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Action of *Asparagus racemosus* Against Streptozotocin-Induced Oxidative Stress

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Abstract – Antidiabetic treatment with tubers of *Asparagus racemosus* (Liliaceae) extract (100 and 250 mg/kg body weight) for three weeks showed significant reduction in thiobarbituric acid reactive substances (TBARS) and glutathione reductase (GSH-R) in both liver and kidney. The treatment with *A. racemosus* significantly altered the glutathione (GSH) and GSH-R to be comparable with the control group. *A. racemosus* and glibenclamide treated rats showed decreased lipid peroxidation that is associated with increased activity of superoxide dismutase (SOD) and Catalase (CAT). The ability of *A. racemosus* on tissue lipid per oxidation and antioxidant status in diabetic animals has not been studied before. The result of this study thus shows that though, *A. racemosus* possesses moderate antidiabetic activity, but it exhibits potent antioxidant potential in diabetic conditions.
Keywords – *Asparagus racemosus*, Liliaceae, Antioxidant, Antidiabetic

Introduction

The elevated levels of blood glucose in diabetes produce oxygen free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation (Baynes, 1991). Glucose auto-oxidise in the presence of transition metal ions generates oxygen free radicals which make the membrane vulnerable to oxidative damage. The lipid peroxidation of the cell membrane has been associated with a number of pathological phenomenon such as increased membrane rigidity, decreased cellular deformability and lipid fluidity in erythrocytes. The action of diabetes inducing agents produces reactive free radicals, which have been shown to be cytotoxic to the β cells of the pancreas (Heikkila *et al.*, 1976). As the diabetogenic action can be prevented by the SOD, CAT, and other hydroxyl radical scavengers such as ethanol, dimethyl urea, there is evidence to suggest that the incidence of diabetes involves superoxide anion and hydroxyl radicals. The harmful effects of superoxide anion and hydroxyl radicals can be counteracted by antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (GSH-px). In addition to these enzymes, GSH-R and glutathione-S-transferanse (GST) provide GSH and help to neutralize toxic electrophiles respectively. There are clear cut evidence to show the role of free radicals in diabetes and studies indicate that tissue injury in diabetes may be due to free radicals (Grankvist *et al.*, 1981). Since *Asparagus racemosus* has been used in a number of formulations in Indian system of medicine as a adaptogen, and as an immunomodulator (Agarwal & Singh, 1999), but antidiabetic activity or the level of the antioxidant enzymes in diabetic rats have not been reported before.

Asparagus racemosus Willd. (Liliaceae), commonly known as 'Shatavari' (Hindi and Sanskrit), is a tall climber under-shrub found all over India. Almost all parts of this plant are used by the Indian traditional system of medicine (Ayurved and Unani) for the treatment of various ailments in human beings. In particular, the roots are used in dysentery, diarrhoea, tuberculosis, leprosy, skin diseases, epilepsy, inflammations, and as an expectorant (Jain, 1985; Nadkarni, 1976). Antioxidant properties of the *Asparagus racemosus* against damage induced by gamma-radiation in rat liver mitochondria has been reported (Kamat *et al.*, 2000). Chemically the plant has been reported to contain isoflavone (Saxena and Chourasia, 2001), polycyclic alkaloids, asparagine (Sekine *et al.*, 1994).

Thus keeping in view the above, the present study was undertaken to study the level of the antioxidant enzymes SOD, GSH, GSH-R, and CAT along with lipid peroxidation in rats after induction of diabetes by streptozotocin. The blood glucose level was also measured to study the

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antidiabetic activity.

Materials and Methods

Plant material – Root tubers of *Asparagus racemosus* were procured from Lucknow market, authenticated by was authenticated by Dr. A.K.S. Rawat and deposited in the departmental herbarium of National Botanical Research Institute (Voucher No. LWG 224831). The material was then coarsely powdered. The powder (500 g) was extracted with aqueous ethanol (50%) and concentrated under reduced pressure to obtain 10.46 g (5.23%) of the extract.

Animals – Male sprague Drawley rats (160 – 180 g) were purchased from the animal house of the Central Drug Research Institute, Lucknow, India. These were kept in the departmental animal house at 26 ± 2°C and relative humidity 44 – 55% light and dark cycles of 10 and 14 h respectively for one week before the experiment. Animals were provided with rodent diet (Amruth, India) and water *ad libitum*. All studies were conducted in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals".

Experimental induction of diabetes – Rats were rendered diabetic by injecting a freshly prepared aqueous solution of Streptozotocin (STZ, 50 mg/kg, i.p.) Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration after 96 h after the administration of STZ. The rats with blood glucose level above 200 mg/dl were considered to be diabetic and were used in the experiment.

