Global Journals LaTeX JournalKaleidoscope™
Artificial Intelligence formulated this projection for compatibility purposes from the original article published at Global Journals. However, this technology is currently in beta. *Therefore, kindly ignore odd layouts, missed formulae, text, tables, or figures.*

Effect of Diallyl Disulphide on Protein and Lipid Glycation, and Lipid Peroxidation in Brain of Alloxan Diabetic Rats

ijay V[1] and Vickram[2]

[1] Vijayanagara Institute of Medical Sciences

Received: 7 December 2012 Accepted: 3 January 2013 Published: 15 January 2013

Abstract

Non enzymatic glycosylation of proteins and lipids is the main initiating factor for the pathophysiology of chronic diabetic complications. This glycation is more prevalent in insulin independent tissues like brain, kidney, RBCs, etc. Diallyl disulphide (DADS), the principle compound of garlic oil, is well known for its antihyperglycemic, antihyperlipidemic, anticarcinogenic and antibiotic properties. Hence a study was undertaken to assess the anti-glycation properties of DADS, in alloxan diabetic brain tissue, thereby to establish any usefulness of DADS in prevention of central nervous system complications in diabetes mellitus like diabetic dementia or diabetic encephalopathy. The current study showed a significant decrease ($p<0.001$) in glycated proteins, glycated lipids and total TBARS levels in brain tissue of DADS treated diabetic rats as compared to diabetic control rats. Hence it can be concluded that DADS helps in reducing glycation of brain proteins and lipids as well as lipid peroxidation and thus may be useful in prevention of CNS diabetic complications like diabetic encephalopathy.

Index terms— diallyl disulphide, protein glycation, lipid glycation, diabetic encephalopathy.

1 Introduction

on enzymatic glycosylation of proteins and lipids will be normally proportional to available free glucose in the tissues. It can be expected that a consistent hyperglycemia in diabetic subjects may induce hyperglycation of tissue proteins and lipids, and this is high in tissues which are not dependent on insulin for glucose transport like kidney, brain, RBCs, optic lens, etc [1][2][3] . It is shown that the main initiating factor for the pathophysiology of chronic diabetic complications like diabetic nephropathy is non-enzymatic glycosylation of kidney proteins and lipids 4 . There are evidences for glycation to occurs in brain tissue of diabetic animals like studies of Miyazawa A 5 have established increased lipid glycation in neurons of diabetic animals whereas studies of Jingsheng H 6 have similarly established protein glycation in brain of diabetic rats. Few studies have shown that lipid glycation occurs faster than protein glycation 7 . Since brain has rich content of lipids, lipid glycation is of significance in diabetes induced CNS complications. Glycation of proteins and lipids probably results in increased formation of advanced glycation end products (AGEPs) and advanced glycated lipid products (AGLPs), which leads to formation of various oxidants (like lipid peroxidation products, example Malonaldehyde, etc) resulting in tissue damage [8][9][10][11][12] . These AGEPs and AGLPs are indicated in late diabetic CNS complications like Alzheimers disease 13 , diabetic dementia 5 and diabetic encephalopathy 14 .

Among the various biological activities of the medicinal plants, the hypoglycaemic and hypolipidemic activities have been the most commonly studied. Garlic, (Allium sativum Linn) is well known for its antidiabetic, antihyperlipidemic, antiatherogenic as well as anticarcinogenic properties [15][16][17][18] . DADS, the principle sulphur compound of garlic is probably responsible for the above mentioned beneficial functions of garlic. Studies have shown that DADS crosses blood brain barrier 19,20 and its use in various neurological disorders have been established 21,22 .

Hence a study was undertaken to assess the anti-glycation properties of DADS on brain proteins and lipids in alloxan diabetic rats, thereby to establish the usefulness of DADS in prevention of CNS complications in diabetes mellitus like diabetic encephalopathy.

2 II.

3 Materials and Methods

Alloxan and Diallyl disulphide (DADS) were procured from Sigma Chemical Company. All other chemicals employed were of analytical grade.

