

Effect of garlic component s-allyl cysteine sulfoxide on glycated human serum albumin induced activation of endothelial cells: an *in vitro* study

B.K. NIRALA, N.K. GOHIL

Centre for Biomedical Engineering, Indian Institute of Technology Delhi, New Delhi, India

Abstract. – OBJECTIVE: Alternative medicine or herbal therapies have been in use for blood glucose control in patients with diabetes for considerable time. Effect of garlic, more specifically its biologically active component s-allyl cysteine, on amelioration of hyperglycemia has also been reported. However, the cellular or molecular target of this compound is little known. This study was designed to determine the action of s-allyl cysteine sulfoxide (SACSO) at the cellular and molecular level in the widely reported HUVEC model when activated with advanced glycation end products (AGEs).

MATERIALS AND METHODS: AGE-HSA was derived from non-enzymatic glycation of human serum albumin in the presence of 20 mM glucose. AGE-HSA induced expression of receptors of AGEs, namely RAGE and galectin-3 has been assayed. Activity of endothelial nitric oxide synthase (NOS) denoting normal endothelial function and expression of AGE-RAGE triggered inflammatory marker sICAM-1 is also evaluated in the presence or absence of SACSO.

RESULTS: In presence of SACSO, AGE-HSA induced expression of RAGE was down-regulated, galectin-3 was significantly up-regulated, NOS activity was enhanced and sICAM-1 expression was reduced.

CONCLUSIONS: The data suggest that SACSO exerts an attenuating effect on 20 mM glucose derived AGE-HSA induced inflammation, by selectively inhibiting RAGE while stimulating galectin-3 expression. The former triggers inflammatory pathways while the latter sequesters AGE molecules and prevents AGE-RAGE engagement. This may form the basis for its therapeutic action.

Key Words:

Advanced glycation, Diabetes, Garlic, Endothelial cells, Inflammatory markers, s-allyl cysteine sulfoxide.

Introduction

The alarming rate at which diabetes, particularly type 2 diabetes, is afflicting the human race world-wide¹ and the phenomenal cost involved, has prompted researchers to explore complemen-

tary and alternative medicine for its treatment and control. Prominent side effects of currently used drugs (hypoglycemic agents) is another compelling reason for preferring compounds derived from natural products. Effect of natural herbs of *Allium* species, particularly garlic has been examined on hypertension and hyperlipidemia². A well designed randomized clinical trial of garlic in humans was designed to examine thrombocyte aggregation in non-diabetic individuals whereby a significant decrease in fasting serum glucose was a parallel observation³. The other clinical trial of garlic in patients with type 2 diabetes did not report consistent glucose or insulin responses after 1 month of supplementation⁴. Garlic extract and more specifically its biologically active component, s-allyl cysteine sulfoxide (SACSO) has been investigated in considerable details by various researchers⁵. Its anti-diabetic properties have been validated both in experimental animal diabetic model as well as in human subjects in clinical studies. Effect of different doses of s-allyl cysteine (SAC) on different blood parameters, like blood glucose, glycated hemoglobin, serum uric acid, serum creatinine, and plasma insulin was studied in diabetic rats⁶ and compared with gliclazide (standard oral drug) administration. While reduction in all parameters was observed with SAC, plasma insulin levels were raised significantly. In another study, the extract of raw garlic administered intra-peritoneally in streptozotocin induced diabetic rats caused reversal of proteinuria in addition to reducing blood sugar, cholesterol and triglycerides⁷. Mode of action of allium derivatives include increased secretion and delayed degradation of insulin, and increased glutathione peroxidase activity⁸. The limited data available so far does not provide conclusive evidence for garlic in glycemic control. Further, the lacuna in these studies lies in unraveling the interactions at the cellular and molecular level, which bring

about its therapeutic effect. Detailed mechanistic studies of the effects produced by individual bio-active metabolites of garlic on endothelial cell function are of utmost importance.

