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Histopathological and Biochemical Effects of *Allium Sativum* Oil Administration on Type 1 Diabetic rats

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ABSTRACT

Allium sativum (AS) has been widely recognized as hypoglycemic agent against type 1 and type 2 diabetes mellitus, however, little is known about its effect on damaged pancreatic tissue in insulin dependent type 1 diabetes (IDDM). The research purpose was to experimentally investigate the histological effects of AS oil administration in IDDM with an attempt to find a relation between these histological findings and biochemical effects on damaged pancreatic tissue. We have evaluated with the help of ELISA kits the levels of serum insulin in male Sprague-Dawley rats with streptozocin-induced IDDM in addition to measuring of blood glucose and body weights. All biochemical results were compared with AS effects on pancreatic histological changes. The four groups (6 rats each) under study received or not different intraperitoneal doses of AS for a period of 30 days. Daily intraperitoneal administration of AS (either low dose 10 mg/kg or high dose 20 mg/kg) for up to 30 days to type1 diabetic rats effectively reduces levels of blood glucose with significant effect on rats body weights, in addition, reduced levels of serum insulin due to damaged Langerghans islet cell was significantly increased in the serum due to repairing tissue process in pancreatic tissues. These experimental results suggest that AS treatment has therapeutic protective effects against biochemical changes occur in IDDM with significant repairing histological effects on damaged pancreatic tissue.

Keywords: Allium sativum; Type1 diabetes mellitus; Serum insulin, blood glucose, histological effect; pancreas.

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INTRODUCTION

Type 1 Diabetes mellitus or insulin-dependent diabetes mellitus (IDDM) 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 IDDM 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 IDDM 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]. Although, the people who have suffered from diabetes, medicinal therapy is insulin which is the only treatment for IDDM patients, but because this treatment is a biochemical agent so leads to many side effects.

Allium sativum(AS), commonly known as garlic, is a species in the oinion genus, with a history of human use of over 7000 years. AS is almost certainly one of the first known medicinal plants, has become popular preventative and treatment alternatives [3]. Also is a hardy, perennial bulb which is native to the Mediterranean regions, Africa and Europe and for thousands of years amazing magical and medicinal powers have been attributed to garlic [4]. There are many components inside garlic, such as APDS -allyl propyl disulphide, Allicin-diallyl disulphide oxide, flavonoids and many others.[5-6].

AS is still employed in folklore medicine today in many part of the world both for prophylaxis and for the healing. Natural substances in AS are very beneficial because it can work as antibacterial, antiviral including the common cold virus and anticancer [7]. AS is regarded as one of the most effective remedies to lower blood pressure. There have been numerous clinical studies which have employed various preparations of AS to treat hypertension [8]. AS has been proven to be hypolipidemic, hypocholestrolemic [9], hypoglycemic in type 2 diabetes and decrease the effect of diabetic retinopathy, diabetic nephropathy [10], in addition, could ameliorate the impaired renal function, inhibit liver damage and induced free radicals associated with experimental diabetes. [11]

Many studies have examined the hypoglycemic effect of AS in both types of diabetes [12-14]. The probable mechanism underlying AS hypoglycemic in type 2 diabetes mellitus most likely is improved insulin secretion and sensitivity [15]. However, till now the mechanism has not been reported regarding type 1 diabetes mellitus. Therefore, our present study was carried out in UiTM Malaysia to investigate the potential effects of AS oil administration on the histological and biochemical findings in IDDM.

MATERIALS & METHODS

Experimental Animals

The animals were used for the duration of the experiments are male Sprague-Dawley rats with an average weight of 150-250g and an average age of 12-16 weeks, obtained from Scientifacts 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). 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 was maintained in the quarantine.

Plant Material and Chemicals

Ready Allium sativum oil was purchased from Nano Life Quest Company (sigma). The AS 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 Nano Life Quest Company (Sigma). All other chemicals used in this study were obtained from same brand Nano Life Company.

Study Protocol

Type 1 diabetic was induced in overnight fasted animal group by intraperitoneal injection with a single dose of STZ (65mg/kg body weight) [16]. STZ was dissolved in sodium citrate buffer solution (PH 4.5) immediately before used. 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 (citrate buffer, 65 mg/ Kg body weight) ; Group B (GB; untreated STZ-diabetic rats) ; Group C (GC; STZ-diabetic rats treated with10 mg/ kg, i.p., AS oil) ; Group D (GD; STZ-diabetic rats treated with 20 mg/ kg, i.p., AS oil).

