effectively increased levels of T, B lymphocytes and innate cells markers, meanwhile, the administration of garlic oil especially in high doses to the T1DM rats leads to a significant decreases in the levels of all immunological parameters assessed in the study. Results of this study suggest that garlic oil administration could affect the autoimmune disease process of T1DM and leads to increase insulin production, hence it has immunomodulatory effects against this disease

Keywords: Garlic; Type1 diabetes mellitus; autoimmune disease; CD markers, ELISA test

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INTRODUCTION

Type 1 Diabetes mellitus or insulin-dependent diabetes mellitus (T1DM) is a multi-factorial autoimmune disease characterized by insulin deficiency, due to T-cell mediated damage of pancreatic cells [1]. The mechanisms of onset for T1DM are proposed. The first mechanism suggests that environmental factors trigger the autoimmune process, most often in childhood before 10 years of age, although the diagnosis of T1DM is usually preceded by only a few weeks of known symptoms, in fact clinical disease becomes evident only after a long period characterized by the gradual destruction of pancreatic beta cells [2]. The second mechanism suggests that a super antigen reaction results in rapid destruction of pancreatic beta cells within a few weeks to a month, leading to the onset of clinical disease [2].

The disease process in T1DM is primarily caused by the destruction of pancreatic beta cells that is thought to result mainly from the action of T-lymphocytes which the key players in autoimmune disease development [3]. Meanwhile majority of children diagnosed with type 1 diabetes have high levels of islet cell antibodies (ICA). Moreover, these autoantibodies appear during the preclinical period of β-cell destruction before the clinical manifestation of diabetes [3]. The hallmark of the autoimmunity of T1DM 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 [4].

Studies with animal models 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. Between these cells, B-cells are essential in the onset and progression of type 1diabetes [5, 2] and although it is not fully understood when and how these cells participate in T1DM, but it is known that they produce ICA autoantibodies against many ß-cell autoantigens and act as antigen-presenting cells [6] in contrast, the production of specific ICA autoantibodies directly correlates with the progression of T1DM in both humans and laboratory animals [2, 6].

Although, the people who have suffered from diabetes, medicinal therapy "insulin" which is the only treatment for T1DM patients, but because this treatment is a biochemical agent so leads to many side effects.

Garlic (Allium sativum) is a member of the liliaceae family, which is almost certainly one of the first known medicinal plants, has become popular preventative and treatment alternatives [7]. Also is a hardy, perennial bulb which is native to the Mediterranean regions of Africa and Europe and for thousands of years amazing magical and medicinal powers have been attributed to garlic [8]. There are many components inside garlic, such as APDS -allyl propyl disulphide, Allicin -diallyl disulphide oxide, flavonoids and many others [9]. Garlic is very beneficial because it can work as antibacterial, antiviral including the common cold virus [10], beside there have been numerous clinical studies which have employed garlic to treat hypertension, hypolipidemic and other cardiovascular diseases [11] , immunomodulatory [12], and antioxidant [13].

Many studies have examined the hypoglycemic effect of garlic in both types of diabetes [14-16]. The probable mechanism underlying garlic hypoglycemic in type 2 diabetes mellitus most likely is improved insulin secretion and sensitivity. However, till now the mechanism has not been reported regarding type 1 diabetes mellitus.

The present study was carried out in UiTM Malaysia to investigate the potential effects of garlic oil administration on some immunological parameters of type 1 diabetes mellitus.

MATERIALS & METHODS

Male Sprague-Dawley rats were used in the study with an average weight of 150-250g and an average age of 12-16 weeks, obtained from Nano life quest company. The animals were feed with rodent pellet diet and tap water *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from University Animal Ethics Committee (ACUC), Faculty of Medicine, Universiti Teknologi MARA (UiTM) Malaysia. The rats were acclimatized for a period of 21 days. Standard environmental conditions such as temperature (20-22C), relative humidity (45-55%) and 12 hrs dark/light cycles were maintained in the quarantine.

Ready Garlic oil (Allium sativum) was purchased from Nano Life Quest Company, Malaysia. The Garlic was administered once a day by intraperitoneal injection (i.p) at two doses (low dose of 10 mg/kg and high dose of 20mg/kg) for 30 days. Streptozotocin (STZ) used in the present study was purchased from sigma. All other chemicals used in this study were obtained from same brand Nano Life Company.

