Natural Product Sciences
13(4) : 295-299 (2007)

Protective Effect of Methanol Extract of *Swietenia macrophylla* Seeds on Oxidative States Associated with Streptozotocin Induced Diabetic Rats

Anup Maiti*, Saikat Dewanjee, Mintu Kundu, and Subhash C. Mandal

Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032.

Abstract – The methanol extract of seeds of *Swietenia macrophylla* King. (MESM) was studied for its antidiabetic activity in streptozotocin induced diabetic rats. It was principally aimed to correlate the efficacious role of MESM on reduction of oxidative state associated with diabetes. The extract was found to be potent antidiabetic evidenced by significant reduction of blood glucose level in diabetic rats (47.96% reduction of blood glucose level, at 300 mg/kg, on day 10). It was found that, MESM at 300 mg/kg, significantly decreased TBARS (35.03 and 22.22%) whilst increased GSH (86.75 and 31.45%), SOD (93.05 and 45.88%) and CAT (56.99 and 68.46%) levels in liver and kidney respectively in diabetic rats.
Keywords – Antioxidant, diabetes, streptozotocin, *Swietenia macrophylla*

Introduction

There is the concept of toxic effects by oxygen free radicals (Hughes, *et al.*, 1998), especially in diabetic condition (Feillet and Coudry, 1998). Diabetes mellitus is a multifunctional disease characterized by hyperglycemia and lipoprotein abnormalities (Scoppola, *et al.*, 2001). It causes the damage of cell membrane and in turn production of the oxygen centered free radicals are called reactive oxygen species (ROS). ROS includes superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), nitric oxide ($NO^{\cdot}$) and hydrogen peroxide (H_2O_2) and other molecules (Huang & Manton, 2004). In hyperglycemia, there is not only an increase in the production of ROS but also affects antioxidant reaction catalyzed by ROS scavenging enzymes (Uchimura, *et al.,* 1999). All organisms possess antioxidant enzymes like reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) are responsible for scavenging ROS (Ugochukwu, *et al.*, 2003). A number of research observations has indicated that, the risk of chronic diseases like diabetes in human is markedly reduced by dietary antioxidants chiefly present in foodstuff. Thus the aim of present investigation was to investigate the protective role of a traditional antidiabetic herb on augmented oxidative state associated with diabetes mellitus.

The plant *Swietenia macrophylla* King. (Meliaceae) is a beautiful, lofty, evergreen large tree (Rastogi, *et al.,* 1990). The seed of *S. macrophylla* has been reported for its anti-inflammatory, antimutagenecity, antitumor and antidiabetic activity (Guevera, *et al.*, 1996; Maiti, *et al.*, 2007). Phytochemical studies reported the presence of swietenine, swietenolide (Guha, *et al.*, 1951), swietemahonin, khayasin, andirobin, augustineolide, 7-deacetoxy-7-oxogedunin, 6-deoxy swietenine, proceranolide, 6-*O*-acetyl swietonolide, 2-hydroxy swietenine (Mootoo, *et al.,* 1999) in seeds of the plant. The aqueous extract of the seeds of *S. macrophylla* is widely used in Indonesia as folk medicine to cure diabetes (Shigetoshi, *et al.*, 1990). The rural people of East Midnapore, West Bengal, India, also use the aqueous extract of the seeds for the treatment of diabetes. The aim of present investigation was lied on evaluation of antidiabetic activity of methanol extract of *S. macrophylla* seeds on streptozotocin induced diabetic rats as well the establishment of efficacious role of extract to alleviate the augmented oxidative state associated with diabetes mellitus in term of TBARS, GSH, SOD and CAT activities in liver and kidney.

Experimental

Plant material – Seeds of *Swietenia macrophylla* King. (Meliaceae) were collected in the month of December and January, 2006 from Midnapore (East),

*Author for correspondence
Fax: +0091-33-28371078; E-mail: anup_phytochem@yahoo.com

West-Bengal, India. The plant was authenticated by H J Chowdhury, Joint Director, Botanical Survey of India, Shibpur, Howrah, India. A voucher specimen CNH/1-1(64) was deposited at our laboratory for future reference.

Preparation of methanol extract – The powdered seeds of *S. macrophylla* (600 g) were extracted exhaustively with methanol using Soxhlet apparatus. The resulting extract was concentrated using rotary vacuum evaporator and finally lyophilized. The yield obtained was 15% (w/w). The extract was stored in a refrigerator at 0 - 4 ^{o}C for further use.