Albino rats of both sexes, weighing 300-350g were randomly selected from Central Animal House, BMCH, Chitradurga and were used for the present investigation. The animals were maintained on a standard rat feed supplied from Amrut rat feeds, Bangalore. The experiments were conducted according to the norms approved by Ministry of Social Justice and empowerment, Government of India, and Institutional Animal Ethics Committee (IAEC) guidelines. The animals were fasted overnight and Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan (150mg/kg body wt.) 23 , in sterile normal saline. The animals were considered diabetic if their blood glucose were consistently above 300mg/dl and urine showed consistent glucosuria. The treatment was started on 5 th day after alloxan injection and was considered as first day of treatment. The rats were divided into three groups comprising six rats in each group as follows:

Group I: Normal rats -which were fed on 30 ml of normal saline per kg body weight, through gastric intubation, daily for 90 days.

Group II: Diabetic Control rats -which were fed on normal saline 30ml / kg body weight, through gastric intubation, daily for 90 days.

Group III: Diallyl disulphide (DADS) treated Diabetic rats -which were fed on DADS (100mg/ kg body weight) prepared in normal saline, given as 30ml / kg body weight suspension, through gastric intubation, daily for 90 days.

On completion of the stipulated period, the rats were anaesthetized by anaesthetic ether and were sacrificed by cervical dislocation. Blood was collected in heparinized tubes from internal jugular vein. Whole brain was dissected and net weight was noted. Immediately the brain was processed as follows. One part of whole brain was homogenized with 9 parts of cold Phosphate buffer (pH 7.4) using Potter Elvehjam homogeniser and the extract was used for estimation of total proteins 24 and carbohydrate content of these protein [Glycated protein] 25 . A second part of brain was homogenized with 9 parts of Chloroform methanol (1:1 v/v) mixture using Potter Elvehjam homogeniser and the extract was used for total lipids 26 and carbohydrate content of this lipids [Glycated lipids] 25 . And another part of whole brain was homogenized with 9 parts of trichloroacetic acid (10%) and extract was used for the estimation of thiobarbituric acid reactive substances (TBARS) levels 27 . Whole blood was employed for glycated hemoglobin estimation 25 . A part of whole blood was centrifuged at 3500 rpm for 6-8mins and the free separated plasma was used for glucose estimation 28 . The free sugar content of phosphate buffer extract was estimated by Folin Wu method 29 and the value obtained was deducted from the total carbohydrate content of phosphate buffer protein to calculate glycated protein content.

The results were expressed as mean + SD. Statistical analysis was done by using student ' t' test.

III.

4 Results

The results obtained are given in table 1 and 2. Table 1 gives the glycated Hb levels, plasma glucose levels, body weight and ratio of brain to body weight in normal rats (group 1), alloxan diabetic rats (group II), as well as in DADS treated alloxan diabetic rats (group III).

As seen from the table, there is a significant increase in plasma glucose levels ($p<0.001$), glycated hemoglobin levels ($p<0.001$) body weight and ratio of brain to body weight ($p<0.001$) in group II as compared to group I rats. A significant decrease is seen in the above parameters ($p<0.001$) in group III rats as compared to group II rats. Further no significant alteration is observed in plasma glucose levels in group III rats as compared to group II rats.

Tables 2 shows the levels of brain tissue total proteins, glycated brain proteins, brain tissue total lipids and glycated brain lipids in group I, group II and group III rats. A significant raise in glycated brain proteins ($p<0.001$), glycated brain lipids ($p<0.001$) and brain total lipids ($p<0.001$) were observed in group II rats as compared to group I rats whereas a significant decrease in brain total proteins ($p<0.05$) was observed in group II as compared to group I. A significant decrease in glycated brain proteins ($p<0.001$), glycated brain lipids ($p<0.001$) and brain total lipids ($p<0.001$) is observed in DADS treated diabetic rats (group III) as compared to diabetic control rats (group II).

