

Protective effect of *Asystasia gangetica* reduced oxidative damage in the small intestine of streptozotocin-induced diabetic rats

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SUMMARY

Oxidative stress plays an important role in the pathogenesis of various diabetic complications and small intestine is vulnerable to damage resulting in morphological and functional changes. In this study, the effects of *Asystasia gangetica* leaf extract (AGLE) on oxidative stress status in small intestine of diabetic rats were examined. The leaves of *Asystasia gangetica* was extracted with 70% ethanol. Oral administration of AGLE once daily (100 mg/kg and 200 mg/kg b.w.) for 28 days to diabetic rats significantly ($P < 0.05$) increased antioxidant levels of catalase, superoxide dismutase, glutathione peroxidase, glutathione, GSSH, carbohydrate metabolizing enzyme, glucose-6-phosphate dehydrogenase. The increased levels of protein carbonyl content, lipid peroxidation and xanthine oxidase/xanthine dehydrogenase in diabetic rats were reverted back to near normal levels on treatment with AGLE. Both doses of AGLE offered significant activity ($P < 0.01$) against oxidative damage and were comparable with standard, glibenclamide. The results revealed the occurrence of oxidative stress in small intestine during diabetes and suggest the potential of AGLE as an antioxidant in protecting the tissue defense system against oxidative damage in streptozotocin-induced diabetes.

Key words: Oxidative stress; Reactive oxygen species; Diabetic complications; Antioxidant status; Streptozotocin

INTRODUCTION

Diabetes causes increased oxidative damage through generation of ROS, which is thought to play an important role in the pathogenesis of various diabetic complications. Damage of cellular macromolecular components during hyperglycemia-induced oxidative

stress can trigger a series of responses like the activation of protein kinase C, transcription factor NF- κ B, glucose autooxidation, non-enzymatic glycation and over production of ROS by mitochondria (Konishi *et al.*, 1997; Koya *et al.*, 1998). Diabetes mellitus can inflict damage to the entire gastrointestinal tract from the oesophagus to colorectal region (Zhao *et al.*, 2002). These deleterious effects leads to hyperplasia and hypertrophy of epithelial cells, elevates levels of digestive enzymes, increased absorption of sugars and amino acids, enhanced endogenous synthesis of total cholesterol and

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triglycerides ultimately suppressing the fluidity of the brush border membrane (Sharma and Sivakami, 1998; Bhoj et al., 2004; Shirpoor et al., 2006). Gastrointestinal mucosa is constantly exposed to ROS generated by luminal contents such as oxidized food debris, transition metals like iron and copper, bacterial metabolites, bile acids and salivary oxidants (Halliwell et al., 2000).

The use of herbs in the treatment of diabetes is on the rise worldwide. Many traditional treatments have recommended medicinal plants in the complementary and alternative system of medicine but its mechanism, therapeutic efficacy, safety are not completely validated. Search for novel type of compounds with both hypoglycemic and antioxidant property from plant materials has achieved considerable attention in recent years and would serve as antidiabetic agents (Elder, 2004). *Asystasia gangetica* (L) T. Anders (Acanthaceae) commonly called as Tropical Primrose is a perennial herb usually found along road side and river banks climbing among bushes (Kirthikar and Basu, 1998). The tender leaves and shoots of this plant is used as a source of food as it contains rich amounts of proteins, vitamins, carbohydrates, minerals, lipids and fibers (Yeoh and Wong, 1993). The tribal peoples of Irular group applied the root paste of this plant to cure skin allergies (Senthilkumar et al., 2006). Juice of this plant is used in India as anthelmintic, to treat swellings and rheumatism (Kirthikar and Basu, 1998). In Nigeria the leaves of *A. gangetica* was claimed to be effective in the treatment of asthma and the anti-asthmatic property of the leaf extract is also reported (Akah et al., 2003). Bronchospasmolytic activity of the extract and fractions of *A. gangetica* is also documented (Ezike et al., 2008). In African traditional human and veterinary medicine the leaves of *A. gangetica* is used in the treatment of snake bite, fever, epilepsy, stomach aches, heart disease, gonorrhoea, enlargement of spleen, help child birth and the decoction of leaves given orally to treat internal parasitism, anthelmintic and vermifuge (Krief et al., 2005). A 5,11-epoxymegastigmane

glucoside (asysgangoside) was isolated from the aerial parts of *A. gangetica* along with the known compounds, salidroside, benzyl β -d-glucopyranoside, (6S,9R)-roseoside, ajugol, apigenin 7-O- β -d-glucopyranoside, apigenin 7-O-neohesperidoside, and apigenin 7-O- β -d-glucopyranosyl (16)- β -d-glucopyranoside (Kanchanapoom and Ruchirawat, 2007).