Type 1 diabetic was induced in overnight fasted animal group by i.p injection with a single dose of STZ (65mg/kg body weight) [17]. Rats with blood glucose above 13.9 mmol/L (250 mg/dL. which lasted for at least three days were considered diabetic. 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 ; Group B (GB; untreated STZ-diabetic rats) ; Group C (GC; STZ-diabetic rats treated with10 mg⁄ kg, i.p., Garlic oil) ; Group D (GD; STZ-diabetic rats treated with 20 mg⁄ kg, i.p., Garlic oil).

The treatment by plant was started from the same day to the groups C and D for a period of 30 days. During this period, animals in all groups were having free access to standard diet and water until 6pm. None of the rats in all groups was treated with insulin at any time during the experiment. Animals were sacrificed at 30^{th} day of experiment immediately measuring blood sugar [16]. Blood glucose levels were estimated at 8 am from a tail of 14 h fasting animals on each day of the treatment. A drop of blood was used for the blood glucose test with the help of a One Touch Glucometer (Roche, USA).

At 30th days, the animals were anaesthetized. Blood samples fasting were collected by cardiac puncture. Each blood sample was kept in tubes containing heparin (10ml of blood). Blood samples were let to clot at room temperature for at least 30 minutes before they were

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centrifuged. Then samples were centrifuged for 10 min at 3000 rpm separate serum from the blood. Separated serum was then stored in new Eppendrof tubes using micro pipette also all tubes were labeled accordingly. Freezing of serum having of the rats were stored at -80ºC until required for analyses and submitted to determining immunological study. 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 from (USCNK, CHINA). The data are expressed as mean ± SE. There are two ways analysis of variance (ANOVA) was carried out using SPSS 16 software to assess the overall effects and interaction of treatment and time on parameters. 0.05 was taken to indicate significance.

RESULTS

Induction of diabetes with streptozotocin was associated with the characteristic development of lower serum insulin levels than in the control rats. Meanwhile, levels of the insulin were significantly higher compared to those of the control group at the end of the experiment (p=0.001). (Figure 1)

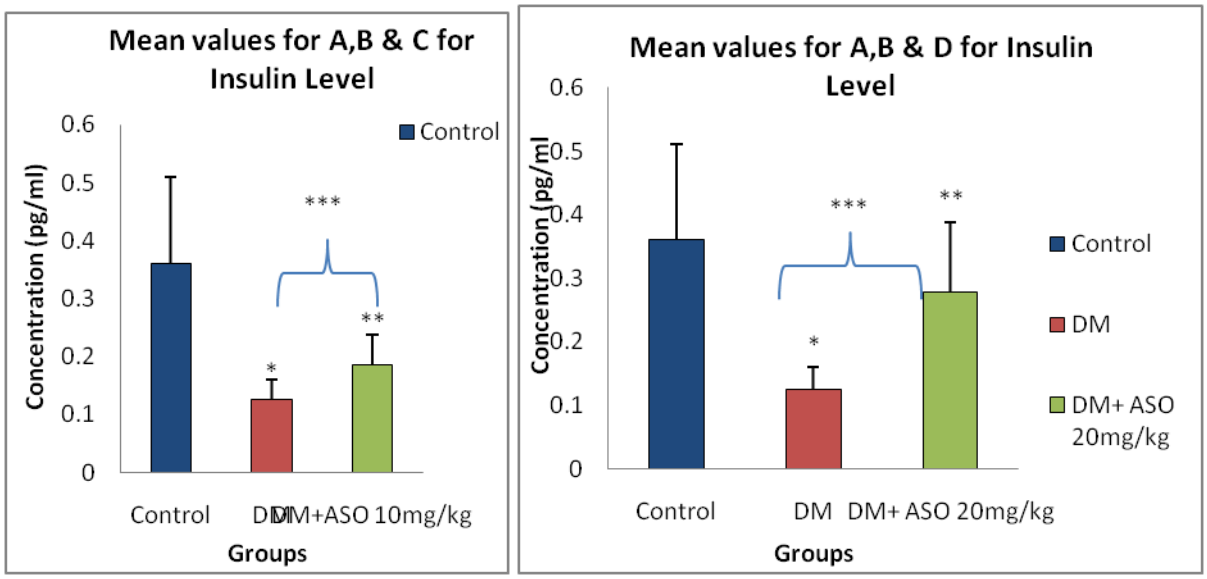

Figure 1. Effects of garlic oil treatment on serum insulin levels of diabetic rats (low dose 10 mg/kg and high dose 20 mg/kg)