Animals – Healthy adult Wister strain albino rats of both sex between 2 - 3 months of age and weighing 180 - 240 g were used for the study. Animals were acclimatized for a period of 2 weeks in our laboratory environment prior to the study. Rats were housed in polypropylene cages (4 animals per cage), maintained under standard laboratory conditions (i.e. 12 : 12 hour light and dark sequence; at an ambient temperature of 25 ± 2 ºC; 35 - 60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai, India) and water *ad libitum*. The principles of Laboratory Animals Care (NIII, 1985) were followed and instructions given by our institutional animal ethical committee were followed throughout the experiment.

Chemicals – Thiobarbituric acid, nitroblue tetrazolium (NBT) and nicotinamide adenine dinucleotide (NAD) were purchased from Loba Chemie, Mumbai, India. 5,5-dithio bis-2-nitro benzoic acid (DTNB), reduced glutathione (GSH) and Streptozotocin were procured from SISCO Research Lab, Bombay, India. Glibenclamide (Daonil^{TM}, Hoechst, India) tablets were purchased from local medical store, Jadavpur, India. All chemicals and reagents used were of analytical grade.

Induction of diabetes – Hyperglycaemia was induced in overnight fasted adult Wister strain albino rats weighing 180 - 240 g by a single intraperitoneal injection of 65 mg/kg Streptozotocin in a volume 1 mL/kg body weight (Siddque, *et al.,* 1987). Owing to the instability of streptozotocin in aqueous media, the solution was made in citrate buffer (pH 4.5) immediately before injection (Karunanayake, *et al.*, 1974). The elevated glucose level in plasma, determined at 48 hrs after injection, confirmed hyperglycaemia. The rats found hyperglycaemic were screened for the experiment.

Experimental design – One group of six normal rats was kept as normal control and treated with distilled water. Diabetic animals were divided into four groups of six rats in each group. Two groups of diabetic animals were treated with MESM at doses of 200 and 300 mg/kg body weight respectively by oral route; one group of diabetic animals was treated with standard drug glibenclamide at a dose of 10 mg/kg body weight orally (Mandal, *et al.*, 1997) whilst rest group was kept as diabetic control only treated with distilled water. All doses were started forty-eight hours after streptozotocin injection. Fasting blood glucose levels were estimated on overnight fasted rats on day 1 and 10.

After 10 days of treatment, all the rats were sacrificed by decapitation; livers and kidneys were separated and washed thoroughly to clear off blood. The tissues were immediately transferred to ice-cold containers containing 0.9% NaCl and homogenized in 0.1N Tris-HCl buffer (pH 7.4). This homogenized mass was used for the estimation of TBARS activity. For the assay of SOD, CAT and GSH 10% homogenate in 0.2 M phosphate buffer pH 8.0 was centrifuged and the clear supernatant was used for the subsequent assays of the enzymes.

Analytical procedure – Fasting blood glucose was estimated with the help of single touch glucometer (Ascensia Entrust, Bayer Health Care, USA). TBARS in tissues was estimated according to the methods of Fraga, *et al.* 1988 with minor modification. GSH (Ellman, 1959) SOD (Kakkar, *et al.,* 1984) CAT (Sinha, 1972) activities in selected tissues viz. liver and kidney were estimated by standard assay procedures.

Statistical analysis – Data were statistically calculated by utilizing one way ANOVA and expressed as mean ± SEM followed by Dunnett's *t*-test using computerized GraphPad InStat version 3.05, Graph pad software, USA.

Results and Discussion

Administration of streptozotocin induced diabetes in experimental rats evidenced by elevation of fasting blood glucose level. The methanol extract of seeds of *S. macrophylla* was found potent to reduce elevated blood glucose level of diabetic rats in a dose dependant manner (Table 1). The reduction of blood glucose level were found 16.08 ($p < 0.05$) and 47.96% ($p < 0.01$) at 200 and 300 mg/kg respectively, on 10th day, whilst maximal effect of 51.23% reduction of blood glucose level was found on day 10, $p < 0.01$ in glibenclamide treated diabetic rats.

