EVALUATION OF BENEFICIAL EFFECTS OF ANTIOXIDANT PROPERTIES OF AQUEOUS LEAF EXTRACT OF *ANDROGRAPHIS PANICULATA* IN STZ-INDUCED DIABETES

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ABSTRACT

The beneficial effects of antioxidant properties *Andrographis paniculata (Andrographis)* were studied in the diabetic animals. Hyperglycemia, characteristic feature of diabetes mellitus leads to decreased antioxidant defense and hence development of oxidative stress, which in involved in the etiology of development of diabetic complications. The evidences suggest that good glycemic control and/or use of antioxidants may play an important role in the prevention of complications associated with diabetes. Diabetes was induced with single intraperitoneal injection of streptozotocin (45 mg/kg, i.p) dissolved in freshly prepared citrate buffer (pH 4.5), resulted in elevation of blood glucose levels, decrease in the superoxide dismutase and catalase activity. Oral administration of *Andrographis* (400 mg/kg, p.o) resulted in significant decrease in the blood glucose levels and increase in the activity of SOD and catalase. In conclusion *Andrographis* decreased the blood glucose levels in diabetic animals may be due to its antioxidant properties.

Keywords: *Andrographis, diabetes, oxidative stress, antioxidant, streptozotocin.*

INTRODUCTION

Free radicals, also known as reactive oxygen species (ROS), play a role in the etiology of several major diseases, including cancer, atherosclerosis, and diabetes (Kuyvenhoven *et al*., 1999; Kebapci *et al*., 1999). Increase in extra and intracellular concentration of glucose leads to oxidative stress, which can be defined as imbalance between oxidants and antioxidant status (Nedeljkovic *et al*., 2003). Oxidative damage, due to oxidative stress, may play an important role in the pathogenesis of complications of diabetes (Venkateswaran *et al*., 2003; Farhangkhoee *et al*., 2003). Oxidative stress is associated with type-II diabetes leads to generation of free oxygen radicals which will initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alterations in the structure and function of collagen basement and other membranes (Friedman *et al*., 2003 and Lipinski, 2001). Over the past decade, there has been increased interest in oxidative stress and its role in the development of complications of diabetes. Apart from traditional anti diabetic treatment, anti oxidant therapy may benefits in diabetes. Many plants and herbs have potent antioxidant activity, but there is a need to explore these properties in diabetes. *Andrographis* is a shrub found throughout India and other Asian countries. It is been reported that *Andrographis* benefits for preventing heart diseases, helps protecting the liver diseases, stimulates gall bladder contraction (Zhao and Fang, 1991; Zhang and Tan, 2000;Wang and Zhao, 1994).

In the present study, we have evaluated the beneficial effects of aqueous leaf extract of *Andrographis* in diabetes with relation to its antioxidant activity.

MATERIALS AND METHODS

Plant material

Aqueous leaf extract of *Andrographis* was supplied by Green Chem. Laboratories, Bangalore.

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

Male Wistar rats weighing 180-200 grams 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/ 1999CPCSEA). They were kept in clean and dry cages with a bedding of paddy husk, exposed to 12 hr dark and light cycle, fed with normal chow diet, Amrut feeds, India, and water *ad libitum*.

Induction of experimental diabetes

Diabetes was induced in rats by single intraperitoneal injection of streptozotocin (45 mg/kg, i.p) dissolved in freshly prepared citrate buffer (pH 4.5, 0.1M), in overnight fasted rats. The blood glucose levels were measured by glucose oxidase method 96 hrs after the injection. Each animal with blood glucose concentration above 250mg/dl was considered to be diabetic. To overcome the hypoglycemia, which occurred during the

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first 24 h following streptozotocin (STZ) administration, diabetic rats were given 5% glucose solution orally.

