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Protective Effects of *Andrographis paniculata* Against Endothelial Dysfunction in Diabetic Wistar Rats

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Abstract: The aim of the present study was to elicit the therapeutic effect of *Andrographis* extract on oxidative stress in aorta as well as liver and kidney of streptozotocin diabetic rats. Aqueous leaf extract of *Andrographis paniculata* (*Andrographis*) [400 mg (kg body weight)$^{-1}$ day^{-1}] was administered to the animals 30 days before diabetes induction and continued for next 6 months after the diabetes induction. There was a significant decrease in the activity of superoxide dismutase (SOD), catalase and glutathione (GSH) in liver and kidney of the diabetic rats. *Andrographis* administration to diabetic rats resulted in increase in the activity of SOD, catalase and GSH both in liver as well as kidneys. The diabetic rats exhibited endothelial dysfunction as it was evident from the loss of vasodilatory response to the acetyl choline (Ach). This vasodilatory response was restored in the diabetic animals treated with *Andrographis*. Based on these observations, we conclude that *Andrographis* reverses the endothelial dysfunction associated with diabetes. This effect appears to be due to its antioxidant properties.

Key words: Oxidative stress, *Andrographis*, endothelium dependent relaxation, antioxidant, thoracic aorta

INTRODUCTION

Oxidative stress has been defined as a disturbance in the balance between antioxidants and pro-oxidants (free radicals and other reactive oxygen and nitrogen species), with increased levels of pro-oxidants leading to potential damage. Hyperglycemia, defining established diabetes, can induce oxidative stress by various mechanisms; excessive levels of glucose reaching the mitochondria lead to an overdrive of the electron transport chain, resulting in overproduction of superoxide anions normally scavenged by mitochondrial SOD. When this mechanism fails, oxidative stress develops and it is been proposed that this mechanism is responsible for the activation of all major pathways underlying the different components of vascular diabetic complications (Rebecca, 2006). Guerci *et al*. (2001) suggest that antioxidant defense may be lower in diabetes, which includes reduced antioxidant status or free radical scavenging activity and this plays an important role in mediation of endothelial dysfunction accompanying diabetes.

It was also found out that relaxing response of aortic ring to Ach was decreased in untreated diabetic rats (Guerci *et al*., 2001; Baluchnejadmojarad and Roghan, 2003). The studies have clearly indicated that antioxidants may be beneficial in reducing oxidative stress and endothelial dysfunction in diabetes and eventually preventing the vasculopahy, which accounts for the majority of clinical complications in diabetes mellitus (Ting *et al*., 1996; Maritim *et al*., 2003).

Andrographis is a shrub found throughout India and other Asian countries. The benefits of *Andrographis* in preventing heart diseases, protecting the liver diseases, stimulating gall bladder

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contraction have been reported in earlier works (Nalamolu, 2006; Zhao and Fang, 1991; Zhang and Tan, 1997; Wang and Zhao, 1994). Zhang and Tan (2000) have reported for its antioxidant and antihyperglycemic activity when administered to diabetic rats for 14 days.

In the present study, we have determined whether antihyperglycemic or antioxidant properties of *Andrographis* contribute towards normal functioning of endothelial, which was lost during long term diabetes.

Our data suggests that *Andrographis* restored the normal endothelial functioning, prevented the decrease in the antioxidant enzyme activities. However the prevention in the hyperglycemic condition was not comparable with the controls. Hence the protection of endothelial dysfunctioning by *Andrographis* might be because of antioxidant properties and may an effective therapeutic agent in treating the macrovascular complications of diabetes.

MATERIALS AND METHODS

Plant Material

Aqueous leaf extract of *Andrographis* was procured from and authenticated by Green Chem. Laboratories, Bangalore, Karnataka, India.

Drugs and Chemicals

All the drugs and biochemicals used in this study were purchased from Sigma Chemical Company, Inc., St Louis, MO, USA. The chemicals were of analytical grade.

Animals

Female Wistar rats weighing 150-180 g were procured from inbuilt animal house and all the experiments were conducted as per the protocol approved by the institutional animal ethics committee. (Reg. No. 83/1999 CPCSEA). They were kept in clean and dry cages with a bedding of paddy husk, exposed to 12 h dark and light cycle, fed with normal chow diet, Amrut feeds, India and water *ad libitum*.