The purpose of the current study is to investigate the protective effect of *A. gangetica* against oxidative damage in the small intestine of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals

Streptozotocin, Adrenaline tartarate, NADPH, NADP, DTNB was purchased from Himedia, Mumbai. Bovine serum albumin was obtained from Loba Chemi., Mumbai. All other chemicals used in the study were of analytical grade procured from respective manufacturers.

Collection and preparation of plant material

The leaves of *A. gangetica* was collected from Coimbatore district, during the month of May and June 2007. The plant was identified and authenticated by Dr. G.V.S Moorthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University (TNAU), Coimbatore, India and the voucher specimen (BSI/SC/5/23/07-08/Tech-290 dated 05.06.2007) preserved in the department.

Extraction of plant material

The fresh leaves of the plant were collected and air dried under shade at room temperature. The leaves were powdered mechanically and stored in air tight container for experimental purposes. About 40 g of powder was mixed with 150 ml of ethanol and kept on mechanical shaker for 4 h and filtered. The contents obtained after shaking, was filtered through muslin cloth and the filtrate was concentrated under reduced pressure and controlled

temperature to yield a dark gummy solid. The extract was preserved in a refrigerator at 4°C (Subramoniam *et al.*, 1997).

Phytochemical screening

Phytochemical screening of the powdered leaves was performed to check for the presence of alkaloids, phenolics, flavonoids, saponins, carotenoids, carbohydrates and glycosides (Trease and Evans, 2002).

Experimental animals

Healthy adult Wistar albino rats of male sex weighing 150 - 200 g maintained under controlled temperature (20 - 24°C and 12 h light/dark) were fed with standard rat feed (Hindustan Lever Limited, Mumbai, India) and clean drinking water ad libitum. All animal procedures were performed in accordance with the recommendations for the proper care and use of laboratory animals.

Experimental induction of diabetes

The animals were allowed to fast for 12 h and after baseline blood glucose estimation they were administered freshly prepared streptozotocin (STZ), 60 mg/kg b.w. i.p) in 0.1 M cold citrate buffer, pH 4.5. The STZ treated animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. After 5 days of development of diabetes, rats with moderate diabetes having persistent glycosuria and hyperglycemia (blood glucose > 250 mg/dl) were considered diabetic and were used for further experimentation (Pavana *et al.*, 2007).

Experimental design

Animals were divided into six groups, consisting of a minimum of six animals each: (i) Group I, control rats received 10 ml/kg normal saline; (ii) Group II, diabetic control; (iii) Group III, diabetic rats administered 100 mg/kg *Asystasia gangetica* leaf extract (AGLE); (iv) Group IV, diabetic rats administered 200 mg/kg AGLE; (v) Group V, non diabetic rats administered 200 mg/kg AGLE; (vi)

Group VI, diabetic rats administered glibenclamide, 5 mg/kg. All the drugs were administered orally and treatment was continued for 28 days. The doses employed for all drugs were within therapeutic range to suit the experimental animal used.