After the confirmation of diabetes, animals were divided into following groups; Grouping (3 x 6)

Group I: Control (Normal saline)
Group II: Diabetic animals, STZ (45mg/kg, i.p)
Group III: Diabetic animals treated with *Andrographis* (400 mg/kg, p.o)

Group I and Group II animals were fed with normal saline orally, and Group III with *Andrographis* (400mg/kg, orally) for 45 days respectively. Fasting blood samples were collected on day 0, 15th and 45th and estimated for glucose levels.

After 45 days, animals were sacrificed and liver was isolated. Livers from various animals were homogenized in 10 times their weight of buffer, the homogenate was centrifuged for 15 min at 6000 RPM and the supernatants used immediately for measurement of superoxide dismutase (SOD) and catalase (CAT).

Measurement of plasma glucose

Blood was withdrawn by puncturing retro orbital vein on 8, 16 and 24th week of the treatment and assayed for glucose concentration by glucose oxidase method.

Assay of antioxidant enzymes

After the experimental period, the animals were sacrificed, liver was isolated, homogenized in chilled triss buffer at a concentration of 10% (w/v). The homogenate was centrifuge in cold centrifuge (Remi, Pvt. Ltd.) and the supernatant was assayed for SOD and catalase activity.

The catalase activity was assayed by the method of Sinha (1972). In brief, the incubation mixture contained in a final volume of 2.0ml, 0.1ml of diluted homogenate, 1.0ml of phosphate buffer and 0.4ml of distilled water to which 0.5ml of H_2O_2 solution was added to initiate the reaction, while the H_2O_2 solution was left out in control tubes. After incubating for 1 min at 37^{0}C the reaction was stopped by addition of 2 ml of potassium dichromate-acetic acid reagent. The samples were kept in boiling water bath for 15 minutes, finally cooled and the absorbance measured at 570 nm against control.

$$\text{Activity} = \frac{\Delta \text{ OD x Std conc. } (\mu \text{ mol})}{\text{Enzyme (ml) x Std. OD x Protein (mg/ml)}}$$

$$\text{Unit} = \mu \text{ moles/min/mg/protein.}$$

The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar *et al* (1984). Briefly, in a test tube, 0.5ml of supernatant of centrifuged tissue homogenate was taken. To this 1.5ml of carbonate buffer (pH10.2), 0.5ml of EDTA and 0.4µl of epinephrine was added and the OD was taken at 480nm. Epinephrine was added just before taking the OD.

STATISTICAL ANALYSIS

Values are presented as mean ± S.E.M and difference between the treatment and the controls were tested statistically by one way analysis of variance followed by Dunnet's multiple comparison test.

RESULTS

Effects on body weight

The STZ administered (diabetic) animals showed a significant decrease in body weight at different time intervals (table 1) as compared with the control animals. However, the decrease in body weight was prevented significantly ($p<0.01$) in the group of diabetic animals treated with *Andrographis* as shown by the body weights on 15th, 30th and on 45th day of treatment when compared with the diabetic animals. Further the body weights of the group of diabetic animals treated with *Andrographis* was found to be comparable to that of the control group of animals.

Effects on glucose levels

While Streptozotocin administration resulted in significant ($p<0.01$) elevation of serum glucose levels (table 2), as compared with the control animals, the chronic treatment of *Andrographis* resulted in significant decrease in the elevation of serum glucose levels ($P<0.01$) on 15th as well as on 45th day as compared with the diabetic animals. However, the chronic treatment of *Andrographis* could not bring back the sugar to normal levels as seen in control animals.

Effects on Antioxidant enzymes

Table 3 shows the activities of SOD and catalase in liver homogenate. There was significant ($P<0.01$) reduction in the activities of SOD and catalase in diabetic animals as compared to the control. Administration of *Andrographis* to the diabetic animals resulted in significant ($P<0.01$) increase in the activities of SOD and catalase.