Under induction of STZ anti islet cell auto antibodies ICA level was increased significantly in diabetic rats when compared with control groups, meanwhile, after 4 weeks treating rats with garlic especially in high dose 20mg/kg level of the ICA was significantly lower as compared to the control group (Figure 2). The same effect was noticed with other immunological markers; CD19, CD90, and CD11b which increased after diabetic induction. However, at the end of the experiment rats treated with both (10mg/kg-20mg/kg) garlic oil had decreased in all elevated markers level as compared with control animals (Figures 3, 4, and 5 respectively).

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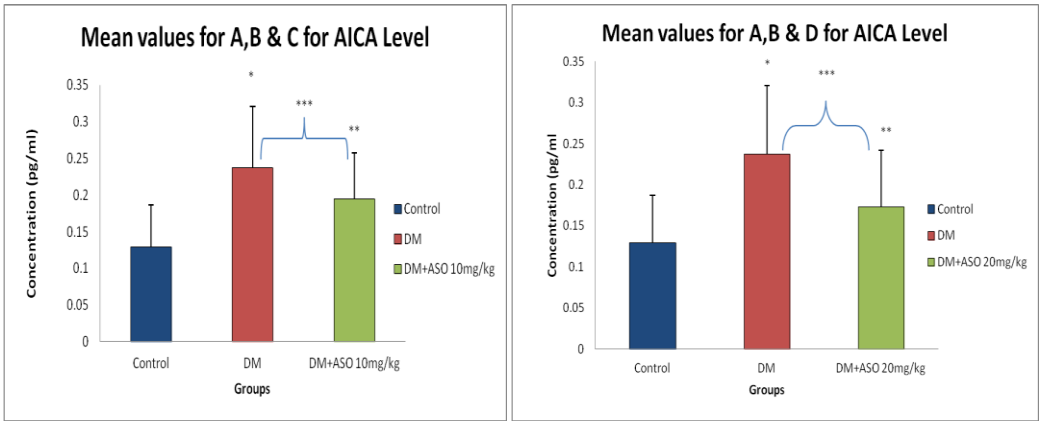

Fig.2. Effect of garlic oil treatment at doses10 &20 mg/kg on levels of ICA

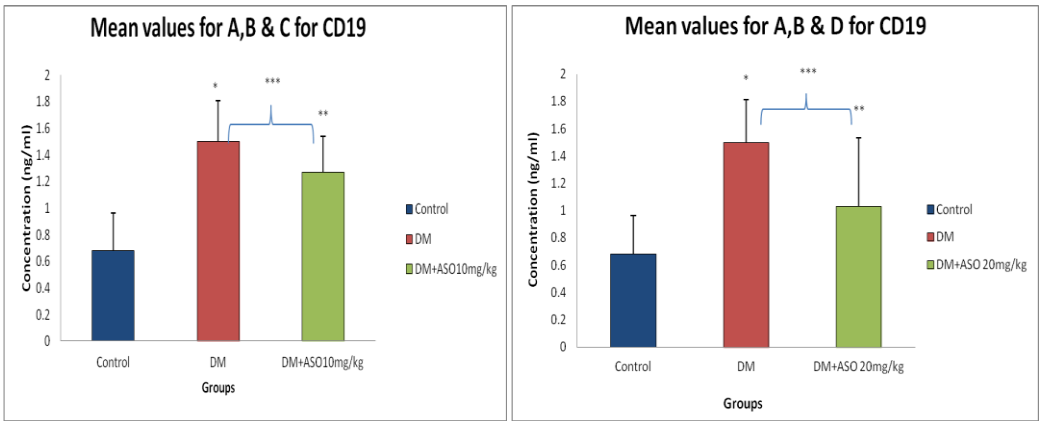

Fig.3. Effects of garlic oil treatment at doses 10&20 mg/kg on the CD19 levels.