Advanced glycated end products (AGEs) of different proteins formed in the body in diabetes, are known to initiate patho-biological events in cells, which are mediated by its receptors^{9,10}. The adverse effects of AGEs on the vasculature of patients with diabetes are caused due to the formation of cross-links between molecules in the basement membrane of the extracellular matrix. Additionally, AGEs block nitric oxide activity in the endothelium¹¹. AGE induced inflammatory response of the endothelial cells exhibits spatial heterogeneity in the vasculature leading to focal atherosclerotic lesions¹².

In this study, we have investigated the effect of s-allyl cysteine sulfoxide (SACSO) on HUVECs exposed to metabolic stress. Cultured monolayers of endothelial cells are exposed to glucose derived glycated human serum albumin. Expression of two receptors of AGEs namely RAGE and galectin-3, activity of endothelial nitric oxide synthase (NOS) and expression of an inflammatory marker sICAM-1 has been measured in the presence or absence of SACSO. The unifying factor is that all these markers are indicators of endothelial function in inflammatory vascular disease. The study reveals that SACSO exerts an ameliorating effect on the stress caused by glycated proteins on some endothelial functions.

Material and Methods

Cell Culture and Treatments

Isolation and Characterization of HUVECs

Human umbilical cord samples were collected from CGHS Maternity and Gynecology Hospital New Delhi, India and analyzed anonymously. Investigation conforms with the principles outlined in the Declaration of Helsinki. As per Indian Council of Medical Research Guidelines, studies involving unidentifiable specimens obtained from the provider, that is prohibited from releasing identifiers by established regulations, qualify for exempt status. Isolation of endothelial cells was carried out as described previously¹³ and grown in EGM-2 growth medium (PromoCell, Heidelberg, Germany) with added antibiotic/antimycotic supplement (Sigma Aldrich, Saint Louis, MO, USA). Cells were incubated in a hu-

midified CO₂ incubator (MMM, Medcenter Einrichtungen, Planegg, Germany). After confluence, they were harvested with 0.1% trypsin EDTA (Sigma Aldrich, USA) for sub-culturing. Cells from 3rd passage, at seeding density of 2×10^5 cells were plated in 60 mm Petri plates coated with gelatin (0.2%) and fibronectin (2 µg/cm²) and used for further experiments upon attaining confluence.

Cell Viability Assay for S-Allyl Cysteine Sulphoxide (SACSO)

A colorimetric MTT assay which measures the reduction of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) into an insoluble formazan product by active mitochondria was performed to determine whether SACSO was toxic to HUVECs. Assay was performed as previously described¹⁴. Briefly, 5 g/L MTT (Millipore, Billerica, MA, USA) was prepared in PBS and filter sterilized with 0.22 µm filter. HUVECs were seeded in 96-well plates with 20,000 cells/well and treated with different concentration of SACSO (0-100 µmol/L) for 24 h in a CO₂ incubator. Subsequently media were discarded, 100 µL of fresh media containing 10 µL of MTT solution was added, cells incubated for further 4h. Finally, media were removed carefully and 100 µL of DMSO was added and plates were read on a microplate reader (Eon, BioTek, Vinnoski, VT, USA) using a test $\lambda = 570$ nm.

Exposure of HUVECS to AGE-HSA and SACSO

AGE-HSA was prepared as described previously¹⁵. 20 mmol/L glucose derived AGE-HSA was used at a concentration of 2 g/L. HUVECs were cultured in 60 mm Petri plates to confluence (4-5 d) and starved overnight in media lacking both growth supplements and serum. Cells were then incubated with complete growth medium containing 40 µmol/L SACSO, (based on the results of MTT assay) for 24 h at 37°C in a humidified CO₂ incubator. Following the removal of medium, cells were exposed to 2 g/L AGE-HSA for 4 h. The control set comprised of HUVECs with either no treatment or no AGE-HSA exposure.

Expression of RAGE, Galectin-3 and sICAM-1 in Response to SACSO

After the treatment of cells with AGE-HSA and SACSO culture media was discarded; cells were washed twice with ice cold phosphate buffer saline (PBS) pH 7.4 and scrapped. Cells

were pelleted down with gentle centrifugation and lysed with 200 μ L of cell lysis buffer (Sigma Aldrich, Saint Louis, MO, USA). Cell lysate was centrifuged at 15000 g at 4°C to pellet down the cell debris, and supernatant was collected into fresh tube. Expression of RAGE (ng/L), galectin-3 (ng/L) and sICAM-1 (μ g/L) in HUVEC was quantified in the cell lysate, using human RAGE ELISA kit (Raybiotech Inc., Norcross, GA, USA), human galectin-3 ELISA kit (Cusabio Biotech, Wuhan, China) and human sICAM-1 ELISA kit (Raybiotech Inc., Norcross, GA, USA) respectively.