At the end of the experimental period, rats were sacrificed by cervical dislocation under mild ether anesthesia and the small intestinal tissue was removed and was flushed with chilled 1.15% (w/v) KCl solution and the mucosa was scraped. A 10% w/v homogenate was prepared in cold potassium phosphate buffer (50 mM, pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 3000 rpm for 10 min, using Remi C-24 refrigerated centrifuge (Torricco *et al.*, 2007). The obtained supernatant were used for the following estimations: catalase (CAT) was estimated following the method of Abei (1984), superoxide dismutase (SOD) was assayed by the method of Kakkar *et al.* (1984), the activity of glutathione peroxidase (GPX) was estimated by using the method of Paglia and Valentine (1967), reduced glutathione (GSH) was assayed according to Sener *et al.* (2007), glutathione reductase (GR) was determined by the method of Racker (1955), glucose-6-phosphate dehydrogenase (G-6-PD) was estimated by using the method followed by Sudnikovich *et al.* (2007), thiobarbituric acid reactive substances (TBARS), xanthine oxidase (XO) and xanthine dehydrogenase (XDH) activities were assayed employing the method of Fraga *et al.* (1988) and Wang *et al.* (2004) respectively. The extent of protein oxidation was determined by adopting the procedure of Kyselova *et al.* (2005) and protein content in tissue homogenate was measured using bovine serum albumin as the standard by the method of Lowry *et al.* (1951).

Statistical analysis

Data are expressed as mean \pm S.E.M. Statistical analysis was done using one-way analysis of variance (ANOVA) and post-hoc comparison was carried out using Dunnett's test. *P* values < 0.05 were

considered as significant.

RESULTS

Phytochemical screening of plant extract revealed the presence of tannins, proteins, carbohydrates, saponins, flavonoids, terpenoids, alkaloids and steroids. Treatment with STZ for 28 days produced a significant ($P < 0.01$) decrease in the enzymatic antioxidants like catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and the non-enzymatic antioxidant, reduced glutathione when compared to normal control. Treatment with AGLE and glibenclamide significantly ($P < 0.05$) restored the levels of both enzymatic and non enzymatic antioxidant enzymes which is almost similar to the control group. The protective role of AGLE against ROS induced oxidative stress in small intestine homogenate was checked using G-6-PD. Treatment of diabetic rats with AGLE and glibenclamide significantly increased the level of G-6-PD when compared to diabetic control rats proving that G-6-PD can support antioxidant enzymes (Table 1).

There was a significant ($P < 0.01$) decrease in the level of total protein in the small intestine of diabetic control animal when compared to normal

control. Treatment with AGLE and glibenclamide for 28 days caused a significant ($P < 0.05$) increase in total protein content (Table 2). A significant ($P < 0.05$) increase in the concentration of the end products of lipid peroxidation, malondialdehyde (MDA) confirm the occurrence of oxidative damage in rats. Administration of AGLE (100 and 200 mg/kg) for 28 days caused a significant ($P < 0.05$) decrease in the malondialdehyde level. In non-diabetic AGLE treated group the level of MDA content was also found to be reduced. Treatment with glibenclamide significantly ($P < 0.05$) reversed the level of MDA when compared to diabetic control (Table 2). Animals treated with STZ produced a significant ($P < 0.01$) increase in XO/XDH enzyme activities in small intestine homogenate compared to control group. Pretreatment of rats with AGLE at a dose of 100 and 200 mg/kg b.w. produced significant ($P < 0.05$) reduction towards XO and XDH when compared with the diabetic control. Highest inhibition of the XO/XDH activities was found at dose of 200 mg/kg and the results were comparable to that of glibenclamide (Table 2). Exposure of rats with STZ resulted in a significant increase in protein carbonyl in small intestine in comparison to the control. Administration of AGLE and glibenclamide to STZ-induced diabetic

Table 1. Effect of *Asystasia gangetica* on the activities of enzymatic and non-enzymatic antioxidant levels