The treatment by plant was started from the same day to the group A and B only for a period of 30 days. During this period, animals in all groups have 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 [17]. 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).

Laboratory Tests

After 30 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

centrifuged. On the other hand some samples took even more time for being completely clotted. 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 biochemical study. Serum was assayed for insulin level using enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (USCNK, CHINA). On the last day of experiment, the tail parts of the pancreas were removed and kept in 10% formaldehyde for histological examination. The tail part (Spelinic) of the pancreas was removed and kept in 10% phosphate buffered. Through the surgical blade was cut Slices 2–3 mm thick for tissues samples. The samples were processed using a tissue processor and embedded in paraffin. Paraffin blocks were cut using a microtome cutter and stained with Haemotoxylin and Eosin (H&E).

Statistical analysis

The data are expressed as mean ± SE. 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. The test to determine the effect of individual treatments on differences among means when the analysis of variance indicated a significant result which follow by repeat one way analysis of variance (ANOVA) with post hoc least significant difference (LSD). $P < 0.05$ was taken to indicate significance.

RESULTS AND DISCUSSION

Biochemical Findings

During the 30 days of the experiment, the fasting blood glucose (FBG) concentration of the control group was significantly changed (figure.1), and body weight of the control group continued to rise during 30 days of experiment (figure.2). Induction of diabetes with streptozotocin was associated with the characteristic development of a slower rate of body weight gain, and higher Fasting blood glucose levels than in the control rats. Compared with that in the AS -treated diabetic groups, the rate of body weight gain were significantly affected by treatment with AS oil .The fasting blood glucose concentration of the diabetic rats was also decreased by treatment with both doses of AS. (figure.1 &2).

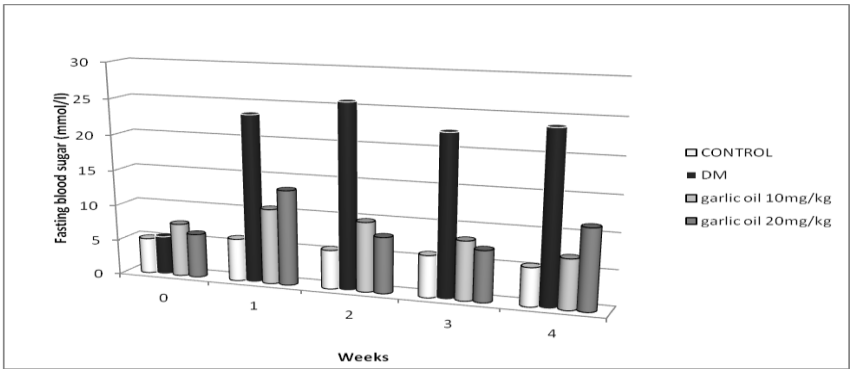

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Figure1: Fasting blood sugar for all rat groups with/without treatment