A significant elevation of lipid peroxides in term of TBARS in liver and kidney was found in diabetes (80.46 and 48.92% respectively in liver and kidney, $p < 0.01$ when compared to the normal group). Administration of MESM significantly lowered TBARS level in diabetic rats particularly at the dose of 300 mg/kg. The reduction

Table 1. Effect of MESM on fasting blood glucose level of normal and diabetic rats

Group	Blood Glucose (mg/dL)	
	1st day	10th day
Normal (Distilled water)	74.17 ± 1.12	92.34 ± 6.06
Diabetic control (Distilled water)	276.67 ± 3.41	235.00 ± 12.57##
Diabetic + MESM (200 mg/kg)	285.00 ± 3.68	197.23 ± 10.42*
Diabetic + MESM (300 mg/kg)	295.67 ± 4.23	122.33 ± 9.22**
Diabetic + Glibenclamide (10 mg/kg)	297.00 ± 6.91	114.67 ± 8.27**

Each value is the mean ± S E M of six determinations.
##P < 0.01 Dunnett test as compared to normal.
*P < 0.05, **P < 0.01 Dunnett test as compared to diabetic control.

Table 2. Effect of MESM on TBARS level in liver and kidney of normal and diabetic rats

Group	TBARS (mM/100 g tissue)	
	Liver	Kidney
Normal (Distilled water)	0.87 ± 0.03	1.39 ± 0.05
Diabetic control (Distilled water)	1.57 ± 0.18##	2.07 ± 0.09##
Diabetic + MESM (200 mg/kg)	1.17 ± 0.11*	1.79 ± 0.15
Diabetic + MESM (300 mg/kg)	1.02 ± 0.15**	1.61 ± 0.14*
Diabetic + Glibenclamide (10 mg/kg)	0.92 ± 0.05**	1.53 ± 0.09**

Each value is the mean ± S E M of six determinations.
##P < 0.01 Dunnett test as compared to normal.
* P < 0.05, **P < 0.01 Dunnett test as compared to diabetic control.

of 35.03%, $p < 0.01$ and 22.22%, $p < 0.05$ respectively in liver and kidney were observed at 300 mg/kg comparable to that of standard glibenclamide (10 mg/kg) which, showed reduction of 41.40 and 26.09% respectively in liver and kidney, $p < 0.01$ (Table 2).

The GSH level was significantly lower in diabetic rats than in normal rats indicated in Table 3. Administration of MESM at 300 mg/kg body weight and glibenclamide increased significantly the GSH levels (increase of 86.75 and 31.45% respectively in liver and kidney, $p < 0.01$ as compared with the levels in diabetic rats).

The enzymatic antioxidants like SOD and CAT were significantly lower in diabetic rats as compared with their values in normal rats. Administration of MESM significantly increased the activities of all the enzymatic antioxidants. For SOD, maximal increase of 93.05 and 45.88% was observed at the dose of 300 mg/kg in liver and kidney tissues respectively, $p < 0.01$ whilst for CAT, maximal enhance of 56.99 and 68.46% was observed at the same dose in liver and kidney tissues respectively, $p < 0.01$. All results are very much comparable to that of standard glibenclamide indicated in Table 3.

Streptozotocin produces diabetes by liberating oxygen free radicals, which cause lipid peroxide mediated pancreatic injury (Rakieten, *et al.,* 1963; Palmer, *et al.*, 1988). At diabetic state, oxidative free radicals (ROS) are generated which cause oxidative stress due to impaired glucose metabolism and protein glycation (Ceriello, *et al.,* 1992; Wolff, *et al.,* 1991). ROS induce oxidative tissue damage plays an important role in many clinical disorders such as heart disease, diabetes, gout and cancer (Slater, 1984; Meneghini, 1988).

Elevated level of lipid peroxidation in tissues and plasma of streptozotocin induced diabetic rats is one of the characteristic features of chronic diabetes (Maxwell, *et al.,* 2001; Cho, *et al.*, 2002, Aydin, *et al.*, 2001). Oxidative stress associated with peroxidation of cellular lipids is determined by TBARS assay. The result showed that the administration of MESM reduced liver and kidney peroxide, which is an indication of inhibition of