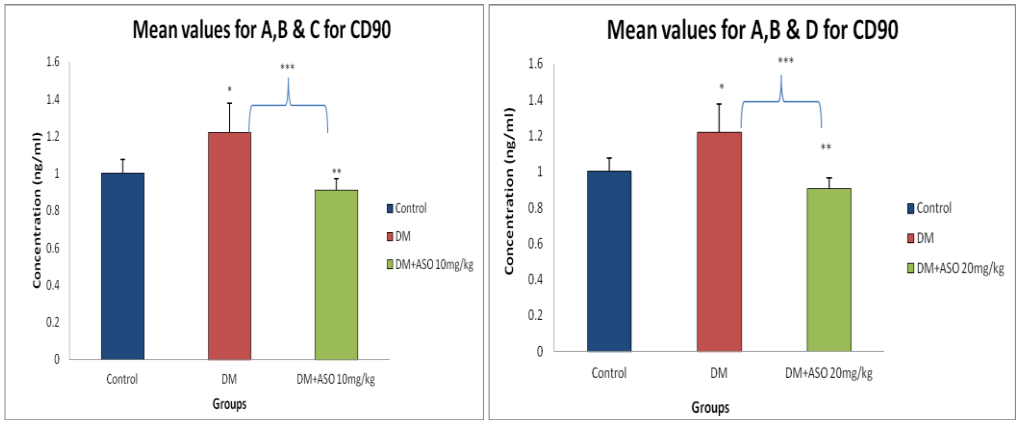

Fig.4. Effects of garlic oil treatment at doses 10&20mg /kg on CD90 levels.

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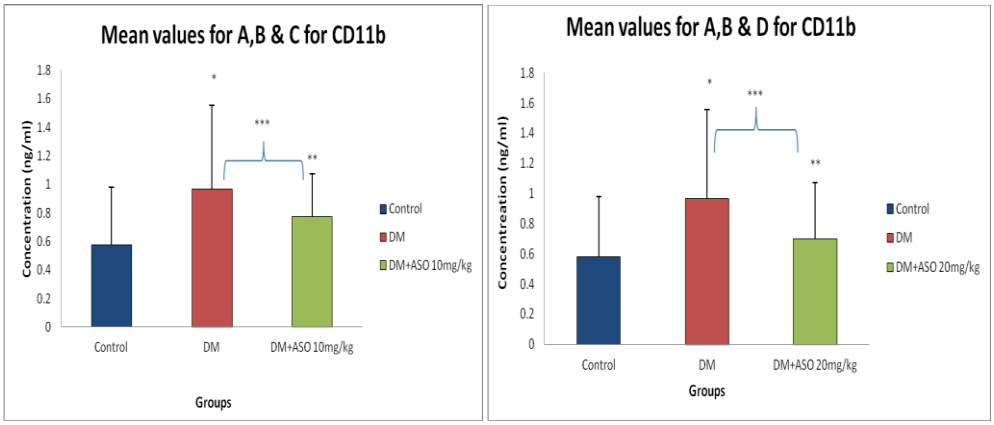

Fig.5. Effects of garlic oil treatment at doses 10& 20mg/kg on CD11b levels

DISCUSSION

We examined the effect of garlic oil on some immunological parameters in T1DM after induction of the disease in rats. The diabetic animals showed that the levels of anti islet cell auto antibodies level higher as compare to control group, however, when garlic oil was administrated to the rats in a dose of 10 mg / kg resulted that ICA level has been reduced however, with high dose (20 mg/kg) there was declined on ICA level. Same was noticed with other immunological markers; the elevated pan B cell marker (CD19), elevated pan T cell marker (CD90) and elevated pan innate cells marker(CD11b) levels, which increased after diabetic induction due to disease process, but all markers were reduced under the effect of garlic oil treatment in both two doses but significantly with high dose. These effects maybe caused stopping of the disease process and leads to increase the production of serum insulin later.

Many studies reported that garlic possesses a variety of medicinal properties such as anti hyperglycemic agent. However the results of the present study showed that daily i.p administration of low dose 10mg/kg & high dose 20mg/kg of garlic oil for 30 days reduced insulin level in the streptozotocin-induced type1 diabetic rats which was significantly increased in the serum after garlic treatment. This study consisted with other studies which have mentioned that garlic is hypoglycaemic in type2 of diabetes through biochemical evidences [18-20].

