

Effect of Kyolic® aged garlic extract on glycaemia, lipidaemia and oxidative stress in patients with type 2 diabetes mellitus

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Abstract

Background: Diabetic patients with hyperglycaemia show oxidative stress and increased formation of advanced glycation endproducts (AGEs) which increases their susceptibility to chronic complications. Aged garlic extract has antioxidant properties and prevents the formation of AGEs *in vitro*. This study investigated the effects of dietary intervention with Kyolic® aged garlic extract on glycaemia, lipidaemia and oxidative stress in diabetic patients.

Methods: Blood samples were collected from 48 diabetic patients on recruitment, after one month and then monthly following an intake of 3000 mg of aged garlic extract daily over a period of 3-months. Samples were analysed for glucose, glycated haemoglobin and lipid profile using automated analyses. Low molecular weight AGEs were measured using a fluorometric method. Lipid hydroperoxides and total antioxidant status were determined using colorimetric kit methods.

Results: Intervention with aged garlic extract did not affect blood glucose, glycated haemoglobin or the lipid profile but serum triacylglycerol concentrations declined after 3-months of intervention ($P < 0.05$). Aged garlic extract intake did reduce levels of serum AGEs although this was not significant. Lipid hydroperoxide, an indicator of oxidative stress, was significantly reduced following intake of aged garlic extract ($P < 0.05$).

Conclusion: Aged garlic extract may therefore protect against the harmful effects of AGEs and oxidative stress thus further investigations are needed to fully evaluate the benefits of long-term consumption of aged garlic extract, in particular its effects on tissue AGEs and oxidative stress.

Keywords: Diabetes mellitus, advanced glycation endproducts, aged garlic extract, antioxidant, lipid peroxidation, hyperglycaemia.

Background

Diabetes mellitus is characterised by hyperglycaemia, dyslipidaemia and increased oxidative stress. A consequence of hyperglycaemia is increased protein glycation and formation of advanced glycation endproducts (AGEs). Accumulation of AGEs alters the structure and functions of biomolecules particularly long-lived proteins [1,2]. Increased glycation and AGE formation are also accompanied by oxidative stress and are major factors underlying the pathogenesis of diabetic complications [1,2]. The inhibition of AGE formation is thought to play a role in the prevention of diabetic complications thus, the development of clinically effective anti-glycation compounds with antioxidant properties may have therapeutic potential [3,2].

Numerous studies have reported the beneficial effects of using garlic (*Allium sativum*) in the prevention of disease processes largely attributed to its high content

of organosulphur compounds and resultant antioxidant activities [4,5]. The use of garlic as a health supplement has received much interest, particularly because of its beneficial effects in protecting against coronary heart disease [5]. Fresh garlic has a pungent taste and to some people, an unpleasant smell. However, studies have shown that garlic does not have to be fresh to be effective nor is its smell required for its health benefits. Commercially, garlic is available in different preparations, one of which is aged garlic extract. This extract is odourless and richer in antioxidants than the fresh bulbs [6,7].

Clinical trials have shown that aged garlic extract is safe and is effective in providing health benefits to humans [5,8]. Aged garlic extract is a potent antioxidant with established lipid-lowering effects [9]. Its antioxidant activity is attributed largely to a key ingredient called S-allylcysteine (SAC), which is a potent antioxidant and free radical scavenger [10]. Previous studies have shown that

both aged garlic extract and SAC are effective inhibitors of AGE formation *in vitro* [11,12]. However, it is not known whether aged garlic extract can prevent formation of AGEs *in vivo* hence the need for the present study. The aim of this study was to investigate the protective effects of a dietary intervention with aged garlic extract against glycaemia, lipidaemia, and oxidative stress over a period of three months in patients with type 2 diabetes mellitus.