Table 3. Effect of MESM on tissue GSH, SOD, and CAT of normal and diabetic rats

Parameters	Normal control (Distilled water)	Diabetic control (Distilled water)	Diabetic + MESM (200 mg/kg)	Diabetic + MESM (300 mg/kg)	Diabetic + Glibenclamide (10 mg/kg)
GSH – Liver [a]	43.48 ± 3.25	21.28 ± 1.63##	27.23 ± 1.12	39.74 ± 1.17**	41.32 ± 2.76**
GSH – Kidney [a]	19.55 ± 0.79	3.28 ± 0.13##	8.54 ± 1.17*	18.15 ± 2.45**	25.37 ± 1.14**
SOD – Liver [b]	7.32 ± 0.26	3.17 ± 0.23##	5.52 ± 0.13**	6.12 ± 0.16**	6.35 ± 0.28**
SOD – Kidney [b]	12.14 ± 0.29	6.56 ± 0.24##	8.72 ± 0.32*	9.57 ± 0.53**	10.07 ± 0.67**
CAT – Liver [c]	83.16 ± 3.21	42.76 ± 4.23##	61.13 ± 4.57*	67.13 ± 4.65**	71.16 ± 5.31**
CAT – Kidney [c]	33.18 ± 2.67	17.31 ± 2.24##	27.12 ± 2.21*	29.16 ± 2.63**	31.07 ± 1.70**

Each value is the mean ± S E M of six determinations.
##P < 0.01 Dunnett test as compared to normal.
*P < 0.05, **P < 0.01 Dunnett test as compared to diabetic control.
Units - [a] mg/100 g wet tissue.
[b] U/mg of protein, one unit is defines as the enzyme concentration require to inhibit the OD at 560 nm of chromogen produced 50% in 1 min.
[c] µmoles of H_2O_2 consumed/min/mg protein.

oxidative damage of hepatic and renal tissues.

GSH is a major endogenous antioxidant which counters the balance of free radical mediated damage. The decrease in liver GSH levels represents increased utilization due to oxidative stress (Anuradha, *et al.,* 1993). Impairment of the GSH redox cycle is an important mechanism of cell protection against ROS (Inouye, *et al.*, 1998). Increase in GSH content in liver and kidney after MESM treatment may be a function of lipid peroxidation inhibition.

Reduced activities of SOD and CAT in liver and kidney were observed during diabetes and this might result in a number of deleterious effects due to accumulation of super oxide radicals and hydrogen peroxide (Searle and Wilson, 1980). After treatment of MESM there was the increase in SOD and CAT levels in liver and kidney. The above observations indicated that the MESM seems to be effective for reducing oxidative stress associated with diabetes. This seeds of *S. macrophylla* have been reported to be rich in triterpenoids are well known antioxidants (Leelavinothan and Muniappan, 2004) that might scavenge the free radicals generated during diabetes. Now our intention is guided to isolate bioactive triterpenoid from extract and to substantiate its effectiveness against oxidation dependant diabetic pathogenesis.

Acknowledgement

Authors are thankful to All India Council for Technical Education, New Delhi for their financial support.

References

Anuradha, C.V. and Selvam, R., Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan induced diabetic rats. *J. Nutr. Biochem.* **4**, 212-217 (1993).

Ceriello, A., Quatro, A., and Giugliano, D., New insights on non enzymatic glycosylation may lead to therapeutic approaches for the prevention of the diabetic complications. *Diabet. Med.* **9**, 297-299 (1992).

Ellman, G.L., Tissue sulphydryl groups. *Arch. Biochem. Biophys.* **82**, 70-77 (1959).

Feilled, C., Rock, E., Coudray, C., Grzelkowska, K., Azais- Brasco, V., Darduct, D., and Mazeer, A., Lipid peroxidation and antioxidant status in experimental diabetes. *Clin. Chim. Acta.* **284**, 31-43 (1999).

Fraga, C.G., Leibovita, B.E., and Toppel, A.L., Lipid peroxidation measured as TBARS in tissue slices: Characterisation and comparison with homogenates and microsomes. *Free Radic. Biol. Med.* **4**, 155-161 (1988).

Guevera, A.P., Apilado, A., Sakarai, H., Kozuka, M., and Tokunda, H., Anti-inflammatory, antimutagenecity and antitumor activity of mahagony seeds *Swietenia macrophylla* (Meliaceae). *Phill. J. Sc.* **125**, 271-278 (1996).

Guha Sircar, S.S.G., and Chakraborty, T., Tetranortriterpenoid from *Swietenia macrophylla. J. Ind. Chem. Soc.* **28**, 207 (1951).

Huang, H. and Manton, K.G., The role of oxidative damage in mitrochondri during aging, a review. *Front Biosciences* **9**, 1100-1117 (2004).

Hughes, K., Choo, M., Kuperan, P., and Ong, C.N., Cardiovascular risk factors in non-insulin dependent diabetes compared to non diabetic controls: A population Based survey among Asians in Singapore. *Atherosclerosis* **136**, 25-31 (1998).