Effect of SACSO on NOS Activity

Sample for the NOS activity was prepared in a similar manner as described, and enzyme activity was determined by Nitric Oxide Colorimetric Assay Kit (Oxford Biomedical Research, Rochester Hills, MI, USA). Protein was quantified by Bradford assay method. Activity of NOS was expressed in terms of pmol/ (L. mg. min).

Statistical Analysis

Results were analyzed by Student's *t*-test for paired sample comparison through Vassarstats software; $p < 0.05$ was considered statistically significant. Experiments were repeated three times; data are shown as mean $\pm$ standard deviation (SD). Multiple comparisons were made with ANOVA.

Results

In diabetic individuals body cells are exposed to prolonged elevated glucose concentrations, leading to formation of non-enzymatic glycated end products of proteins and lipids (AGE and ALE). While blood glucose levels *in-vivo* are variable, depending on the physiological state, AGEs once formed, persist in the circulation, thus interacting with the cells all the time¹¹. In this study, we present the effect of anti-diabetic agent s-allyl cysteine sulfoxide on AGE induced expression of marker molecules in HUVECs.

We hypothesized that the anti-diabetic activity of SACSO manifests through perturbation of AGE-RAGE engagement which is the main cause of the downstream inflammatory effects. This was evaluated in terms of expression of two specific receptors of AGEs, RAGE and galectin-3. We also examined effect of SACSO on nitric oxide synthase activity, which is compromised in diabetes induced endothelial dysfunction. Expression of sICAM-1 was measured as an index of altered inflammation. Viability test (MTT assay) of cells after exposure to SACSO at different concentrations (10-100 μ M) for up-to 24 hours indicates that cells were not significantly affected (Figure 1). Based on this observation, experimental groups were treated with a moderate concentration of 40 μ M SACSO. Previous

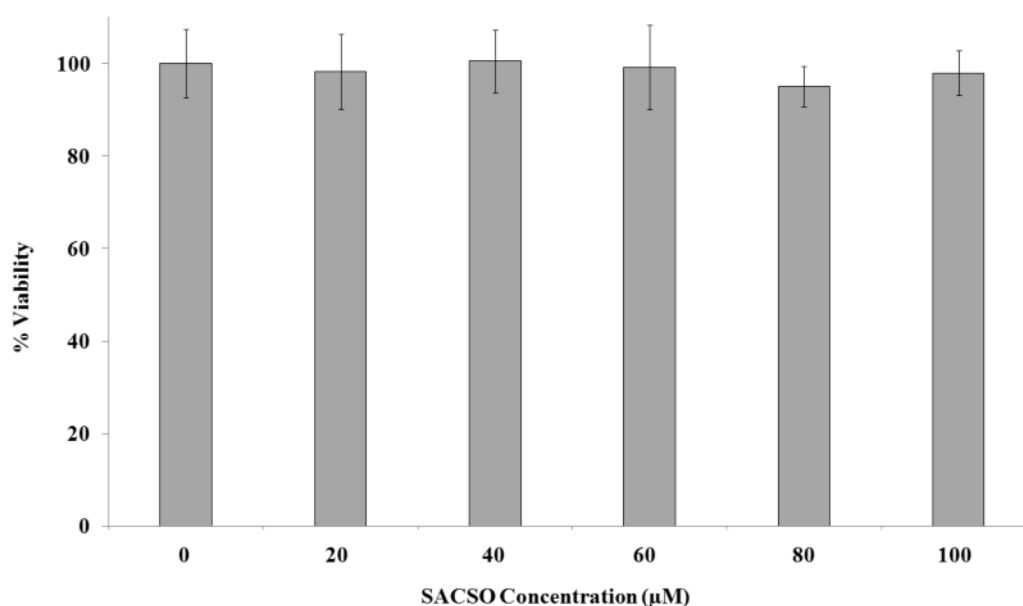


Figure 1. Effect of SACSO at different concentrations (0-100 μ M) on viability of HUVECs. Difference was not statistically significant.

studies on the effect of various garlic derived organo-sulfur compounds on DNA damage have reported that it stabilizes around this concentration^{16,17}.