Materials and methods

Kyolic® aged garlic extract one a day tablets were a generous gift from Wakunaga of America Co., Ltd. Kyolic® aged garlic extract is prepared by soaking fresh sliced garlic at room temperature in 15-20% ethanol for 20-months. The extract is then filtered and concentrated under reduced pressure at low temperature. The content of water-soluble compounds is relatively high whereas that of oil-soluble compounds is relatively low. This process increases its antioxidant concentration and converts harsh unstable compounds, such as allicin, to stable health-promoting substances such as SAC and S-allylmercaptocysteine. The extract has 305 g/l of extracted solids and SAC is the most abundant water-soluble organosulfur compound present at a concentration of 1.47 g/l. Other components of aged garlic extract include N-fructosyl glutamate, N-fructosyl arginine, tetrahydro-beta-carboline derivatives, allixin and selenium.

Subjects

A total of 48 diabetic subjects (25 males and 23 females) were recruited from the clinics of King Abdul Aziz University Hospital, Jeddah, Saudi Arabia. This study was approved by the Ethics Committee of King Abdul Aziz University Hospital. All subjects gave informed consent before participation in the study. All patients were interviewed and a questionnaire completed to collect information such as their age, smoking habits, duration of diabetes, type of medication used for treatment, and the presence of clinical complications. All subjects were taking oral hypoglycaemic agents for treatment of their diabetes. Patient's heights, and weights were measured and their body mass index (BMI) was determined. The inclusion criteria were individuals diagnosed with type 2 diabetes on medication and with normal serum creatinine. Exclusion criteria were those with high creatinine and suffering from diabetic complications such as nephropathy, neuropathy, retinopathy or heart disease.

Blood collection

Overnight fasting venous blood samples were taken from all patients at recruitment, one month following recruitment and before any aged garlic extract intake. Immediately thereafter, each patient was given three tablets of Kyolic® aged garlic extract (each containing 1000 mg of aged garlic extract) with their meals on a daily basis for a period of 3-months and blood samples were taken as before at the

end of the first, second and finally the third month. Serum was separated immediately and frozen at -80°C until needed for assay. Samples were analysed for concentrations of serum glucose, glycated haemoglobin, serum low molecular weight-AGEs (LMW-AGEs), lipid profile, total antioxidant status and lipid hydroperoxides.

Biochemical methods

Glucose: Serum glucose concentrations were measured using an automated method on the Dade Behring Dimension RXL Clinical Chemistry Analyzer and based on a hexokinase-glucose 6-phosphate dehydrogenase method [13].

Glycated haemoglobin: This was determined using a turbidimetric inhibition immunoassay using the automatic Dade Behring Dimension RXL Clinical Chemistry Analyzer and based on an established procedure [14].

Advanced glycation endproducts: Serum LMW-AGE concentrations were determined by a fluorometric method as described previously [15]. Briefly, a total of 20 µl of serum was mixed with 480 µl of 0.15M trichloroacetic acid to precipitate out the large-*Mr* serum proteins, and 100 µl of chloroform was added to extract the lipid fractions. After vortex mixing to ensure complete precipitation of the large-*Mr* proteins, the samples were centrifuged and the aqueous supernatant removed. The fluorescence was measured at 440 nm after excitation at 247 nm, using a LS 45 fluorescence spectrometer (Perkin-Elmer, USA). Due to the lack of a commercial standard for this product, the results were reported in arbitrary units per mg of protein (AU/mg).

Serum creatinine: Serum creatinine concentrations were measured by a modification of the Jaffe reaction [16] using the automatic Dade Behring Dimension RXL Clinical Chemistry Analyzer.

Cholesterol: Serum total cholesterol, LDL and HDL cholesterol measurements were determined colorimetrically using the automatic Dade Behring Dimension RXL Clinical Chemistry Analyzer and based on an established enzymatic method [17]. Serum triacylglycerol was also measured using the same analyser and based on an established procedure [18].

Lipid hydroperoxides: Lipid hydroperoxides concentrations were determined directly by utilizing redox reactions of lipid hydroperoxides with ferrous ions using the lipid hydroperoxide assay kit (Calbiochem, USA) and based on an established method [19].

Total antioxidant status: Serum total antioxidant status was determined using a method based on the quenching of 2,2'-Azino-di(3-ethyl benzthiazolin sulphonate) radical cation (ABTS^{•+}) by antioxidants using a Randox total

antioxidant assay kit as described previously [20].