Inouye. M., Hashimoto, H., Mio, T., and Sumino, K., Levels of lipid peroxidation product and glycated hemoglobin A1C in the erythrocytes of diabetic patients. *Clini. Chimi. Acta* **276**, 163-172 (1998).

Kakkar, P., Das, B., and Visvanathan, P.N., A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.* **2**, 130-132 (1984).

Karunanayake, E.H., Hearse, D.J., and Mellows, G., The synthesis of ^{14}C Streptozotocin and its distribution and excretion in rat. *Biochem. J.* **142**, 673-685 (1974).

Leelavinothan, P. and Muniappan, L., protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipidperoxidation in stz diabetic male wistar rats. *BMC Complementary and Alternative Medicine* **4**, 16-23 (2004).

Maiti, A., Bhattacharya, P., and Mandal, S.C., Effect of *Swietenia macrophylla* seeds on normal and alloxan induced diabetic rats. *Oriental Pharmacy on Experimantal Medicine* (Accepted, will be published on **7**(4), 2007).

Mandal, S.C., Mukharjee, P.K., Saha, K., Das, J., Pal, M., and Saha, B.P., Hypoglycaemic activity of *Ficus racemosa* L. (Moraceae) leaves in streptozotocin induced Diabetic Rats. *Nat. Prod. Sci.* **3**(1), 38-41 (1997).

Maxwell, S.R.J., Thomson, H., Sander, D., Leguem, C, Bastex, A.M., Throp, G.H.G., Jones, A.F., and Bannette, A.M., Poor glycemic control is associated with reduced serum free radical scavenging (antioxidant) activity in non-insulin dependent diabetic mellitus. *Annal. Clin. Biochem.* **34**, 638-644 (1999).

Meneghini, R., Genotoxicity of active oxygen species in mammalian cells. *Mutat. Res.* **195**, 215-230 (1988).

Mootoo, B.S., Allisha, A., Motilal, R., Pingal, R., Ramlal, A., Khan, A., Reynolds, W.F., and McLean, S., Limonoids from *Swietenia macrophylla* and *S. aubrevilleana. J. Nat. Prod.* **62**(11), 1514-1517 (1999).

Rakieten, N., Rakieten, M.L., and Nadkarni, M.V., Studies on the diabetogenic action of streptozotocin. *Cancer Chemother. Rep.* **29**, 91-98 (1963).

Rastogi, R.P. and Mehrotra, B.N., Compendium of Indian Medicinal Plants. New Delhi, India, 1990.

Scopplola, A., Montechi, F.R., Mezinger, G., and Lala, A., Urinary mavalonate excretion rate in Type-2 diabetes: role of metabolic control. *Atherosclerosis* **156**, 357-361 (2001).

Searle, A.J. and Wilson, R., Glutathione peroxide effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. *Int. J. Radiat. Bio.* **37**, 213-217 (1980).

Shigetoshi, K., Lamik, M., Tohru, K., and Hisao, E., Constituents of the seeds of S. mahagony Jacq, isolation, structure and ^{1}H and ^{13}C NMR signal Assignments of new tetranortriterpenoids related to swietenine and swietenolide. *Chem. Pharm. Bull.* **38**(3), 639-651 (1990).

Siddque, M., Sun, Y., Lin, J.C., and Chien, Y.W., Facilitated transdermal transport of insulin. *J. Pharm. Sci.* **76**, 341-345 (1987).

Sinha, K.A., Colorimetric assay of catalase. *Annal. Biochem.* **47**, 389-394 (1972).

Slater, T.F., Free-radical mechanisms in tissue injury. *J. Biochem.* **222**, 1-15 (1984).

Uchimara, K., Nagasaka, A., Hayashi, R., Makino, M., Nagata, M.,

Kakizawa, H., Kobayashi, T., Fugiwara, K., Kato, T., Iwase, K., Shinohara, R., Kato, K., and Itoch, M., Changes in superoxide dismutase activities and concentrations and mycloperoxidase activities in leukocytes from patients with diabetes mellitus. *J. Diabat. Complications* **13**, 264-270 (1999).

Ugochukwu, N.H., Babady, N.E., Cobourn, M., and Gasset, S.R., The effects of *Gangronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *J. Biosci.* **28**, 1-5 (2003).

Wolf, S.P., Jiang, Z.Y., and Shunt, J.U., Protein glycation and oxidative stress in diabetic mellitus and aging. *Free Rad. Biol. Mol.* **10**, 339-352 (1991).

(Accepted September 27, 2007)