IV.

5 Discussion

In the present study, administration of alloxan (150mg/kg body weight) induced hyperglycemia in the albino rats as evidenced by elevated plasma glucose levels and glycated hemoglobin levels in group II rats (refer table 1).

The levels of glycated hemoglobin have been shown to be an important parameter of chronic glycemic control in diabetes. The decrease in body weight of diabetic rats is due to increase in the protein catabolism mainly in skeletal muscles that helps to channel amino acids for gluconeogenesis, decrease in protein uptake as well as insulin deficiency induced lipolysis 30 .

There is substantial epidemiological evidence that, besides the long-term complications of diabetes mellitus, which include accelerated atherosclerosis, retinal microvascular damage, renal failure caused by glomerular injury, and peripheral neuropathy, the disease also has multiple effects on the central nervous system. Diabetic patients have at least twice the risk of stroke 31 and may show performance deficits in a wide range of cognitive domains 32 . The mechanisms underlying this gradually developing end-organ damage, known as diabetic encephalopathy, are only partially understood and can involve vascular changes and direct damage to neuronal cells by glucose 33,34 . Although the high level of glucose in the brain cortex of diabetic rats has been questioned 35 , it has recently been reported that glucose levels increase by up to three times in the hippocampus of diabetic rats compared with controls 36 . Emerging evidence suggests that increased glycation leads to the overproduction of superoxide by the respiratory chain and consequent oxidative stress play a role in the pathogenesis of diabetes complications 14 .

Many studies have shown that garlic and its compounds exhibit diverse biological activities like anti-tumorigenic, anti-atherosclerosis, detoxification, antiinflammatory, antioxidant etc. 21,37,38 . Also, garlic oilderived organosulfur compounds such as diallyl trisulphide, diallyl disulphide, and diallyl sulphide provide significant protection against carcinogenesis, and this protection is likely related with their antioxidant properties 39 . Moreover, the lipophilic characteristics of these compounds allow crossing the blood-brain barrier as follows: dialyl sulfide crosses the blood-brain barrier easier than diallyl disulphide > diallyl trisulfide > Sallylcysteine 20,22 .

DADS, the principle sulphur compound of garlic oil is well known to possess hypoglycemic, hypolipidemic action 15,16 as well as anti-glycation activity 4,40 . It is known that DADS may enhance the half life of insulin probably by decreasing the activity of insulinase enzyme by a sulphydryl exchange reaction 41 . The results given in table 2 indicates, the glycated protein and lipid levels in brain are significantly decreased in DADS treated diabetic rats as compared to diabetic control rats suggesting that DADS may interfere in the non-enzymatic glycation process. This in part may be due to increased glucose oxidation or due to decreased gluconeogenesis, hence resulting in lesser availability of glucose, thus lowering glycation, as DADS has been suggested to possess hypoglycaemic action. DADS is a disulphide, may be involved in sulphydryl exchange reactions with proteins or enzymes 42, **??**3 similar to any other disulphide as follows: R1-S-S-R1 + R2 -SH ——> R1-S-S-SR2 + R1-SH Such non-enzymatic glycation in tissue proteins and probably in tissue lipids may induce an alteration in three dimensional structure of tissue proteins and thereby making the protein thiol (-SH) groups vulnerable for oxidative damage **??**4 . DADS decreases tissue protein glycation as well as tissue lipid glycation, thereby may decrease sulphydryl protein/lipid oxidation and hence preventing the possible tissue damage. This is evidenced by a decrease in brain tissue TBARS levels in DADS treated diabetic rats as compared to alloxan diabetic control rats (refer table **??**I).

V.