Expression of RAGE and Galectin-3

In general the magnitude of expression of galectin-3 in HUVECs was found to be much higher than that of RAGE, both in presence and absence of AGE-HSA.

The expression of RAGE was up-regulated by 20 mM glucose derived AGE-HSA (2 mg/ml) in absence of SACSO compared with control ($p < 0.001$, Figure 2a,b); increase was by 118%. Treatment with SACSO reduced AGE induced RAGE expression significantly by 27% ($p < 0.05$, Figure 2b,d). No difference was observed in expression in controls in the presence or absence of SACSO. Data analysis indicates that the difference in RAGE expression was significant between treatments in the absence and presence of SACSO. This implies that the anti-diabetic effect of SACSO may be delivered through inhibition of RAGE expression and consequent lowering of AGE-RAGE engagement.

The expression of galectin-3 another receptor of AGEs, was also up-regulated by 20 mM glucose derived AGE-HSA (2 mg/ml) in absence of SACSO compared with control ($p < 0.005$, Figure 2e,f); increase was by 90%. Treatment with SACSO further increased AGE induced galectin-3 expression significantly by 134% ($p < 0.001$, Figure 2e,h) with respect to control. An increase by 23% was also observed in AGE induced expression when compared between absence and presence of SACSO ($p < 0.05$, Figure 2f,h). SACSO clearly acts differentially on the two receptors.

Nitric Oxide Synthase Activity

In HUVECs, reduction in the activity of nitric oxide synthase was observed by 26% in presence of 20 mM glucose derived AGE-HSA (2 mg/ml) which was significant (Figure 3B a,b $p < 0.01$). This reduction in NOS activity was ameliorated in presence of SACSO (increase by 26%; Figure 3B b,d) although activity still remained lower than control. It is not evident if a higher concentration of SACSO may have lowered it to control level. SACSO showed a stimulatory effect on

