The potential effect of garlic extract against complication accompanied with induced diabetes in rats

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A B S T R A C T

The present study was to determine the effect of aged garlic extract (AGE) on alleviating diabetic cardiomyopathy (DCM) in rats. An experimental diabetic rat model was induced by a single dose (60mg/kg body weight) of I.P injection of streptozoticin (STZ). AGE was orally administrated at a dose of 500 mg/kg body weight. Metabolic profiles include serum glucose, insulin, total cholesterol and triacylglycerols and myocardial enzymes activities as creatine kinase-isoenzyme (CK-MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were determined. The obtained results indicated that administration of AGE increased the body weight and insulin concentration, meanwhile, significant decrease in serum glucose concentration, CK-MB and LDH activities when compared with untreated induced diabetic group. Total cholesterol, triacylglycerols and AST showed non-significant changes in serum values in AGE treated diabetic rats in comparing with STZ-induced diabetic group. From the obtained results it could be concluded that, AGE has great potential therapeutic effect in the diabetic cardiomyopathy and other complications of coronary heart disease.

Keywords: Diabetic cardiovascular diseases, aged garlic extract, myocardial enzymes.

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1. INTRODUCTION

Diabetes mellitus (DM) is a spectrum of metabolic disorders of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action, and it was believed that it became a major health challenge worldwide (American Diabetes, 2011; Nammi et al., 2003). It is associated with hyperglycemia and oxidative stress which generally cause several tissue damage and subsequently degenerative complications in many organs such as the heart (Adeghate, 2001; Jemai and Sayadi, 2015). Individuals with diabetes and with chronically poor metabolic control may have microvascular and macrovascular complications leading to a significant burden (Harris and Zimmet, 1997). This burden includes direct costs of medical care and indirect costs, such as loss of productivity as a result of diabetes – related morbidity and premature mortality (American Diabetes Association, 2008). Cardiovascular diseases (CVDs) is the major complication of diabetes; it is the leading cause of death in individuals with diabetes (50% of deaths) and occurs 2- to 4 fold more often than in people without (DM) (Nammi et al., 2003; Thent et al., 2012). Epidemiological studies have shown that diets rich in spices are associated with a low risk of cardiovascular diseases, and might offer a natural key to unlock diabetic complications. Aged garlic extract which is an odorless alternative source of garlic, rich in antioxidants; has been shown to modulate cardiovascular risk factors in both clinical and preclinical settings (Borek, 2006; Morihara et al., 2011; Steiner and Li, 2001).

Therefore, the aim of the present study was to determine the ameliorative effect of aged garlic extract as a phyto-medicinal on experimentally induced diabetic cardiomyopathy in rats.

2. MATERIAL & METHODS

2.1. Streptozotocin (STZ):

STZ was obtained from MP Biomedical, LLC, USA Reorder, and Stored at -20°C.

2.2. Aged garlic extract (AGE, Kyolic) Preparation:

Sliced raw garlic was dipped into aqueous ethanol 15-20 % and extracted for 20 months at

room temperature in stainless steel tanks after separation of the solution the extract was generally concentrated and used (Amagase et al., 2001).

2.3. Experimental animals:

Fifty-four white male albino rats weighing between (230±20g) were purchased from the animal house unit (Benha University, Faculty of Veterinary Medicine, Animal Breeding and Research Center). The rats were kept under a 12h light-dark cycle and ambient temperature was maintained at 25 ˚C. Animals were allowed free access to water and were fed on uniformly basal diet. The animals were kept under hygienic conditions for at least two weeks for acclimatization before the beginning of the experiment.

2.4. Induction of diabetes:

Rats were subjected to hyperglycemia by intraperitoneal injection of freshly prepared STZ (dissolved in 0.1 M citrate buffer, pH 4.5) as a single dose (60 mg/kg body weight) in a volume of 1ml/kg body weight. three days after STZ injection; the blood samples were collected from retro-orbital venous plexus of eyes by using fine capillary glass tubes and used directly for blood glucose determination. Rats with blood glucose level ranged from 280–350 mg/dl were considered diabetic and included in the study (Cam et al., 2003; Ganda et al., 1976).