6 Conclusion

The present study suggests that DADS reduces glycation of brain protein and lipids as well as lipid peroxidation in alloxan diabetic brain tissue thus may be effective in prevention of CNS complications in diabetes mellitus like diabetic encephalopathy, diabetic dementia, etc. 43. Augusti [1] [2] [3] [4]

1

	Plasma glucose (mg/dl)	Glycated Hb (%)	Body weight (Gms)	Brain wt / body weight ratio
Group I	112.26	3.9	323.81	0.0062
(n=6)	19.6 +	1.2 +	55.65 +	0.004 +
Group II	623.66***	16.2***	217.85****	0.0087*
(n=6)	102.08 +	1.5 +	31.40 +	0.005 +
Group III	565.00	12.5***	210.16	0.0089
(n=6)	135.01 +	1.9 +	50.32 +	0.004 +

Note: 1.

Figure 1: Table 1 :

2

	Brain Total Proteins (mg/g)	Brain Glycated Protein (%)	Brain Total Lipids (mg/g)	Brain Glycated Lipids (%)	Brain Tissue TBARS (µ mol/g)
Group I (n=6)	85 21.21 + 75	8.26 0.98 + 9.97****	62.18 4.09 + 78.30****	6.49 2.22 + 27.98****	7.12 1.67 + 13.17****
Group II (n=6)	+ 15.43 75 + 15.43	+ 0.98 9.12** + 1.37	+ 12.66 77.57 + 6.54	+ 5.06 17.66**** + 1.59	+ 2.13 9.34**** + 1.88

Note:
1.

Figure 2: Table 2 :

[1]( ) B Effect of Diallyl Disulphide on Protein and Lipid Glycation, and Lipid Peroxidation in Brain of Alloxan Diabetic Rats

[2]( ) B Effect of Diallyl Disulphide on Protein and Lipid Glycation, and Lipid Peroxidation in Brain of Alloxan Diabetic Rats

[3]© 2013 Global Journals Inc. (US) © 2013 Global Journals Inc. (US)

[4]( )B

.1 This page is intentionally left blank

[Romanian and Biophys] , J Romanian , Biophys .

[Maldonado et al. ()] , P D Maldonado , D Limón , S Galván-Arzate , A Santamaria , J Pedraza-Chaverri . 2009.

[Silva-Islas et al. ()] , Carlos Silva-Islas , Ricardo A Santana , Ana L Colín-González , Perla D Maldonado . 2012. p. 2.

[Activation, an Innovative Therapeutic Alternative in Cerebral Ischemia, Advances in the Preclinical Study of Ischemic Stroke, D *Activation, an Innovative Therapeutic Alternative in Cerebral Ischemia, Advances in the Preclinical Study of Ischemic Stroke, Dr. Maurizio Balestrino*,

[Aguilera ()] 'Aged Garlic Extract Delays the Appearance of Infarct Area in Cerebral Ischemia Model, an Effect Likely Conditioned by the Celular Antioxidant System'. P Aguilera . *Phytomedicine* 2010. 17 (4-3) p. .

[Makimattila ()] 'Brain metabolic alterations in patients with type 1 diabetes-hyperglycemiainduced injury'. S Makimattila . *J Cereb Blood Flow Metab* 2004. 24 p. .

[Mccall ()] 'Cerebral glucose metabolism in diabetes mellitus'. A L Mccall . *Eur J Pharmacol* 2004. 490 p. .

[Choudary ()] K Choudary . *Biochemical techniques*, 1989. p. .

[Dubois et al. ()] 'Colorimetric methods for determination of sugars and related substances'. M Dubois , K A Gilles , J K Hamilton , P Rebers , F Smith . *Anal.Chem* 1956. 28 p. .

[Puchowicz et al. ()] 'Comparison of glucose influx and blood flow in retina and brain of diabetic rats'. M A Puchowicz , K Xu , D Magnes , C Miller , W D Lust , T S Kern , J C Lamanna . *J Cereb Blood Flow Metab* 2004. 24 p. .

[Varley et al. ()] 'Determination of Blood glucose (Folin Wu method'. H Varley , A Gowenloch , M Bell . *London Heimann Professional publishing Ltd* 1991. 1 p. . (Practical Clinical Biochemistry.)