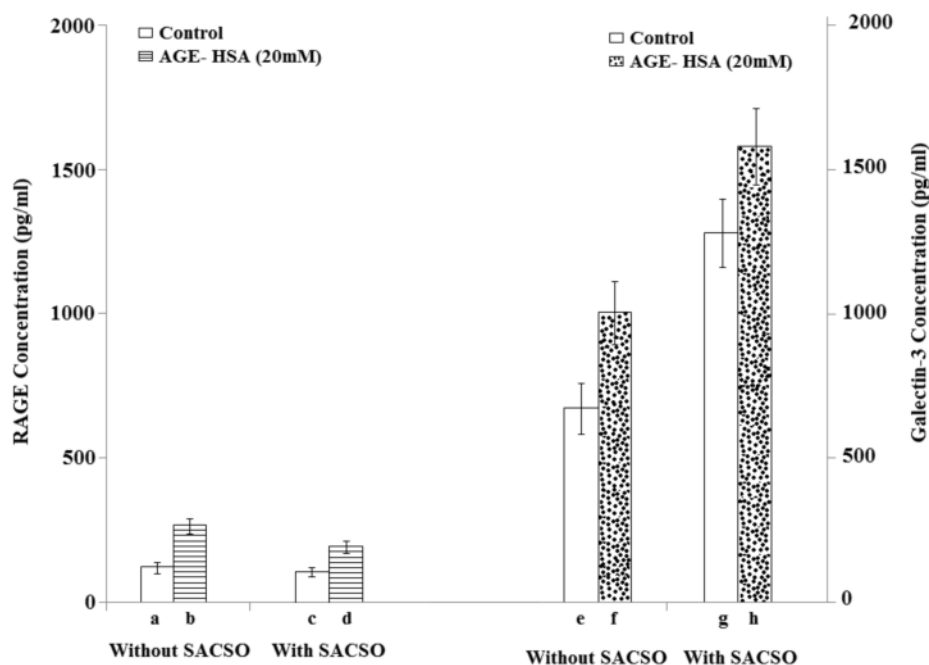


Figure 2. Effect of SACSO on control and AGE-HSA (2 mg/ml) treated HUVEC on the expression of RAGE and galectin-3 protein. Expression of RAGE, (a) control (without any treatment), (b) AGE-HSA, (c) control exposed to SACSO (40 μ M), (d) AGE-HSA along with 40 μ M SACSO. Expression of galectin-3 (e) Control (without any treatment), (f) AGE-HSA, (g) control exposed to SACSO (40 μ M), (h) AGE-HSA along with 40 μ M SACSO. Data are expressed as means $\pm$ standard deviation, $n=3$; ($p < 0.05$ was considered significant).

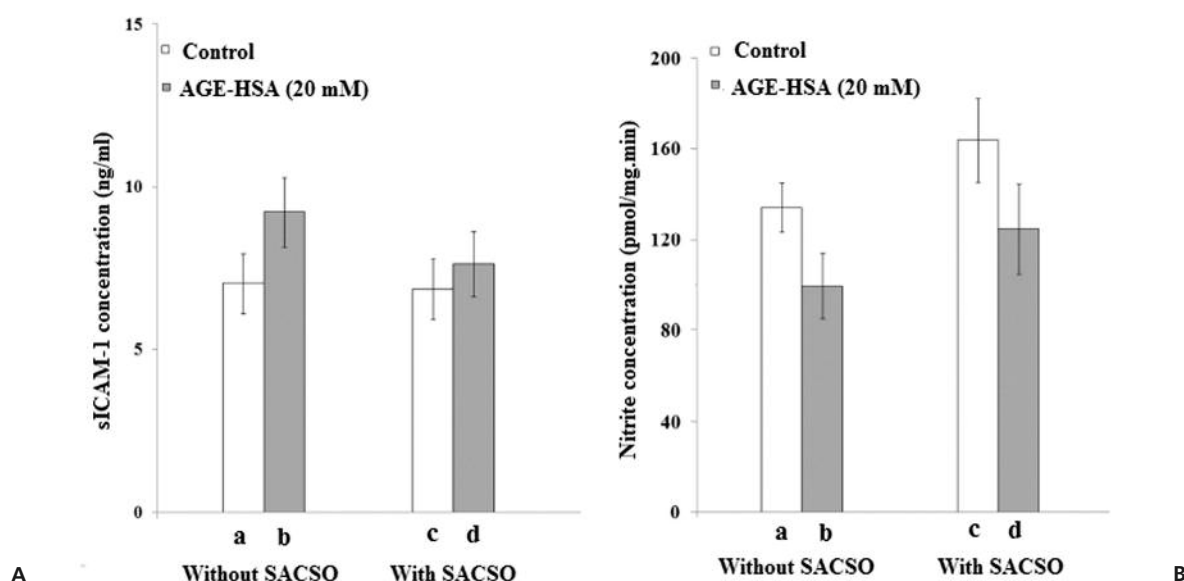


Figure 3. Effect of SACSO on control and AGE-HSA treated HUVEC on **(A)** NO synthase activity and **(B)** Expression of sICAM-1 protein (a) Control (without any treatment), (b) AGE-HSA (2 mg/ml), (c) control exposed to SACSO (40 μ M), (d) AGE-HSA along with 40 μ M SACSO. Data are expressed as means $\pm$ standard deviation, $n=3$; ($p < 0.05$ significant).

NOS activity even in controls. Data analysis indicates that the difference in enzyme activity was significant between treatments in the absence and presence of SACSO ($p < 0.005$).

Expression of sICAM-1

SACSO shows no effect on sICAM-1 expression in controls. In 20 mM glucose derived AGE-HSA treated cells, an increase by 31% was observed in its expression (Figure 3A a,b $p < 0.05$) which was brought down by 14% in presence of SACSO (Figure 3A b,d). It is possible that a higher concentration of SACSO may have reduced it further. The unifying factor in the above responses is that they denote endothelial dysfunction in the inflammatory milieu created by AGEs and the ameliorating effect of SACSO as a preventive measure.