DISCUSSION

In the present study, we have evaluated the benefits of antioxidant properties of *Andrographis* in the STZ induced diabetes in rats. Oxidative stress is an imbalance between the oxidants and antioxidant status (Noda *et al*., 2000; Laight *et al*., 2000). It plays an important role in pathogenesis of diabetes complications. In addition, sustained hyperglycemia has been identified as a principal mediator of increased reactive oxygen species generation

Table 1: Effects on the body weights (grams) in STZ induced diabetes

Group	Mean body weight ± SEM				
	Day 1	7th day	15th day	30th day	45th day
Control	187.5±6.5	187.6±2.1	189.5±2.5	191.2±2.9	195.4±4.7
STZ(45mg/kg)	198.6±7.9	197.8±8.5	162.5±8.5[a]	157.8±10.5[b]	147.6±11.4[b]
Andro(400mg/kg)	183.7±3.7	185.6±4.5	191.5±7.8**	197.5±5.4**	200.8±9.7**

Values expressed as Mean ± SEM (n=6), *P < 0.05, **P < 0.01 as compared with diabetic animals, a-p<0.05, b-p<0.01 as compared with control animals.

Table 2: Blood glucose levels in *Andrographis* treated diabetic animals

Group	Blood glucose levels (mg/dl)		
	0th Day	15th Day	45th Day
Control	101.6±3.756	89.31±8.775	114.3±9.846
STZ(45mg/kg)	106.3±2.856	364.6±22.55[c]	389.5±29.10[c]
STZ+Andro(400mg/kg)	98.6±6.154	208.7±4.004**	252.6±16.690**

Values expressed as Mean ± SEM (n=6), * P < 0.05, ** P < 0.01, as compared with diabetic animals, c-p<0.001 as compared with control animals.

Table 3: Effects on the activities of liver SOD and catalase

Group	SOD (U^a/mg protein)	CAT (U^b/mg protein)
Control	10.5±1.55	85.3±5.80
STZ (45mg/kg)	4.65±0.19[c]	39.78±1.36[c]
STZ+ Andro (400mg/kg)	8.56±1.82**	71.20±1.48**

Values are given Mean ± SD (n=6), ** P < 0.01, as compared with diabetic animals, c-p<0.001 as compared with control animals
U^a: one unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in 1 min
U^b:µmol of hydrogen peroxide consumed/min

in diabetes (Kayamori and Igarashi, 1994; Santakumari *et al*., 2003).

STZ treatment results in diabetes because of destruction of beta cells and hence increases in the generation of ROS, leading to tissue damage. This diabetic condition can be overcome by antioxidant enzymes viz SOD, catalase and other hydroxyl radical scavengers. In our study we aimed at utilizing the antioxidant properties of *Andrographis* in oxidative stress mediated diabetes.

Administration of STZ (single dose, 45mg/kg, i.p) resulted in significant increase in the blood glucose levels, decrease in the antioxidant enzymes (SOD & catalase) activity, and loss of body weight.

The animals treated with *Andrographis* showed significant decrease in fasting blood glucose levels on 15th and 45th day of diabetes as compared to diabetic animals.

The treatment also increased the activities of SOD and catalase as compared to the diabetic animals.

The *Andrographis* treatment significantly improved the body weight loss at different time intervals throughout the period of experiment as compared to the diabetic animals which indicates its anti diabetic activity.

As it is evident that hyperglycemia is an independent factor for increased in the oxidative stress in diabetes, *Andrographis* which caused increased in the antioxidant enzyme activities i.e., SOD and catalase showed significant decrease in the fasting blood glucose levels might be because of its antioxidant properties.

In conclusion, our study demonstrates that *Andrographis* (400mg/kg, p.o) showed potential antioxidant and antidiabetic activity without causing hypoglycemic state. These properties may offer great benefit in the management of diabetes mellitus involving increased oxidative stress. Further investigation is in progress to find out its mechanism of action and to establish its potential in the treatment of macro vascular complications of diabetes.

ACKNOWLEDGEMENT

The author is thankful to Indian Council of Medical Research, New Delhi, for the research fellowship.

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