[Varley et al. ()] 'Determination of Blood glucose (O' Toluidine method'. H Varley , A Gowenloch , M Bell . *London Heimann Professional publishing Ltd* 1991. 1 p. . (Practical Clinical Biochemistry.)

[Varley et al. ()] 'Determination of Serum proteins'. H Varley , A Gowenloch , M Bell . *London Heimann Professional publishing Ltd* 1991. 1 p. . (Practical Clinical Biochemistry.)

[Lukovits et al. ()] 'Diabetes mellitus and cerebrovascular disease'. T G Lukovits , T M Mazzone , T M Gorelick . *Neuroepidemiology* 1999. 18 p. .

[Beeri et al. ()] 'Diabetes mellitus in midlife and the risk of dementia three decades later'. M S Beeri , U Goldbourt , J M Silverman , S M Noy , J Schmeidler , R Ravona-Springer , A Sverdlick , M Davidson . *Neurology* 2004. 2004. 63 p. .

[Kim et al. ()] 'Dietary S-allyl-L-Cysteine Reduces Mortality With Decreased Incidence of Stroke and Behavioral Changes in Stroke-Prone Spontaneously Hypertensive Rats'. J M Kim , N Chang , W K Kim , H S Chun . *Biotechnology, and Biochemistry* 2006a. 70 (8) p. . (Bioscience)

[Vijay et al. ()] 'Effect of Diallyl disulphide protein and lipid glycation in alloxan diabetic rat kidney'. V Vijay , Vickram , R T Kashinath . *Journal of Advance Researches in Biological Sciences* 2010. 2 (2) p. .

[Augusti ()] 'Effect of long term feeding of the aqueous extracts of Onion (Allium cepa Linn) and garlic (Allium sativum Linn) on normal rats'. K Augusti , MathewP . *Ind. J. Exp. Biol* 1973. 11 p. .

[Levi ()] 'Effect of phosphotidylethanolamine glycation on lipid-protein interactions and membrane protein thermal stability'. Valeria Levi . *Biochem J* 2008. 416 p. .

[Atangwho ()] 'Effect of Vernonia amygdalina del leaf on kidney function of diabetic rats'. I J Atangwho . *Int.J. Pharmacol* 2007. (2) p. .

[Vijay et al. ()] 'Effects of Diallyl disulphide on renal glycated proteins and plasma sialic acid levels in alloxan Diabetic rats'. V Vijay , Vickram , R T Kashinath . *Global Journal of Medical Research* 2011. 11 (3/1) p. .

[Inge ()] 'Endothelial dysfunction, inflammation and apoptosisin Diabetes mellitus'. A M Inge . *Mediators of inflammation* 2010. 1 p. .

[Teruo et al. ()] 'Evidence of Biomembrane Lipid Glycation'. M Teruo , S Naoki , N Kiyotaka . *BUNSEKI KAGAKU* 2006. 55 (12) p. .

[Amir and Nisar ()] 'Glucosylated Glycerophosphoethanolamines are the major LDL Glycation Products and Increase LDL Susceptibility to Oxidation'. R Amir , Arnis K Nisar , A S . *Arterioscler Thromb Vasc Biol* 2000. 20 p. .

[Miyazawa ()] 'Glycation of brain lipid and its inhibitor'. A Miyazawa . *Uehara Kinen Seimei Kagaku Zaidan Kenkyu Hokokushu* 2004. 18 p. .

[Hu et al. ()] 'Hyperglycemia induces protein non-enzymaticglycosylation in brain neurons of diabetic rats at early stage'. Jingsheng Hu , Xueyi Ma , Shuli Sheng . *Neural Regeneration Research* 2007.

[Amadu et al. ()] 'Hypolipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two month period'. I Amadu , P K Joseph , K T Augusti . *Experentia* 1982. 38 p. .

[Fisher ()] 'Induction of Drug Metabolizing Enzymes by Garlic and Allyl Sulfide Compounds Via Activation of Constitutive Androstane Receptor and Nuclear Factor E2-Related Factor 2. Drug Metabolism and Disposition: The Biological Fate of'. C D Fisher . *Chemicals* 2007. 35 (6) p. .