Statistical analysis

Statistical analyses were accomplished using the statistical software package SPSS (version 15; SPSS Inc., Chicago). Results are expressed as mean \pm standard deviation (SD). Differences between groups were analysed with analysis of variance (ANOVA) for multiple comparisons. Paired comparison student t-test between intervals was undertaken, thereafter, where ANOVA results indicated a significant difference. The limit of statistical significance was set at $P < 0.05$.

Results

The subjects studied suffered from type 2 diabetes mellitus and their descriptive details are given in **Table 1**. Most of the 48 subjects were overweight and indeed their mean BMI is close to the value of 30 kgm^{-2} , above which individuals are classified as being obese. Only 6 of the 48 subjects (13%) were smokers.

Table 1. The descriptive characteristics of subjects used in this study. Data are presented as mean \pm SD (n= 48).

Age (years)	49.2 \pm 7.88
Weight (kg)	78.42 \pm 16.05
Height (cm)	162 \pm 7.79
Body mass index (kgm^{-2})	29.68 \pm 5.23
Duration of diabetes (years)	11 \pm 7.17
Males	25
Females	23
Smoker	6
Non-smoker	42

Table 2 shows the mean concentrations \pm SD of serum glucose, glycated haemoglobin and serum LMW-AGEs after taking 3000 mg of Kyolic[®] aged garlic extract each day over a period of 3-months during the intervention period. The results showed that throughout the intervention study no significant changes occurred in the concentration of serum glucose, or glycated haemoglobin. The serum LMW-AGE concentration does decrease over the intervention period but this decline is not significant.

Table 2 also shows the mean concentrations \pm SD of the serum lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triacylglycerol) after taking 3000 mg of Kyolic[®] aged garlic extract each day over a period of 3- months. The results show no significant changes in the concentrations of total cholesterol, LDL cholesterol or HDL cholesterol throughout the intervention study. However, serum triacylglycerol concentrations declined significantly after the 3-month intervention period compared to the base line value ($P < 0.05$).

Table 2 shows the mean concentrations \pm SD of lipid hydroperoxide. Serum lipid hydroperoxide concentrations showed a gradual and significant decrease in concentration compared with basal values ($P < 0.05$). Indeed the decline occurs after the first month of the intervention and was maintained over the 3-months of study. In contrast, there was no significant change in serum total antioxidant status between intervals.

Discussion

The subjects in this study were middle aged, overweight with a high BMI and typical of type 2 diabetic patients. This study has found no significant changes in fasting serum glucose and glycated haemoglobin throughout the different time intervals used. Studies on the hypoglycaemic effect of garlic have been controversial [21]. Several studies have shown that garlic can reduce blood glucose concentrations in streptozotocin-induced diabetic animals,

Table 2. The biochemical parameters of patients with type 2 diabetes mellitus at (A) recruitment, (B) one month after recruitment and before Kyolic[®] aged garlic extract intake, (C) one month (D) two months and (E) three months after Kyolic[®] aged garlic extract (3000 mg/day) intake. Results are presented as mean \pm SD (n=48) * $P < 0.05$ vs control (A).

	A	B	C	D	E
Serum Glucose (mM)	10.60 \pm 4.01	11.02 \pm 3.61	10.45 \pm 3.69	10.64 \pm 3.32	10.51 \pm 3.97
Glycated Haemoglobin (%)	8.28 \pm 1.60	8.41 \pm 1.65	8.76 \pm 1.77	8.71 \pm 1.68	8.77 \pm 1.81
LMW-AGEs (AU/mg)	7.09 \pm 5.40	6.86 \pm 5.06	6.14 \pm 5.76	6.32 \pm 5.05	5.63 \pm 4.47
Serum Creatinine (μM)	74.50 \pm 26.30	76.10 \pm 30.84	74.92 \pm 26.49	73.96 \pm 24.69	74.84 \pm 23.78
Total Cholesterol (mM)	5.30 \pm 1.10	5.23 \pm 0.98	5.12 \pm 0.97	5.16 \pm 0.96	5.25 \pm 0.86
HDL Cholesterol (mM)	1.24 \pm 0.29	1.21 \pm 0.26	1.20 \pm 0.28	1.21 \pm 0.24	1.20 \pm 0.26
Serum Triacylglycerol (mM)	2.02 \pm 1.25	2.07 \pm 0.94	2.00 \pm 1.00	2.07 \pm 1.15	1.88 \pm 1.06*
LDL Cholesterol (mM)	3.05 \pm 0.75	2.97 \pm 0.75	2.95 \pm 0.77	2.97 \pm 0.71	3.00 \pm 0.69
Serum Total Antioxidant Status (mM)	1.25 \pm 0.21	1.31 \pm 0.26	1.29 \pm 0.2	1.27 \pm 0.17	1.29 \pm 0.23
Lipid Hydroperoxide (μM)	7.52 \pm 2.68	7.10 \pm 2.77	6.13 \pm 3.80*	4.81 \pm 3.33*	5.44 \pm 3.44*