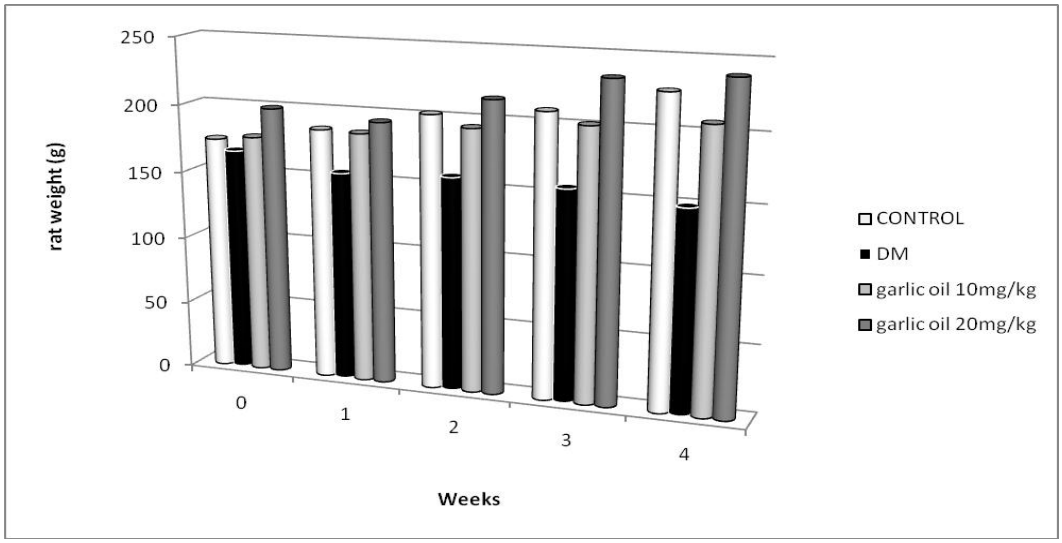
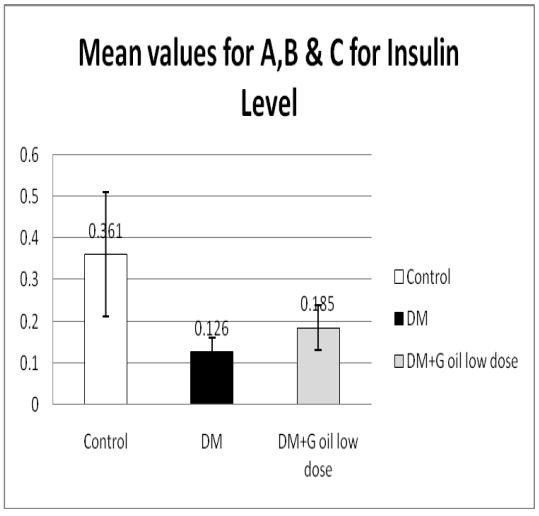


Figure2. Body weight for all rat groups with/without treatment

Among diabetic rats group without treatment (GB), the serum insulin levels were significantly decreased when compared with control group (GA), meanwhile, treating rats with AS specially in high dose: levels of the insulin were significantly higher compared to those of the control groups at the end of the experiment (p=0.001). (Figure 3 a and 3b)



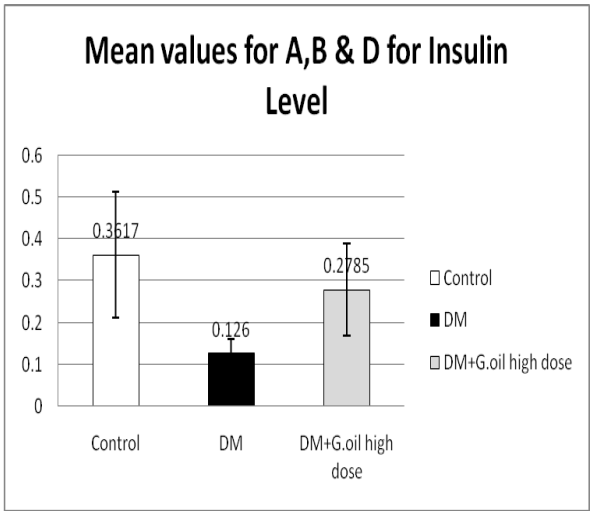


Fig 3b: Effect of treatment of diabetic rats with garlic high dose 20 mg/kg on serum insulin levels.
Fig 3a: Effect of treatment of diabetic rats with garlic low dose 10 mg/kg on serum insulin levels.

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Histopathological findings

Pancreatic sections stained with hematoxylin and eosin (H &E) obtained from normal control rats (Group A) showed normal histologicy (Figure 4a). Meanwhile, pancreatic tissue obtained from untreated diabetic rats (Group B) showed severe degenerative changes of the pancreatic islets, particularly the cells in the center of the islets, relative reduction in the size and number of the islets (Figure 4b).