2.5. Experimental design:

The rats were randomly divided into 3 equal groups (18 rats each) as the following: group 1: was injected with citrate buffer saline only and was served as a control normal for all experimental groups. Group 2: (STZ-Diabetic) was given saline by an oral gavage and served as non-treated diabetic group. Group 3: AGE treated diabetic group was administered orally at a dose of (500 mg/kg body weight/day) (Shiju et al., 2013). After fifty-six days of treatments (the end of the experimental period) and in the basal fasting state, experimental rats of each group were weighed separately and average body weights were recorded. Random blood samples were collected from retro-orbital venous plexus of eyes in clean, dry screw capped tubes. Samples were allowed to coagulate at room temperature for 30 minutes and centrifuged at 3000 rpm for 15 minutes. The clean, clear serum was aspirated by pasture pipettes and received in dry sterile sample tubes, processed directly for glucose determination then kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

2.6. Biochemical Analysis:

Serum glucose, insulin, total cholesterol, triacylglycerols, creatine kinase-MB, lactate dehydrogenase, aspartate amino transferase were determined according to the methods described by Allain et al. (1974); Baba (1979); Schettler and Nussel (1975); Trinder (1969); Urdal and Landaas (1979) Kornberg (1955) and Reitman and Frankel (1957), respectively.

2.7. Statistical analysis:

All data were presented as the mean ± Standard Error (SE). The data was evaluated by a one-way ANOVA using SPSS (ver.16). The means were assessed for differences through Least significant difference (LSD). The differences were considered statistically significant at $p<0.05$.

3. RESULTS

STZ-induced diabetic rats showed significant decrease in the body weight and serum insulin concentration accompanied with marked increase in glucose, triacylglycerols, CK-MB, LDH and AST serum values. Meanwhile, there was a non-significant change in serum total cholesterol concentration when compared with the control normal rats (Table 1). Regarding AGE treated – diabetic rats, the obtained results showed significant elevation in the body weight and serum insulin concentration accompanied with significant reduction in serum values of glucose, CK-MB and LDH. However, non-significant effect on serum values of total cholesterol, triacylglycerols and AST as compared with STZ-induced diabetic rats were recorded (Table 1)

4. DISCUSSION

Diabetes is a metabolic disorder affects 1–2 % of the population worldwide, its global prevalence is expected to reach 360-380 million between 2025 and 2030 (Wild et al., 2004). Hyperglycemia plays an important role in the pathogenesis of long-term complications, affect organs where cells do not require insulin for glucose uptake, such as those of the nervous system, heart, kidneys and small blood vessels resulting in retinopathy, nephropathy, neuropathy, cardiomyopathy, cataract, and atherosclerosis. Diabetic patients with poor glycemic control are at risk of various diseases, morbidity and mortality among people with diabetes mellitus are mostly triggered by cardiovascular disease (American Diabetes, 2010; Rutter et al., 2003).

Table (1): The effect of aged garlic extract on body weight and serum level of (glucose; insulin; total cholesterol; triacylglycerol; creatine kinase-isoenzyme (CK-MB); lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) in streptozotocin-induced diabetic rats

Parameter	Group (1)	Group(2)	Group(3)
Body weight (gm)	402 ±14.98[a]	214±18.04[c]	266±12.93[b]
Glucose(mg/dl)	117.50±4.40[b]	329.50±42.86[a]	156.83±28.06[b]
insulin (µl/ml)	3.75±0.11[a]	1.10±0.18[c]	2.84±0.40[b]
Total cholesterol (mg/dl)	98.50±4.15	112±17.18	87.67±5.47
Triacylglycerols (mg/dl)	115±1.35[b]	144±11.4[a]	143±3.33[a]
CK-MB (U/L)	129±5.59 [d]	604±12.85[a]	482±9.46 [b]
LDH (U/L)	1118±26.59[d]	4739±120.17[a]	3045±36.91[b]
AST (U/L)	87.50± 1.89[c]	174 ± 16.10[a]	149 ± 14 [ab]

Groups1, 2 and 3: Control group, diabetic group, and aged garlic extract treated diabetic group, respectively. Data are represented as (Means ±S.E.). Values with different letters within the same row significantly differed at ($p< 0.05$)

The data recorded 3 days after intra-peritoneal injection of 60 mg/ kg body weight of (STZ) and at the end of the experiment (8 weeks) came in agreement with Eileen Dolan (1997) and Wei et al. (2003) who reported that, STZ is a permanent diabetes inducing drug and the course of the condition for (8 weeks) is necessary to study the mechanisms of the chronic changes in the cardiovascular system that accompany STZ-induction in experimental rats. Eleazu et al. (2013) referred to the toxic action of (STZ) to its selective uptake into β cells via its low affinity glucose transporter (GLUT2) present in the plasma membrane, leading to inhibition of insulin secretion of beta cells, impair the pancreas and glucose metabolism.