Experimental Design

Animals were divided into 3 groups: normal control; diabetic control, in which diabetes was induced by single administration of STZ (45 mg kg^{-1}, i.p.), dissolved in freshly prepared 0.1 M citrate buffer, pH 4.5; the third group of animals were given *Andrographis* (400 mg kg^{-1} p.o.) for a period of 4 weeks before inducing the diabetes. The animals having blood glucose levels >250 mg dL^{-1} were considered as diabetic. Drug treatment was continued for next 6 months. The animals had free access to standard chow diet and drinking water.

Measurement of Plasma Glucose

The blood (about 0.5 mL) was collected by retro orbital vein puncture. The plasma glucose levels were measured after 8, 16 and 24 weeks after the induction of diabetes by using standard glucose oxidation method.

Measurement of SOD, Catalase and GSH Activity

After the experimental period i.e., 6 months, the animals were anaesthetized with the mixture of ketamine and xylazine, liver and kidneys were collected and assayed for SOD (Kakkar *et al*., 1984), catalase (Sinha, 1972) and glutathione (Manea *et al*., 2004) in liver and kidney tissue homogenates.

Before collecting the tissues, rat aorta was isolated and the rings prepared were mounted on blood vessel chamber to study the endothelial dependent vascular relaxations.

Measurement of Isometric Force

Aortic Ring Isolation

After the experimental procedure, the animals were anaesthetized using a mixture of ketamine (50 mg kg^{-1}, i.p.) and xylazine (8 mg kg^{-1}, i.p.). The thoracic aorta (from the arch of aorta to diaphragm) was quickly excised and placed in ice cold carbogenated (95% O_2 + 5% CO_2) Krebs-Henseleit Buffer (KHB). The aorta was cleaned of adhering fat and tissue and cut into 3-4 mm segments. Due care was taken not to stretch the tissue or damage the endothelium. The composition (mM) of KHB was NaCl: 136, KCl: 5.4, $MgSO_4.7H_2O$:0.5, $CaCl_2$:2.7, KH_2PO_4:0.45, $NaHCO_3$:11.9 and glucose: 5.6 per liter (Turcotte *et al*., 2002).

Relaxation of the Aorta Precontracted with Phenylephrine

Each ring was suspended by a pair of 's' shaped stainless steel hooks in a water jacketed organ bath (10 mL capacity), filled with 10 mL of carbogenated KHB maintained at 37°C. A resting tension of 2 g was applied to the aortic rings. After 30 min, the rings were exposed to sub maximal dose of Phenylephrine (PE) i.e., 10^{-6} M and the response was recorded, which were then allowed to equilibrate for further 60 min during which they were washed every 15 min with the KHB. After the equilibrium period, the rings were precontracted with sub maximal dose of PE (10^{-6} M) and vasorelaxant effects were recorded with cumulative addition of acetyl choline (10^{-9} to 10^{-6} M). The isometric tension responses were measured on a 4-channel polyrite (Recorders and Medicare Systems (P) Ltd., Ambala, India) using an isometric force transducer (T-305, Recorders and Medicare Systems (P) Ltd., Ambala, India). Responses were expressed as percentage relaxation of PE induced contraction.

Statistical Analysis

Statistical difference between the treatment and the controls was tested by one way analysis of variance followed by Tukey's multiple comparison test. A probability level of $p<0.05$ being regarded as significant. Relaxant responses are given as percentage relaxation relative to pre contraction levels to PE. Data are shown as mean±SEM of experiments.

RESULTS

STZ administration produced the characteristic signs of diabetes such as increased intake of water and food, failure to gain the body weight (loss of weight) and increased blood glucose concentration during the 24 weeks of observation.

All control age matched female wistar rats (n = 10) survived the 24 week period, while in the diabetic control animals, the survival rate was 100 (n = 10), 60 (n = 6) and 50% (n = 5) during 8, 16 and 24th weeks, respectively. In the diabetic animals treated with *Andrographis*, the survival rate of animals was 100 (n = 10), 70 (n = 7) and 40% (n = 4) during 8, 16 and 24 weeks, respectively.

Body Weights

The STZ administered (diabetic) animals showed a significant decrease in body weight at different time intervals as compared with the control (treated with normal saline) animals. The treatment with *Andrographis* prevented this decrease in the diabetic animals significantly ($p<0.01$). Further the body weights of the group of diabetic animals treated with *Andrographis* (400 mg kg^{-1}) was found to be comparable to that of the control group of animals (Table 1).