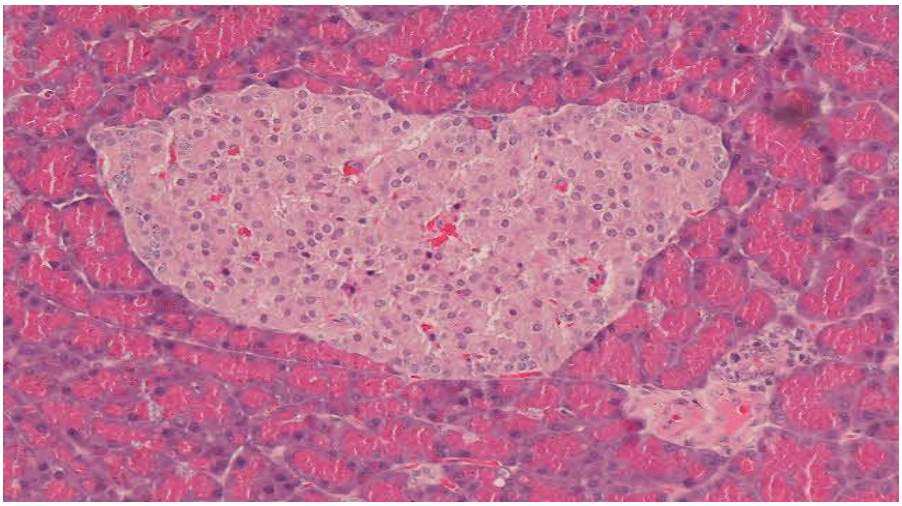

Figure 4a: Histological appearance of pancreas related to normal control rats group. (X 20, H & E).

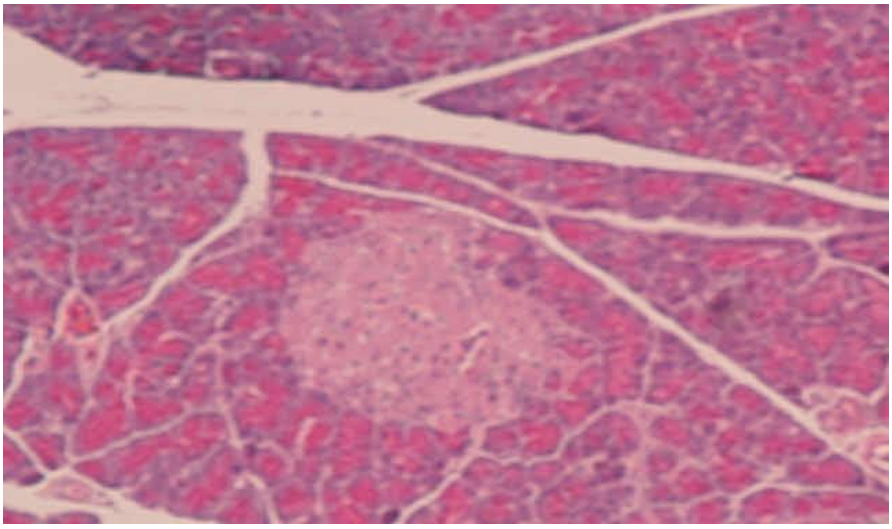

Figure 4b: Histological appearance of pancreatic tissue related to diabetic rats group without treatment. (X 20, H & E).

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Microscopic examination of the Pancreas of the treated rats with low dose AS oil showed that the shape of pancreatic islets cells, relatively irregular, with some normal cells and some others still appeared degenerated. (Figure 4c) Meanwhile, the group, which consumed AS high dose, showed considerable effects of AS on the diabetic histological changes of the pancreas. There were noted for regeneration and more improved in islets cells. (Figure 4d).

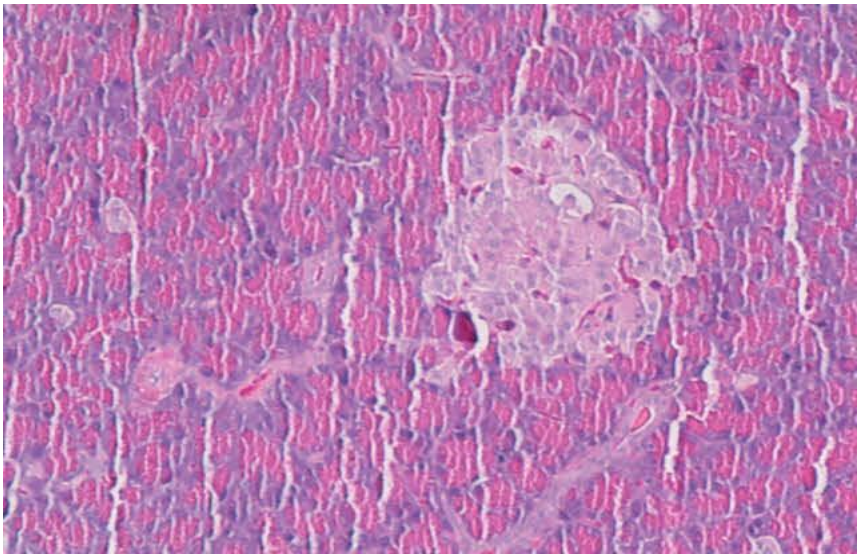

Figure4c: Histological appearance of pancreas related to diabetic rats group treated with low dose of AS (10mg/kg). (X 20, H & E).