Group	Treatment	CAT ($\mu\text{M}/\text{min}/\text{mg}$ protein)	SOD ($\mu\text{M}/\text{min}/\text{mg}$ protein)	GSH ($\mu\text{M}/\text{min}/\text{mg}$ protein)	GR ($\mu\text{M}/\text{min}/\text{mg}$ protein)	GPX ($\mu\text{M}/\text{min}/\text{mg}$ protein)	G6PD ($\mu\text{M}/\text{min}/\text{mg}$ protein)
I	Control (Saline 10 ml / kg)	100.22 \pm 8.13	0.45 \pm 0.01	2.81 \pm 0.10	0.62 \pm 0.04	3.52 \pm 0.09	3.17 \pm 0.40
II	Diabetic control (STZ-60 mg/kg)	58.93 \pm 4.85**	0.31 \pm 0.44**	1.63 \pm 0.1**	0.39 \pm 0.04**	2.98 \pm 0.17**	2.07 \pm 0.24**
III	Diabetic + AGLE (100 mg/kg)	85.24 \pm 3.7*	0.44 \pm 0.0**	2.32 \pm 0.04*	0.63 \pm 0.05*	3.58 \pm 0.10*	2.91 \pm 0.12*
IV	Diabetic + AGLE (200 mg/kg)	87.47 \pm 11.29*	0.52 \pm 0.04*	2.62 \pm 0.16*	0.73 \pm 0.09*	3.63 \pm 0.17*	3.22 \pm 0.18*
V	Non diabetic + AGLE (200 mg/kg)	86.30 \pm 4.78*	0.47 \pm 0.03*	2.78 \pm 0.36*	0.60 \pm 0.05*	3.68 \pm 0.81*	2.91 \pm 0.12*
VI	Diabetic + Glibenclamide (5 mg/kg)	85.09 \pm 1.37*	0.44 \pm 0.02*	2.46 \pm 0.05*	0.64 \pm 0.02*	3.58 \pm 0.16*	3.16 \pm 0.75*

All values are expressed as mean \pm S.E.M.; (n = 6) animals in each group. * $P < 0.05$, ** $P < 0.01$, Group II rats were compared with Group I, animals in Group III, IV, V and VI were compared with Group II.

Table 2. Effect of *Asystasia gangetica* on the levels of lipid peroxidation, xanthine oxidase, xanthine dehydrogenase and total carbonyl content

Group	Treatment	Protein ($\mu\text{g}/\text{mg}$)	TBARS ($\text{nmol}/\text{min}/\text{mg}$ protein)	XO (nmol/uric acid formed/ min/mg protein)	XDH (nmol/uric acid formed/ min/mg protein)	Carbonyl content ($\text{nmol}/\text{min}/\text{mg}$ protein)
I	Control (Saline 10 ml / kg)	217.67 \pm 15.11	0.19 \pm 0.01	1.53 \pm 0.12	1.34 \pm 0.13	0.81 \pm 0.22
II	Diabetic control (STZ-60 mg/kg)	161.71 \pm 16.63**	0.62 \pm 0.07**	2.10 \pm 0.21**	0.79 \pm 0.04**	1.38 \pm 0.34**
III	Diabetic + AGLE (100 mg/kg)	247.64 \pm 15.11*	0.20 \pm 0.02*	1.36 \pm 0.10*	1.21 \pm 0.10*	0.88 \pm 0.50*
IV	Diabetic + AGLE (200 mg/kg)	252.80 \pm 13.56*	0.15 \pm 0.008*	1.37 \pm 0.07*	1.22 \pm 0.99*	0.72 \pm 0.39*
V	Non diabetic + AGLE (200 mg/kg)	243.01 \pm 16.15*	0.13 \pm 0.01*	1.52 \pm 0.07*	1.32 \pm 0.78*	0.71 \pm 0.67*
VI	Diabetic + Glibenclamide (5 mg/kg)	237.69 \pm 5.14*	0.17 \pm 0.003*	1.46 \pm 0.03*	1.26 \pm 0.04*	0.74 \pm 0.40*

All values are expressed as mean \pm S.E.M.; (n = 6) animals in each group. * $P < 0.05$, ** $P < 0.01$, Group II rats were compared with Group I, animals in Group III, IV, V and VI were compared with Group I.

rats consistently gave lower protein carbonyl level ($P < 0.01$) in small intestine in comparison to diabetic control. Administration of AGLE to normal rats did not produce any significant change in protein carbonyl content (Table 2).

DISCUSSION

The driving force besides the destructive nature of ROS is the unpaired electron residing within cellular structures, making them vulnerable, unstable and highly reactive. Oxidative stress and nitrosative stress occurs in biological system when there is an overproduction of ROS/RNS on one side and insufficiency of antioxidants on the other thus exacerbating the development and progress of diabetes (Valko *et al.*, 2007). STZ is commonly used in chemically induced diabetic animal model. The timing of STZ injection is important and will affect the type of diabetes that subsequently develops. If STZ is injected to adult rats (i.e. 3 months or older) type 1 diabetes results. However, if injected during the first week of birth, the capacity of pancreatic β -cell re generation remains in the animals, type 2 diabetes develops (Szkudelski, 2001).