[Bucala ()] 'Lipid advanced glycosylation: Pathway for lipid oxidation in vivo'. R Bucala . *Proc Natl Acad Sci* 1993. 90 (14) p. .

[Abe et al. ()] 'Lipid glycation occurs faster than protein glycation'. R Abe , O Higuchi , K Nakagawa , S Oikawa , T Miyazawa . *Seikagaku* 2005.

[Dei ()] 'Lipid peroxidation and advanced glycation end products in the brain in normal aging and in Alzheimer's disease'. Rika Dei . *Acta Neuropathol* 2002. 104 p. .

[Nadigar et al. ()] 'Malonyl-dialdehyde levels in different organs of rats subjected to acute alcoholoxicity'. H A Nadigar , S R Marcus , M V Chandrakala , . D Kulkarani . *Ind. J. clin biochem* 1986. 1 p. .

[Baquer et al. ()] 'Metabolic and molecular action of Trigonella foenum-graecum (fenugreek) and trace metals in experimental diabetic tissues'. N Z Baquer , P Kumar , A Taha , R K Kale , S M Cowsik , P Mclean . *J. Biosci* 2011. 36 p. .

[Van Der Graaf ()] 'Metabolic profile of the hippocampus of Zucker Diabetic Fatty rats assessed by in vivo 1H magnetic resonance spectroscopy'. M Van Der Graaf . *NMR Biomed* 2004. 17 p. .

[Mathew and Biju ()] 'Neuroprotective Effects of Garlic a Review'. B C Mathew , R S Biju . *The Libyan Journal of Medicine* 2008. 3 (1) p. .

[Vasudevan and Sreekumari ()] *Pg No 61-72. In textbook of Medical Biochemistry for medical students. 3 rd Edn*, Vasudevan , S Sreekumari . 2001. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. (Regulation of blood sugar and Diabetes mellitus)

[Pãcurar and Krejci] M Pãcurar , & G Krejci . *Medicinal Properties of Garlic: Importance of Its Antioxidant Activity*, (New York, United States) Nova Science Publisher. p. . (Garlic Consumption and Health)

[Kim et al. ()] 'Structure-Activity Relationship of Neuroprotective and Reactive Oxygen Species Scavenging Activities for Allium Organosulfur Compounds'. J M Kim , H J Chang , W K Kim , N Chang , H S Chun . *Journal of Agricultural and Food Chemistry* 2006b. 2006b. 54 (18) p. .

[Augusti ()] 'Studies on the effects of hypoglycemic principle from Allium cepa Linn'. K Augusti . *Ind. J. Med .Res* 1973. 61 p. .

[Block ()] 'The chemistry and health benefits of organosulphur compounds in garlic (Allium sativum): recent findings'. E Block . *Hypernutritious Foods*, (Auburn vale, Finland) 1985. p. .

[Tessier ()] 'The Maillard reaction in the human body. The main discoveries and factors that affect glycation'. F J Tessier . *Pathol. Biol* 2010. 58 p. .

[Mohora et al.] *The sources and the targets of oxidative stress in the etiology of diabetic complications*, Maria Mohora , Maria Greabu , Corina Muscurel , Carmen Du?? , Alexandra Totan .

[Aggarwal ()] 'Therapeutic uses of garlic'. K Aggarwal . *Ind. J. Expt. Bio* 1996. 11 p. .

[Augusti ()] 'Therapeutic values of Onion (Allium cepa Linn) and garlic (Allium sativum Linn)". Ind'. K Augusti . *J. Exp. Biol* 1996. 34 p. .

[Aragno (2005)] 'Up-Regulation of Advanced Glycated Products Receptors in the Brain of Diabetic Rats Is Prevented by Antioxidant Treatment'. Aragno . *Endocrinology* December 2005. 146 (12) p. .