Discussion

Several AGE-binding receptors have been described so far which include the 35-kDa member of the immunoglobulin superfamily called RAGE¹⁸, the macrophage scavenger receptors class A and B^{19,20}, the 60-kDa and 90-kDa proteins AGE-receptor 1 and 2 respectively (AGE-R1 and AGE-R2) and the 32-kDa protein galectin-3 or AGE-R3^{21,22}. AGE receptors have been found

to occur in specific cells like monocytes, macrophages, endothelial cells, pericytes, podocytes, astrocytes and microglia⁹. Cell activation in response to AGE-modified proteins is mediated by their interaction with one or more of these receptors. Some of the cellular events include increased expression of extracellular matrix proteins, vascular adhesion molecules, cytokines and growth factors. The outcome varies according to the cell type and the associated signaling.

Our focus in this study is on endothelial cells, which have a primary role in transducing the signals in blood to components of the vessel wall. Macrovascular complications of diabetes are attributed to prevailing elevated blood sugar levels in blood, which modify both proteins present in blood and those in the vessel wall, mainly collagen. The resulting stiffness of arteries and the pro-inflammatory environment are among the main causes of vascular disease. One of the non-conventional therapies for reducing blood sugar levels is the use of natural products. In this connection garlic has received lot of attention particularly with reference to its cholesterol lowering effects. Some studies have also suggested that it is also effective in controlling hyperglycemia. In the present study we have observed that SACSO, the bioactive compound in garlic extract, affects two of AGE receptors in different ways in HUVECs. It down-regulates glycated serum albumin induced

RAGE expression while up-regulating the expression of galectin-3. AGE-RAGE interaction is directly responsible for generation of reactive oxygen species (ROS) and stimulation of p21(ras)-dependent MAP kinase²³ and its downstream targets NF- κ B and the activator protein-1 complex^{24,25}. Experiments in cultured human aortic endothelial cells have shown that RAGE and its ligands like AGEs induce inflammation through JNK signaling²⁶. Increased oxidative stress associated damage have been implicated as mediators of vascular injury in cardiovascular pathologies, including hypertension, atherosclerosis and ischemia-reperfusion²⁷. Therefore, the RAGE pathway essentially is pro-inflammatory and pro-vascular injury²⁸. Inhibition of RAGE expression by SACSO indicates that its therapeutic action is based on reducing AGE-RAGE interaction and thus reducing the possibility of downstream pro-inflammatory effects. The antioxidant properties of SACSO in this case is likely to be derived from its effect on lowering levels of RAGE as is observed in HUVECs in this study.

Galectin-3 on the other hand is the less studied receptor and has been shown to have multiple functions imparted by its structural properties, one of which is high-affinity binding, internalization and degradation of AGEs^{25,29}. We observe an increase in galectin-3 expression in presence of SACSO, indicating that a competitive interaction may be present between RAGE and Galectin-3 for AGE-HSA. SACSO promotes AGE sequestration by the latter, by up-regulation of its expression such that less AGE-HSA is available for inducing RAGE expression. The outcome is down regulation of RAGE expression. Further, in a previous study S-allyl cysteine (SAC) was shown to cause dose-dependent inhibition of NF- κ B activation in HUVECs stimulated with TNF- α or hydrogen peroxide³⁰.

It appears that, in the present experiments, SACSO exerts a two-pronged approach whereby, it reduces RAGE production and up-regulates galectin-3. In an earlier study activation of signaling pathway by advanced oxidation protein products in HUVECs resulted in over-expression of VCAM-1 and ICAM-1 at both gene and protein levels which was shown to be RAGE mediated by blocking the latter with either anti-RAGE IgG or excess sRAGE³¹. We also observe a similar RAGE-mediated increased expression of sICAM-1 due to AGE induced activation of HUVECs, and this is inhibited in presence of SACSO in our study. We have also observed a con-

comitant increase in endothelial NO-synthase activity in presence of SACSO, which indicates partial restoration of endothelial function. It is also interesting that galectin-3 expression is enhanced even in controls exposed to SACSO (Figure 2e,g; $p < 0.005$).