SOD activity was determined in small intestinal homogenate. Epinephrine can be autooxidised to adrenochrome by superoxide radicals resulting in

the formation of H_2O_2 and molecular oxygen and the ability of SOD to inhibit this autooxidation serve as a basis for the assay. Induction of diabetes decreased the SOD activity and could result from inactivation of H_2O_2 or by glycation of enzymes (Sozmen *et al.*, 2001). The increase in SOD activity by AGLE suppresses the oxidative damage in small intestine and also protects CAT and GPX against inactivation by superoxide anions. CAT is a heme protein which catalyses the reduction of H_2O_2 and protects the tissues from highly reactive hydroxyl radicals. The decreased activity of CAT during diabetes may be due to excess production of H_2O_2 (Yan and Harding, 1997). Drug treatment resulted in a significant elevation of intestinal CAT in diabetic rats proving that AGLE can reduce glycation of enzymes or ROS and improve the antioxidant status.

GSH plays a multifactorial role in antioxidant defense and serve as a co-substrate for peroxide detoxification of GPX. Decreased intestinal GSH level in diabetic rats could be the result of either decreased synthesis or increased destruction of GSH by oxidative damage (Loven *et al.*, 1986). In our study treatment with AGLE and glibenclamide resulted in the increase of GSH and this may be due to either increased biosynthesis of GSH or reduced oxidative stress or both. GPX, a selenium containing enzyme, minimizes oxidative damage

by detoxifying H₂O₂ to H₂O through the oxidation of reduced glutathione. The depletion in GPX during diabetic state may result in the involvement of deleterious oxidative changes resulting in accumulation of toxic products (Kinalski *et al.*, 2000). The observed increase in GPX activity in drug treated groups may be due to decreased glycation of enzyme.

G6PD is a cytoplasmic and rate limiting enzyme of the hexose monophosphate (HMP) shunt pathway is decreased in diabetic conditions. It serves as a supporter by providing coenzymes to the primary antioxidant enzymes by playing a protective role against ROS mediated oxidative damage. Protein carbonyl content, a measure of oxidative damage to proteins, was found to be increased in small intestine of STZ-induced rats in comparison with healthy control, suggesting enhanced protein oxidation during hyperglycemic conditions (Stadtman, 1992). The decrease in protein content in diabetic control rats compared with those in control intestine could be partly due to leakage of proteins and insolubilization. Treatment with AGLE and glibenclamide prevented the increase in carbonyl content and decrease in protein and resulted in a significant activity in controlling the oxidative stress.

The extent of tissue damage can be assessed by the formation of a colored complex between MDA and TBA by measurement of thiobarbituric acid reactive substances. The induction of diabetes with streptozotocin results in an elevated level of TBARS in diabetic rats is due to the overproduction of lipid peroxidation byproducts and diffusion from damaged pancreatic tissues. This pathological consequence increases membrane rigidity, decreased cellular deformability and lipid fluidity (Gutteridge, 1995). Terpenoids and alkaloids were reported to have free radical scavenging activity and antioxidant capacity in diabetes (Jang *et al.*, 2000; Yasuda *et al.*, 2002). AGLE is found to contain alkaloids, flavonoids and terpenoids which may be responsible for scavenging free radicals generated by streptozotocin and thus enhance both enzymatic and non-enzymatic

antioxidants in diabetic rats.

Oxidative stress is implicated in the pathogenesis of various diabetic complications which may contribute to pathological alterations of some of the enzymes in gastrointestinal tract of hyperglycemic rats. Treatment of diabetic rats with AGLE at both doses improved type 1 diabetes induced oxidative stress by significantly decreasing the lipid peroxidation and protein oxidation and by elevating the antioxidant levels by protecting the tissue defense system against oxidative damage in streptozotocin-induced diabetes.

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