STZ- induced diabetic rats showed a significant decrease in the body weight, the results are in a harmony with Thomson et al. (2016) who reported that rats lost weight and became over 50 % lighter after 8 weeks of STZ-inducing diabetes. Reduction in body weight in diabetic rats might result from sever polyuria which accompanied the untreated hyperglycemia. In addition to degradation of fat and structural proteins due to unavailability of carbohydrates for utilization as a source of energy (Juarez-Rojop et al., 2012). Also, STZ-induced diabetic rats developed significant hyperglycemia and hypoinsulinemia. These results agreed with Thomson et al. (2016) who noticed continuous increase in blood glucose level accompanied with approximately 11-fold decrease in serum insulin level in untreated STZ-diabetic rats compared to control normal one after induction of diabetes with STZ throughout the experimental period (8week). Eleazu et al. (2013) regarded the chronic changes that accompany STZ-induced diabetes to releasing of nitric oxide (NO), that mediates carbamoylation and alkylation of cellular components and destruction of pancreatic β islet cells through DNA damage and cell necrosis. While there was a non-significant effect in total cholesterol level. These findings are consistent with Wei et al. (2003) and Zubaidah et al. (2014) who reported that, chronic STZ-diabetic rat mimics many but not all of the chronic complications observed in the diabetic human; with explanation that glucose obtained from food cannot be utilized as an energy source by the body, so that the body uses energy from other sources, one of them lipid; insulin deficiency causes inhibition of lipogenesis and increased lipolysis resulting in mobilization of fatty acids from adipose tissue. Increased mobilization of fatty acids inhibits the glycolytic pathway, fatty acid synthesis, and encourages beta oxidation in liver to acetyl Co-A. High levels of acetyl Co-A in the liver would increase pathways that use acetyl co-A, the ketogenesis pathway and fatty acid synthesis.

Cardiac biomarkers: CK-MB; LDH and AST showed a significant increase in serum level of these three cardiac enzymes. The current study agreed with those of Suanarunsawat et al. (2016) who found that DM impaired the liver, kidney and cardiac functions of the diabetic rats by augmenting serum levels of AST, ALT, creatinine, blood urea nitrogen, LDH and CK-MB. Also, Badole et al. (2015) reported that, any serious insult to the heart muscle will enhance the release of AST, CK-MB, and LDH enzymes into the serum of diabetic animals. Likewise, Feng et al. (2008) have been suggested that peak rise in LDH is proportional to the extent of injury to the myocardial tissue.

Concerning to the effect of Aged garlic extract (AGE) treatment on the body weight, results showed recovery in body weight after an initial loss

following STZ injection. The obtained results are in a harmony with Shiju et al. (2013) and Thomson et al. (2016) who noticed that the STZ-diabetic rats treated with the 300,500 or 600 mg/kg doses of AGE stabilized their weight after an initial weight loss, with improving polydipsia, polyphagia and polyuria. On the other hand, we found a significant reduction in serum fasting glucose level accompanied with a significant elevation in insulin level in AGE-treated diabetic group.

Our finding agreed with Eileen Dolan (1997), who reported that treating diabetic rats with either 300 or 600 mg/kg of AGE significantly decreased blood glucose and markedly increased serum insulin. In this regard, Cam et al. (2003) and Wild et al. (2004) reported that garlic may acting as an insulin secretagogue, release of bound insulin or increase of insulin sensitivity.

While Thomson et al. (2016) found that increasing in SAC and polyphenol compounds during aging of garlic could be responsible for stronger antioxidant activity of AGE which resulted in its hypoglycemic effect. While there was a non-significant decrease on each of total cholesterol and triacylglycerol levels.

The findings agreed with Borek (2006) who detected that treatment of type 2 diabetic patients with 3000 mg of AGE daily had no effect on serum cholesterol and triacylglycerol after 3 months of treatment. After the oral administration of AGE to STZ- diabetic rats, results showed significant decrease in serum cardiac enzymes (CK-MB and LDH).

While there was a non-significant decrease in AST. These findings are indicative to the fact that of extent of cardio protection offered by the drug is associated with significant attenuation of plasma creatine kinase and LDH levels (Majithiya and Balaraman, 2005; Nammi et al., 2003). Interestingly, Capasso (2013) and Thomson et al. (2016) reported that, increasing in SAC and polyphenol compounds during ageing causes increasing in AGE antioxidant properties; involving the ability to scavenge reactive oxygen (ROS) and nitrogen (RNS) species; increase of enzymatic and non-enzymatic antioxidants levels; activating Nrf2 factor; or inhibiting some prooxidant enzymes (xanthine oxidase, cyclooxygenase, and NADPH oxidase).

5. CONCLUSION

The present study revealed that AGE has potential therapeutic in the treatment of DCM by attenuating metabolic and myocardial enzymes disorders in diabetic rat.

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