whereas such effects were not found in humans [22,23]. Daily administration of an ethanolic extract of garlic (50 mg/kg) to streptozotocin-induced diabetic rats for 4-weeks caused a significant reduction in their blood glucose concentrations [24]. Similarly, another study showed that streptozotocin-induced diabetic rats treated daily with 500 mg/kg of aqueous garlic extract have a significant reduction in serum glucose concentrations after 7-weeks of treatment [25]. Liu *et al.*, (2006) investigated the effects of garlic oil and diallyl disulfide on glycaemic control and renal function in streptozotocin-induced diabetic rats [21]. A dose of 100 mg/kg body of garlic oil or 40-80 mg/kg body weight of diallyl disulfide for 16-weeks after the induction of diabetes was used but neither affected blood glucose concentrations during this period. Eidi *et al.*, (2006) found that oral administrations of the ethanol garlic extract (0.1-0.5 g/kg) for 14-days in normal and streptozotocin-induced diabetic rats significantly decreased serum glucose in diabetic but not normal rats [26]. A small pilot study in humans indicated that daily intake of 4 ml aged garlic extract in patients with known coronary artery disease over one year did not affect their serum glucose concentrations [27]. A clinical trial reported that treatment of healthy adults with standardized garlic at 900 mg/day for 3-months produced no significant changes in serum glucose [28]. A further study showed that daily administration of four capsules of ethyl acetate garlic extract for 3-months had no effect on glucose concentrations in patients with coronary artery disease [29]. Also, the consumption of fresh garlic (approximately 3g daily) by male volunteers for 16-weeks did not affect the concentrations of serum glucose [30]. These findings are consistent with this study which was unable to demonstrate a reduction in blood glucose following aged garlic extract intervention in diabetic patients. The discrepancy between the human and animal studies might be accounted for by differences in physiology but also due to differences in the type of preparation, concentration of garlic used, and the duration of study. In animal studies, the dose and the duration of medication can be controlled, but not so in humans as in the latter it is difficult to be certain that all patients are taking the recommended dose.

The ability of aged garlic extract and its constituent SAC to inhibit formation of AGEs *in vitro* has been reported previously by our group [11,12]. However, studies investigating the effects of aged garlic extract *in vivo* have not been reported previously. The anti-glycation activity of aged garlic extract is believed to be due to its antioxidant constituent SAC which could prevent autoxidative and glycoxidation reactions involved in AGE formation. Furthermore, the amino groups on SAC could react with and block carbonyl groups from reducing sugars, Amadori products and dicarbonyl intermediates preventing their conversion to AGEs [12]. The present study reports for the first time a reduction in serum LMW-AGEs in diabetic patients following aged garlic extract intake

but this decline was not significant. This finding may be attributed to the slow formation of AGEs and the fact they tend to accumulate more readily on long-lived proteins such as collagen as opposed to short-lived proteins such as albumin, the major serum protein. Thus measurement of tissue AGEs in diabetic subjects may reveal a reduction in their content following consumption of aged garlic extract. Intake of higher doses of aged garlic extract for a longer period of time may also reduce the concentration of serum AGEs. In this study, serum LMW-AGEs were measured using fluorescence spectroscopy which is a crude method and subject to interference from other non-AGE fluorescent substances in the serum [15,31]. Furthermore, not all AGEs have fluorescent properties [32].