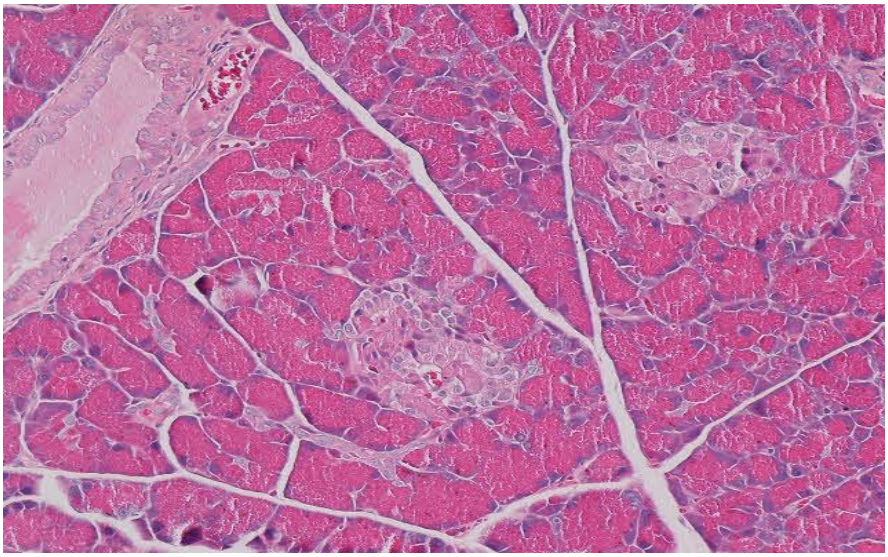

Figure 4d: Histological appearance of pancreas related to diabetic rats group treated with high dose of AS (20mg/kg). (X 20, H & E)

DISCUSSION

Many studies reported that Allium sativam (garlic) possesses a variety of medicinal properties such as anti hyperglycemic agent but in type2 diabetes. However the results of the present study showed that daily i.p administration of low dose 10mg/kg and high dose 20mg/kg of AS ol for 30 days decreased the blood glucose concentrations into normal range in the streptozotocin-induced type1 diabetic rats. These results are consistent with findings of previous research that garlic had a significant effect in reducing blood sugar in type 2 diabetes [18-19]. This hypoglycemic action of garlic may possibly be due to an increase in pancreatic secretion of insulin from β-cells, after tissue healing due to garlic treatment.

In the present study, IDDM rats had the lowest body weight change during experiment. Similarly, many studies showed that type2 diabetic animals had significantly lower weight gain than the control groups [20-21]. The results of our study agreed with the previous studies which showed that garlic oil treatment generally normalizes body weight rate in streptozotocin diabetic rats.

In our experiment, there was decreasing in the serum insulin level due to the effect of STZ induction. However, after the AS oil was given i.p during a period of four weeks increased serum insulin levels in treated diabetic rats as compared with control diabetic rats. This was consistent with other studies that have shown AS may act as an antidiabetic agent by increasing either the pancreatic secretion of insulin from the β-cells or release of bound insulin and antioxidant effect of S-allyl cysteine sulfoxide [14, 19].

These biochemical finding are consistent with the histological findings that obtained in this study. The pancreatic ß cells were destroyed after induction of IDDM by using of STZ. STZ has a destructive effect on the beta cells of the pancreas [22]. Histopathological study of diabetic untreated rats (GB) showed degeneration of pancreatic islet cells. However, this probably leads to insulin deficiency which causes excessive elevation of blood glucose [23]. AS treatment specially at high dose to the diabetic rat groups lead to increased volume density of islets and increased percentage of beta cells, which may be a sign of regeneration. Signs of regeneration of ß cells, potentiation of insulin secretion from surviving ß cells of the islets of Langerhans and decrease of blood glucose have been reported following consumption of this plant [24-25].

CONCLUSION

Allium sativum (garlic) is able to normalize the blood glucose levels in type 1 diabetic rats with increase in serum insulin levels due to histological repair of damaged pancreatic tissue occurred due to the disease process. These findings give additional evidence about AS to be used in management and prevention of IDDM.

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