Scientists have well known several antibodies that seem to be related to the development of type 1 diabetes, such as islet cell antibodies (ICA). Islet cell antibodies were the primary auto antibodies discovered in patients with diabetes. These antibodies fight unwanted proteins in and on the islet beta cells. The autoimmune attack of these antibodies appears to destroy ß cells selectively [3,5]. Furthermore, numerous studies have considered that ICA serum autoantibodies are an important hallmark of this disease, and assays for these islet cell antibodies have facilitated the examination and understanding of several facets in the pathogenesis of autoimmune diabetes [2-3].

Available data from many researchers reported that garlic may be a promising candidate as a biological immune response modifier, stimulating necessary functions and suppressing unnecessary functions [21-24]. So, modification of immune function by garlic as resulted in the present study may contribute to treatment and prevention of many diseases caused by immune dysfunction, for example autoimmune diseases and tumour [21-24. 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 [21-24].

CONCLUSION

Results of this study suggest that garlic oil administration could stop the autoimmune disease process of T1DM, therefore, garlic oil has protective immunomodulatory effects against this disease. Garlic oil could be recommended as excellent candidate in the clinical management of T1DM.

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Disclosure: There are no conflicts of interest to declare.

REFERENCES

[1] Hanan F Al-Mutairi., Ameer M Mohsen and Zaidan M Al-Mazidi. Kuwait Medical Journal. 2007; 39(2): 107-115.
[2] Pietropaolo M and Pugliese A. The target organ: Embryology, biochemistry and physiology. In: Eisenbarth GS, physiology. Lafferty KJ, eds. Type 1 diabetes. Molecular, cellular and clinical immunology. Oxford University Press 2000; 53-75.
[3] Notkins AL, Lernmark A. J Clin Invest 2001; 108: 1247.
[4] Rabinovitch, A, Suarez-Pinzon WL. Biochem Pharmacol 1998; 55: 1139-1149.
[5] Bach JF. Transplant Proc 1994; 26: 3188-3190.
[6] Kikutani H, Makino S. Adv Immunol 1992; 51: 285-322.
[7] Metwally M. World J Fishd Marine Sci 2009; 1(1): 56-64.
[8] Eidi A, Eidi M, Esmaeili E. Phytomed 2006; 13: 624-629.
[9] Mor Oron-Herman, Talma Rosenthal, David Mirelman, Talia Miron,Aharon Rabinkov, Meir Wilchek, Ben-Ami Sela. Atherosclerosis 2005; 183: 238-243.
[10] Gruenwald J. Allicin in Garlic, nature's antibiotic; New Straits Times, Body & Soul. 2005.

[11] Banerjee S and Maulik S. Nut J 2002; 1(4): 1475-2891.
[12] Tooba Ghazanfaria, Zuhair M. Intl Immunopharmacol 2002; 2: 1541-1549.
[13] Ohaeri O. Biosci Rep 2001; 21: 1.
[14] Padiya R, Khatua T, Bagul P, Kuncha M and Banerjee S. Nutr Metabol 2011; 8-53.
[15] Sally SS, Mustafa, Nihad I Eid, SA Jafri, Hekma A, Abd El-Latif and Helmy MS Ahmed. Pakistan J Zool 2007; 39(5): 279-284.
[16] AHM Nurun Nabi, Laila N Islam, Mohammad Mahfuzur Rahman, and Kazal Boron Biswas. J Biochem Mol Biol 2005; 38: 661-667.
[17] Alison M Gurney and Frank C Howarth. Cardiovascular Diabetology 2009; 8(4): 1475.
[18] Rizwan Ashraf, Rafeeq Alam Khan and Imran Ashraf. J Med Plants Res 2011; 5(13): 2922-28.
[19] Birdee GS, Yeh G. Clin Diabetes 2010; 28(4): 147-155.
[20] Cheng-Tzu Liu A, Hunry Hsea, Chong-Kuei Liia, Phi-Sam Chena and Lee-Yan Sheen. European J Pharmacol 2005; 516: 165-17.
[21] Kikai Kyo, Naoto Uda, Shigeo Kasuga and Yoichi Itakura. The J Nutr 2001; 131: 1075-1079.
[22] Donald L Lamm and Dale R Riggs. The J Nutr 2001; 131: 1075-1079.
[23] Chandrashekar PM, Venkatesh YP. J Ethnopharmacol 2009; 124: 384-90.
[24] Clement F, Siddanakoppalu NP, Yeldur PV et al. Intl Immunopharmacol 2010; 10: 316-324.