Conclusions

Even though the results are from *in vitro* set up it has implication indicating that this compound can act as a protective sequestering agent for any circulating AGEs *in vivo* which are present at low levels in the body, arising from de-novo non-enzymatic glycation and dietary sources. Selective pharmacologic inhibition/stimulation of RAGE and galectin-3 mediated cell activation observed with SACSO in these experiments may form the basis for therapeutic action in diabetes where AGE accumulation is one of the many etiological factors causing vascular complications of diabetes.

Acknowledgements

This work has been supported by Institutional grants (NPN05). Bikesh K Nirala is supported by a student fellowship from University Grants Commission, India.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- 1) DANAEI G, FINUCANE MM, LU Y, SINGH GM, COWAN MJ, PACIOREK CJ, LIN JK, FARZADFAR F, KHANG YH, STEVENS GA, RAO M, ALI MK, RILEY LM, ROBINSON CA, EZZATI M. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet* 2011; 378: 31-40.
- 2) YE H GY, EISENBERG DM, KAPTCHUK TJ, PHILLIPS RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care* 2003; 26: 1277- 1294.
- 3) KIESEWETTER H, JUNG F, PINDUR G, JUNG EM, MROWIETZ C, WENZEL E. Effect of garlic on thrombocyte aggregation, microcirculation, and other risk factors. *Int J Clin Pharmacol Ther Toxicol* 1991; 29: 151-155.
- 4) SITPRUA S, PLENGVIDHYA C, KANGKAYA V, BHUVAPANICH S, TUNKAYOON M. Garlic and diabetes mellitus phase II clinical trial. *J Med Assoc Thai* 1987; 2: 223-227.