Table 1: Effects of *Andrographis* on body weights at different time intervals in normal and experimental animal groups

Groups	0th day	30th day	60th day	90th day	120th day	150th day	180th day
Control	150.0±2.3	160.0±2.8	158.5±3.1	159.0±2.9	161.0±3.8	165.0±3.9	169.0±5.4
STZ treated	156.4±2.47	178.5±2.58	154.0±4.2	140.2±3.81*	143.6±3.03**	107.0±1.22***	104.0±1.87***
STZ+*Andrographis*	144.0±3.48	179.5±3.11	168.0±5.83	181.4±3.22**	187.1±2.64**	159.0±11.2	181.0±12.98**

The values represent the means±SEM. *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ as compared to control group, respectively

Table 2: Effects of *Andrographis* treatment in diabetic animals on glucose levels (mg dL^{-1})

Groups	96 h after STZ administration	8 weeks	16 weeks	24 weeks
Control	87.63±2.91	90.1±3.85	89.1±4.1	102.6±3.75
STZ	324.40±16.7	350.6±11.4	324.2±7.85$^{(n=6)}$	298.6±6.75$^{(n=5)}$
Andrographis	334.80±9.24^{a}	223.7±13.4^{a}	199.0±10.2$^{a\ (n=7)}$	188.4±9.85$^{b\ (n=4)}$

The values represent the means±SEM. ^{a}: $p<0.05$ and ^{b}: $p<0.01$ as compared to diabetic control group, respectively

Table 3: Effect of *Andrographis* (Andro) on the activities of SOD, Catalase and GSH in liver and kidney of normal and experimental animals

	Liver			Kidney		
Groups	GSH (mg g^{-1} protein)	SOD (U^{1} mg^{-1} protein)	CAT (U^{2} mg^{-1} protein)	GSH (mg g^{-1} protein)	SOD (U^{1} mg^{-1} protein)	CAT (U^{2} mg^{-1} protein)
Control	49.83±0.598	9.39±0.312	80.90±1.140	34.76±0.623	14.90±0.505	42.72±1.814
STZ	25.00±2.667	3.99±0.406	39.66±2.578	21.80±0.729	8.62±0.483	24.82±1.224
Andrographis	48.51±0.79^{c}	6.62±0.256^{c}	61.55±0.785^{c}	33.15±1.404^{c}	11.23±0.356^{b}	36.05±0.622^{b}

The values represent the means±SD, ^{b}: $p<0.01$ and ^{c}: $p<0.001$ as compared to diabetic control group, respectively. U^{1}: One unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in 1 min, U^{2}: µmol of hydrogen peroxide consumed/min

Plasma Glucose Levels

The diabetic animals treated with *Andrographis* showed significant decrease in the glucose level at 8, 16 and 24th weeks (Table 2) as compared to the diabetic animals. Even though a significant ($p<0.05$) antihyperglycemic effect was evident from 8th week onwards, the decrease in the blood glucose level was highly significant ($p<0.01$) at 24th week as compared with the diabetic animals. However the *Andrographis* treatment could not restore glucose levels to normal.

Effects on Antioxidant Enzymes

The GSH content was decreased both in liver and kidney of the diabetic animals as compared with the controls. However the diabetic group of animals treated with *Andrographis*, showed significant ($p<0.001$) reversal in the GSH content, the values being comparable with the controls significantly. The diabetic animals exhibited significant reduction in the activities of SOD and catalase in liver and kidney. Treatment with *Andrographis* showed significant increase in the liver ($p<0.001$) and kidney ($p<0.01$) SOD and catalase activity, respectively (Table 3).

Vascular Reactivity

Addition of sub maximal dose of Phenylephrine (10^{-6}M) to organ bath resulted in contraction of aorta of all groups. Isolated aortic rings precontracted with Phenylephrine (10^{-6}M) showed dose dependent relaxation to acetyl choline (10^{-9} to 10^{-6} M). The magnitude of relaxations was significantly ($p<0.001$) reduced (19.78% Vs 92.57%) in diabetic rats when compared with control groups Fig. 1. The aortic rings of diabetic animals treated with Andrographis showed significant relaxation ($p<0.001$) as compared to the diabetic aortic rings (95.94% vs 19.78%) respectively, which was comparable with the control.