Experimental design – After induction of diabetes, the rats were divided into 5 groups.

Group I - Control rats received vehicle solution (2% gum acacia)
Group II - Diabetic control
Group III - Diabetic rats treated with *Asparagus racemosus* extract 100 mg/kg b.w. in 2% gum acacia
Group IV - Diabetic rats treated with *Asparagus racemosus* extract 250 mg/kg b.w. in 2% gum acacia
Groups V - Diabetic rats treated with glibenclamide 600 μg/kg b.w. in aqueous solution

The vehicles and the drugs were administered orally using intra gastric tube daily for three weeks. After three weeks of treatment the rats were fasted overnight, the blood samples were analyzed for blood glucose content by using O-toluidine method (Sasaki *et al.*, 1972) with optical density measured by visible spectrophotometer at 520 nm. Then the animal was sacrificed by cervical decapitation. The liver and kidney was exposed and perfused with cold phosphate buffer saline of pH 7.4. Blood free liver was taken out and homogenized in a glass Teflon homogeniser (10% w/v). Incubations were done at 37°C under controlled conditions.

Antioxidant assay – The degree of lipid peroxidation was assayed by estimating the TBARS by using the standard method (Okhawa *et al.*, 1979) with minor modifications (Govindarajan *et al.*, 2003). GSH was estimated using Beutler *et al.* (1967), GSH-R was estimated using standard method of Horn (1963). SOD was measured by using the method of Marklund and Marklund (1974) based on the inhibition of autoxidation of pyrogallol. CAT activity was measured by using the rate of decomposition of H_2O_2 by the method of Aebi (1983). All these estimations were made in both liver and kidney.

Statistical analysis – Values were represented as mean ± S.D and data were analyzed by paired-*t*-test using SPSS software package.

Results

There was a moderate decrease in the blood glucose level of diabetic rats upon administration *A. racemosus* (Table 1). Table 2 shows the levels of TBARS, GSH, and GSH-R in liver and kidney of control and experimental animals. A significant elevation in tissues TBARS, GSH, and GSH-R was observed in the diabetic control rats as compared to the normal control rats. Oral administration of *A. racemosus* extract (100 and 250 mg/kg body weight) for three weeks shows significant reduction in TBARS and GSH-R in both liver and kidney. With respect to GSH there was a significant reduction in the glutathione in the liver while no significant reduction was observed in the kidney. The results were comparable to that of the standard drug.

Table 3 shows the activities of the enzymatic

Table 1. Effect of various treatments on blood glucose level

Groups	Blood glucose (mg/dl)		
	0 day	7th day	21st day
Group 1	95.45 ± 1.41	96.21 ± 2.33	93.66 ± 2.76
Group 2	240.78 ± 2.68	235.98 ± 2.87	228.33 ± 3.03
Group 3	242.36 ± 2.97	202.37 ± 3.96	140.98 ± 3.58
Group 4	240.69 ± 2.14	187.99 ± 4.17	119.77 ± 4.22
Group 5	239.59 ± 2.71	160.29 ± 3.49	94.33 ± 3.66

Results are expressed as mean ± S.D

Table 2. Effect of various treatments on lipid peroxidation and activities of glutathione and gultathione reductase

Groups	TBARS (nmoles of MDA/mg protein)		Glutathione (nM of DTNB conjugated/ mg protein)		Glutathione reductase (nM of NADPH oxidized/ mg protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Group 1	18.36 ± 0.41	16.97 ± 0.21	70.11 ± 1.26	46.20 ± 2.10	245.32 ± 6.75	261.35 ± 5.19
Group 2	20.47 ± 0.71	20.42 ± 0.46	120.20 ± 2.43	112.30 ± 4.30	421.88 ± 5.28	504.10 ± 9.45
Group 3	17.80 ± 0.63**	19.12 ± 0.22**	102.20 ± 2.86*	137.60 ± 3.50 (NS)	286.97 ± 7.11**	358.76 ± 6.31**
Group 4	11.14 ± 0.29**	14.22 ± 0.78**	72.40 ± 4.56**	126.20 ± 2.30 (NS)	240.21 ± 5.44**	252.36 ± 4.22**
Group 5	10.75 ± 0.36**	13.49 ± 0.55**	65.12 ± 2.20**	72.30 ± 2.6**	238.69 ± 7.87**	249.63 ± 7.88**