- 5) SHEELA CG, AUGUSTI KT. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. *Indian J Exp Biol* 1992; 30: 523-526.
- 6) SARAVANAN G, PONMURUGAN P, SENTHIL KUMAR GP, RAJAJAN T. Antidiabetic effect of S-allyl cysteine: Effect on plasma and tissue glycoproteins in experimental diabetes. *Phytomedicine* 2010; 17: 1086-1089.
- 7) THOMSON M, AL-AMIN ZM, AL-QATTAN KK, SHABAN LH, ALI M. Anti-diabetic and hypolipidaemic properties of garlic (*Allium sativum* Linn.) in streptozotocin induced diabetic rats. In *J Diab Metab* 2007; 15: 108-115.
- 8) SHANE-MCWHORTER L. Biological complementary therapies: A focus on botanical products in diabetes. *Diabetes Spectrum* 2001; 14: 199-208.
- 9) THORNALLEY PJ. Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Cell Mol Biol* 1998; 44: 1013-1023.
- 10) YAMAGISHI S, IMAIZUMI T. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. *Curr Pharm Des* 2005; 11: 2279-2299.
- 11) GOLDIN A, BECKMAN JA, SCHMIDT AM, CREAGER MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006; 114: 597-605.
- 12) JANDELEIT-DAHM K, COOPER ME. The role of AGEs in cardiovascular disease. *Curr Pharm Des* 2008; 14: 979-986.
- 13) BALA K, AMBWANI K, GOHIL NK. Effect of different mitogens and serum concentration on HUVEC morphology and characteristics: implication on use of higher passage cells. *Tissue Cell* 2011; 43: 216-222.
- 14) MOSMANN T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65: 55-63.
- 15) BALA K, GOMES J, GOHIL NK. Interaction of glycated human serum albumin with endothelial cells in a hemodynamic environment: structural and functional correlates. *Mol Bio Sys* 2011; 7: 3036-3041.
- 16) BELLOIR C, SINGH V, DAURAT C, SIESS MH, LE BON AM. Protective effects of garlic sulfur compounds against DNA damage induced by direct- and indirect-acting genotoxic agents in HepG2 cells. *Food Chem Toxicol* 2006; 44: 827-834.
- 17) XIAO D, CHOI S, JOHNSON DE, VOGEL VG, JOHNSON CS, TRUMP DL, LEE YJ, SINGH SV. Diallyl trisulfide-induced apoptosis in human prostate cancer cells involves c-Jun N-terminal kinase and extracellular-signal regulated kinase-mediated phosphorylation of Bcl-2. *Oncogene* 2004; 23: 5594-5606.
- 18) STERN DM, YAN SD, YAN SF, SCHMIDT AM. Receptor for advanced glycation end products (RAGE) and the complications of diabetes. *Ageing Res Rev* 2002; 1: 1-15.
- 19) HORIUCHI S, HIGASHI T, IKEDA K, SAISHOJI T, JINNOUCHI Y, SANO H, SHIBAYAMA R, SAKAMOTO T, ARAKI N. Advanced glycation end products and their recognition by macrophage and macrophage-derived cells. *Diabetes* 1996; 45: S73-76.
- 20) OHGAMI N, NAGAI R, IKEMOTO M, ARAI H, KUNIYASU A, HORIUCHI S, NAKAYAMA H. Cd36, a member of the class b scavenger receptor family, as a receptor for advanced glycation end products. *J Biol Chem* 2001; 276: 3195-3202.
- 21) LI YM, MITSUHASHI T, WOJCIECHOWICZ D, SHIMIZU N, LI J, STITT A, HE C, BANERJEE D, VLASSARA H. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. *Proc Natl Acad Sci USA* 1996; 93: 11047-11052.
- 22) VLASSARA H, LI YM, IMANI F, WOJCIECHOWICZ D, YANG Z, LIU FT, CERAMI A. Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. *Mol Med* 1995; 1: 634-646.
- 23) LANDER HM, TAURAS JM, OGISTE JS, HORI O, MOSS RA, SCHMIDT AM. Activation of the receptor for advanced glycation end products triggers a p21 (ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J Biol Chem* 1997; 272: 17810-17814.
- 24) BIERHAUS A, ILLMER T, KASPER M, LUTHER T, QUEHENBERGER P, TRITSCHLER H, WAHL P, ZIEGLER R, MULLER M, NAWROTH PP. Advanced glycation end product (AGE)-mediated induction of tissue factor in cultured endothelial cells is dependent on RAGE. *Circulation* 1997; 96: 2262-2271.
- 25) GUIMARAES EL, EMPSEN C, GEERTS A, VAN GRUNSVEN LA. Advanced glycation end products induce production of reactive oxygen species via the activation of NADPH oxidase in murine hepatic stellate cells. *J Hepatol* 2010; 52: 389-397.
- 26) HARJA E, BU DX, HUDSON BI, CHANG JS, SHEN X, HAL-LAM K, KALEA AZ, LU Y, ROSARIO RH, ORUGANTI S, NIKOLLA Z, BELOV D, LALLA E, RAMASAMY R, YAN SF, SCHMIDT AM. Vascular and inflammatory stresses mediate atherosclerosis via RAGE and its ligands in apoE^{-/-} mice. *J Clin Invest* 2008; 118: 183-194.
- 27) TOUYZ RM. Reactive oxygen species in vascular biology: role in arterial hypertension. *Expert Rev Cardiovasc Ther* 2003; 1: 91-106.
- 28) LIN L, PARK S, LAKATTA EG. RAGE signalling in inflammation and arterial aging. *Front Biosci* 2009; 14: 1403-1413.
- 29) ZHU W, SANO H, NAGAI R, FUKUHARA K, MIYAZAKI A, HORIUCHI S. The role of galectin-3 in endocytosis of advanced glycation end products and modified low density lipoproteins. *Biochem Biophys Res Commun* 2001; 280: 1183-1188.
- 30) HO SE, IDE N, LAU BH. S-allyl cysteine reduces oxidant load in cells involved in the atherogenic process. *Phytomedicine* 2001; 8: 39-46.
- 31) GUO ZJ, NIU HX, HOU FF, ZHANG L, FU N, NAGAI R, LU X, CHEN BH, SHAN YX, TIAN JW, NAGARAJ RH, XIE D, ZHANG X. Advanced oxidation protein products activate vascular endothelial cells via a RAGE-mediated signaling pathway. *Antioxid Redox Signal* 2008; 10: 1699-1712.