Intervention with aged garlic extract reduced triacylglycerol but not total, LDL or HDL cholesterol concentrations. These findings are consistent with one published study that found no effects on the concentrations of plasma LDL cholesterol and other lipids in adults with moderate hypercholesterolemia receiving 1.8 g of aged garlic extract 6 days/week for 6-months [33]. Another study demonstrated a 7% and 10% reduction in total and LDL cholesterol respectively following intake of 7.2 g of aged garlic extract daily for 5-months in a group of 56 moderately hypercholesterolaemic men whereas triacylglycerol and HDL remained unaffected [34]. The discrepancy between this and the earlier study may be because their subjects had higher initial total cholesterol values (6.3 ± 0.6 mM) compared to this study (5.3 ± 0.16 mM) and also because of the higher dosage of aged garlic extract used. The diabetic patients in this study had moderate cholesterol concentrations for which pharmaceutical intervention is not required. Furthermore, the duration of this study was shorter than that of the previous one and possibly not enough to detect even modest effects on serum lipid concentrations. The cholesterol lowering effects of aged garlic extract have been attributed to its key component SAC which has been reported to inhibit hepatic cholesterol synthesis [34].

Our data on inhibition of lipid peroxidation following aged garlic extract intake are in agreement with other studies [35,36]. Indeed, smokers receiving 5 mL of aged garlic extract for 14-days had a 35% reduction in plasma 8-*iso* prostaglandin F_{2α}, a specific marker of lipid peroxidation [37]. Similarly, another study found that aged garlic supplementation (10 g /day) for 4-months decreased the level of serum lipid hydroperoxides [38]. An animal study reported that aged garlic extract (0.2 and 0.4 g/kg) significantly decreased ($P < 0.05$) plasma malondialdehyde concentration in rats on a high cholesterol diet compared to control animals on a standard diet [39]. Oxidation of LDL plays a key role in the development and progression of atherosclerosis. Aged garlic extract has been shown to protect LDL against oxidation *in vitro* [40]. Other studies have shown that aged garlic extract and SAC prevent membrane

damage, loss of cell viability, and lipid peroxidation in bovine pulmonary artery endothelial cells exposed to oxidized LDL [38]. The high antioxidant potential of garlic is believed to be due to its high content of sulphur compounds.

The lack of effect of aged garlic extract intake on total antioxidant status in this study contrasts with a previous study where aqueous aged garlic extract supplementation (10 g/day) for 4-months in volunteer subjects improved their plasma antioxidant potential [38]. This discrepancy might be due to the differences in doses, type of garlic used and period of intervention. In animal studies, streptozotocin-induced diabetic rats receiving aqueous extract of garlic prepared from garlic cloves (500 mg/kg daily) for 3-weeks had significantly higher serum antioxidant status compared to controls [41]. Further more rigorous, randomised, placebo-controlled studies are required to assess whether aged garlic extract can reduce AGEs *in vivo* by using higher doses or a longer intervention period accompanied by measurement of tissue as opposed to serum AGEs.

Conclusion

The results of this study have shown that aged garlic extract supplementation over 3-months had no effect on serum glucose, glycated haemoglobin, total cholesterol, LDL and HDL cholesterol and total antioxidant status. However, the study also demonstrated the ability of aged garlic extract to reduce lipid hydroperoxide and lower LMW-AGEs albeit the latter was not significant in type 2 diabetic patients. This suggests that aged garlic extract might be useful as a food supplement in delaying or preventing the onset of diabetic complications. Further human studies are required to establish the protective roles of aged garlic extract in diabetes.

Abbreviations

AGEs: advanced glycation endproducts
ANOVA: analysis of variance
BMI: body mass index
HDL: high density lipoprotein
LDL: low density lipoprotein
LMW-AGEs: low molecular weight advanced glycation endproducts
SAC: S-allylcysteine

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

KB conducted the experimental work and analysed the data. OA and QW were involved in the design of the study and analysis of data. NA was involved in the study design, analysis of data and writing of the manuscript.

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