Results are expressed as mean ± S.D
P values were calculated based on the paired-*t*-test in comparison to group 2.
*$p<0.01$
**$p<0.001$
NS Not significant

Table 3. Effect of various treatments on super oxide dismutase and catalase

Groups	SOD (unit/mg protein)		Catalase (units/mg protein)	
	Liver	Kidney	Liver	Kidney
Group 1	15.8 ± 0.9	7.2 ± 0.63	62.2 ± 1.86	33.09 ± 1.63
Group 2	13.8 ± 0.76	12.5 ± 0.57	36.63 ± 1.57	14.18 ± 2.54
Group 3	13.16 ± 0.13*	6.8 ± 0.27*	52.32 ± 1.98**	22.64 ± 2.89**
Group 4	7.86 ± 0.37**	4.6 ± 0.11**	65.21 ± 2.33**	30.08 ± 2.36**
Group 5	7.79 ± 0.19**	4.3 ± 0.09**	64.22 ± 2.22**	29.81 ± 1.14**

Results are expressed as mean ± S.D.
P values were calculated based on the paired-*t*-test in comparison to group 2.
*$p<0.01$
**$p<0.001$

antioxidants SOD and CAT in liver and kidney. Activities of these enzymes decreased significantly in the diabetic control rats as compared to the normal control. Oral administration of the *A. racemosus* extract (100 and 250 mg/kg body weight) for three weeks significantly reversed these enzymes to near normal values.

Discussion

It has been shown that glucose under physiological conditions produces oxidants that possess reactivity similar to the hdyroxyl free radical. The oxidants hydroxylate benzoic acid, fragment protein and induce peroxidation in phosphatidyl choline and low-density lipoprotein. Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated fatty acids. Under physiologic conditions, low concentrations of lipid peroxides are found in tissues. Karpen *et al.*, (1982) observed an elevated level of lipid peroxides in the plasma of diabetic rats and lipid peroxidation as one of the characteristic features of chronic diabetes. Lipid peroxidation has also been observed in the development of both type 1 and 2 diabetes. Increased levels of lipid peroxides has also been reported in the kidney of diabetic rats and increased levels of TBARS as an index of lipid peroxidation (Nakakimura and Mizuno, 1980). The involvement of free radicals in diabetes and the role of these toxic species in LPO and the antioxidant defense system have been studied. Depletion of tissue glutathione and increase in lipid peroxidation has been observed in diabetes (Mukherjee *et al.*, 1994). It has been proposed that antioxidants that maintain the concentration of reduced glutathione may restore the cellular defense mechanisms, block lipid peroxidation and thus protect the tissue damage against oxidative damage (Rauschar *et al.*, 2000). Our results show that in diabetic control animals the level of TBARS was high due to increased lipid peroxidation. In *A. racemosus* and glibenclamide treated diabetic rats, the TBARS levels decreased both in liver and kidney which may be due to the free radical scavenging action of the active ingredients present the *A. racemosus*. Kamat *et al.* (2000) have reported that *A. racemosus* extract inhibited the radiation induced lipid peroxidation process effectively and that could be attributed to the ability to scavenge the free radicals involved in the initiation and propagation steps.

Reduction of oxidized form of glutathione requires NADPH, a cofactor and enzyme glutathione reductase. The reduced availability of NADPH which could be either due to reduced synthesis or increased metabolism of NADPH through some other pathway, could be responsible for low levels of reduced glutathione in streptozotocin treated rats as compared to control rats. One of the consequences of hyperglycaemia is increased metabolism of glucose by sorbitol pathway. Besides this, other pathways such as fatty acid and cholesterol biosynthesis also compete for NADPH with GSH. The significant increase in the GSH content and GSH-R activity in tissues in diabetic rats indicates an adaptive mechanism in response to oxidative stress (Garg *et al.*, 1996). The treatment with *A. racemosus* significantly altered the GSH and GSH-R to be comparable with the control group.

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical which damages the membrane and biological structures. CAT has been shown to be responsible for the detoxification of significant amounts of H_2O_2. SOD and CAT are two major scavenging enzymes, that remove the toxic free radical *in vivo*. Reduced activities of SOD and CAT in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Santhakumari *et al.*, 2003). *A. racemosus* and glibenclamide treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT.

The ability of *A. racemosus* on tissue lipid peroxidation and antioxidant status in diabetic animals has not been studied before. The result of this study thus shows though, *A. racemosus* possesses moderate antidiabetic activity, but it exhibits potent antioxidant potential in diabetic conditions. Thus the extract can be used in diabetic conditions or as a synergistic agent in herbal diabetic formulations as an antioxidant.

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