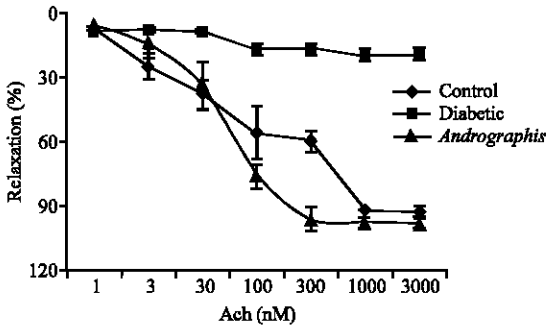

Fig. 1: Effect of *Andrographis* on vascular relaxation as compared with control and diabetic animals

DISCUSSION

In the present study, we have evaluated the protective effects of *Andrographis* on endothelial dysfunction in STZ induced diabetic rats.

Hyperglycemia can induce oxidative stress by various mechanisms; excessive levels of glucose reaching the mitochondria lead to an overdrive of the electron transport chain, resulting in overproduction of superoxide anions normally scavenged by mitochondrial SOD. When this action by mitochondria fails, is responsible for the activation of major pathways viz glycation, PKC activation leading to vascular diabetic complications (Anabela and Carlos, 2006). Matsumoto *et al*. (2004) have suggested that inactivation of Nitric Oxide (NO) by oxygen derived free radicals is responsible for the endothelial dysfunction characterized by impairment of endothelial dependent relaxation associated with diabetes. Involvement of oxidative stress in diabetes and related complications has led to the use of antioxidants as new therapeutic agents and it has been demonstrated that reversing the oxidative stress has improved the functioning of the endothelium (Laight *et al*., 2000).

Short term studies have indicated that the use of anti oxidants may improve endothelium-dependent vasodilation, insulin sensitivity, blood pressure, protein glycation and glucose metabolism. Therefore long term intervention studies are needed to assess the effectiveness of antioxidant therapy in the treatment of diabetes and its complications (Kuyvenhoven and Meinders, 1999).

Yoshida *et al*. (1995) have showed that there was significant increase in the glutathione synthesis from erythrocytes in diabetes after 6 months of treatment with an oral antidiabetic agent.

In the present study, the beneficial effects of the *Andrographis* were studied in hyperglycemia induced oxidative stress condition in STZ diabetic rats for a period of 6 months and also the effects on endothelial dependent vasorelaxation.

There was significant increase in the glucose levels in diabetic animals throughout the experimental period. The treatment with *Andrographis* (400 mg kg^{-1}) showed decrease in the glucose level at different time interval as compared with the diabetic animals significantly ($p<0.001$). However are not comparable with those of control animals.

The increase in the oxidative stress associated with diabetes was implicated by significant decrease in the anti oxidant enzymes viz., SOD, catalase and glutathione both in liver as well as in kidneys in diabetic rats (Table 2). This suggests that antioxidant defense is lower in diabetes and is supported by earlier reports (Laight *et al*., 2000). The treatment with *Andrographis* showed significant ($p<0.01$) prevention in decrease in the levels of these antioxidants.

There is impairment of endothelium-dependent relaxation response to acetyl choline in diabetic rat aorta. Several studies have indicated that the increased production of superoxide anions in vessels

of diabetic animals and it is suggested that this active form of oxygen can inactivate NO to attenuate NO-dependent vasodilatory response in the diabetic rat aorta (Rebecca, 2006; Galen *et al*., 1997; Vriese *et al*., 2000).

We found that the aortic rings of the diabetic animals treated with *Andrographis* showed significant increase in the endothelial dependent relaxation when compared with that of the untreated diabetic animals. The relaxation was comparable with that of the controls. This indicates its protective role against oxidative damage in diabetes.

Although the mechanisms underlying endothelial dysfunction may be multifactorial, there is a growing body of evidence that increased production of free radicals may considerably contribute to this phenomenon. Hyperglycemia, being the characteristic feature of diabetes is one of the reasons for the increase in the oxidative stress in diabetes.

In the present study, *Andrographis* showed decreased oxidative stress (protecting the endothelial layer) owing to its antioxidant properties.

Hence from the above observations, it can be concluded that *Andrographis* reduced the oxidative stress in diabetes which was evident from increase in the anti oxidant enzymes level and thus rendered protection from endothelial damage which is usually associated with diabetes.

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