

# Antidiabetic and Antioxidant potential of *Andrographis paniculata* Nees. leaf ethanol extract in streptozotocin induced diabetic rats

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## ABSTRACT

This study aimed to elucidate the potential of *Andrographis paniculata* (leaves) both as an antidiabetic and as an antioxidant in streptozotocin induced diabetic rats. Diabetes was induced by intraperitoneal injection of streptozotocin. After grouping, diabetic rats were administered the leaf ethanol extract (250 and 500 mg/kg b.w.) for 28 days. Fasting blood glucose, body weight, creatinine and urea levels and histopathological study of pancreas were carried out to evaluate the antidiabetic effects. Enzyme activity of superoxide dismutase, catalase and glutathione peroxidase in the liver homogenate was assayed. After the treatment with *A. paniculata* leaf extract, fasting blood glucose, creatinine and urea levels were found to be decreased in diabetic rats. The extract was found to be non toxic as seen by the normal creatinine and urea levels in the extract fed normal rats. There was an increase in the activity of liver antioxidant enzymes in diabetic treated rats. Histopathological study of pancreas revealed the islet cell restoring and regenerative ability of *A. paniculata* extract. According to our present findings, *A. paniculata* leaves possessed significant antihyperglycemic and antioxidant effect in streptozotocin induced diabetic rat which might be due to its islet cell restoring and regenerative ability as well as the upregulation of antioxidant enzymes.

## INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. As the disease progresses, tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, nephropathy, cardiovascular complications and microbial complications. Thus, diabetes covers a wide range of heterogeneous diseases (Bastaki, 2005). There is an increasing evidence in both experimental and clinical studies which suggests that diabetes is associated with oxidative stress, leading to an increased production of reactive oxygen species, including superoxide radical, hydrogen peroxide and hydroxyl radical (Bagri *et al.*, 2009). Experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes (Matteucci and Gimapietro, 2000) and more importantly in the development of diabetic complications (Sepici-Dincel *et al.*,

2007). The world prevalence of diabetes among adults (aged 20-79 years) was estimated to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030 (Shaw *et al.*, 2010). Out of the two types of diabetes, the incidence of non-insulin dependent diabetes mellitus is much higher than the insulin dependent diabetes mellitus (Hussain, 2002).

Prevention and control of complications associated with diabetes has become one of the key issues in medical research. And effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in diabetic patients (Xie *et al.*, 2003). Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more adverse effects (Bohannon, 2002). Since the existing drugs for the treatment of diabetes mellitus do not satisfy our need completely, the search for new drugs continues. In recent years, herbal remedies for the unsolved medical problems have been gaining importance in the research field. Apart from the currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Devaki *et al.*, 2011).

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India has a rich history of using various potent herbs and herbal preparations for treating diabetes. *Andrographis paniculata* (Burm. f.) Wall. ex. Nees. is an erect annual herb belonging to the family Acanthaceae. It is extremely bitter in taste in all parts of the plant body. *Andrographis paniculata* has traditionally been used over centuries in Asia as a folklore medicine for a variety of ailments or as herbal supplements for health promotion. Indian pharmacopoeia narrates that it is a predominant constituent of at least 26 Ayurvedic formulations and used to treat various diseases (Kumar *et al.*, 2004). The plant has been reported to possess antipyretic, analgesic, antihepatotoxic, antidiabetic, antimalarial, antibacterial, antifertility, anti-inflammatory and immunosuppressive properties (Pandey, 2011). *In vitro* screening of different solvent leaf extracts of *A. paniculata* for antioxidant activities played a strategic role in the selection of ethanol extract for the antidiabetic study. This provides deep insight into the use of extracts for therapeutic purpose and totalitarian approach emphasized in traditional medicine.

The whole plant has been used for preparing various formulations in treating diabetic patients as an antihyperglycemic and as an antioxidant agent. But there are no substantial evidences to prove its efficacy *in vivo*. Hence, this study aimed to elucidate the potential of *A. paniculata* both as an antihyperglycemic and as an antioxidant in streptozotocin induced diabetic rats.

## MATERIAL AND METHODS

### Plant material

Fresh and healthy leaves of *A. paniculata* were obtained from local growers of Mysore. The sample specimen was authenticated at Botanical Herbarium, Department of Botany, University of Mysore, Mysore with an accession number 535.

### Preparation of solvent extract

The leaves of *A. paniculata* were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were subsequently dried in a hot air oven at 40°C for 48 h, powdered to 100-120 mesh in an apex grinder [Apex Constructions, London] and used for extraction. Fifty grams of the powdered material was extracted initially with 300 ml of ethanol for 24 h at 23 ± 2°C. The extract was filtered with sterile Whatman No. 1 filter paper into a clean conical flask. Second extraction was carried out with the same amount of solvent for another 24 h at 23 ± 2°C and filtered. The extracts were later pooled and transferred into the sample holder of the rotary flash evaporator [Buchi Rotavapor R-124, Switzerland] for the evaporation of the solvent. The evaporated extract so obtained was preserved at 4°C in airtight bottle until further use. The suspension of the extract was prepared by dissolving the weighed amount in freshly prepared 1% gum arabic solution.

### Animals

Adult healthy Albino rats of Wistar strain of either sex weighing 120-160 g with no prior drug treatment were used in the

present study. Animals were maintained at 22 ± 2°C with 12 h light and dark cycle. The animals were fed on a standard pellet diet and had free access to water throughout the experiment. Animals that are described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water. Animal study was performed in Central Animal Facility, JSS Medical College, Mysore, with due permission from Institutional Animal Ethics Committee (R.No. JSS/MC/IEC/5064/CPCSEA).

### Induction of diabetes

After initial determination of 16 h fasting blood glucose levels (blood drawn through the tail vein puncture) animals were given single intraperitoneal injection of Streptozotocin at a dose of 45 mg/kg, freshly dissolved in cold 0.9% saline (Venkateswaran and Pari, 2003). Following injection, animals were carefully observed for first 24 h for evidence of allergic reaction, behavioral changes and convulsions.

Fasting blood glucose levels were recorded after 5 days. Animals that developed stable hyperglycemia with fasting blood glucose levels more than 200 mg/dl were selected for the study.

### Treatments

The animals were randomly divided into the 7 groups with six animals in each group. Group A constituted the normal control, group B and group C were the normal controls fed with 250 and 500 mg/kg of the leaf extract respectively, group D was the diabetic control, group E was the drug control and group F and group G were the diabetic rats treated with 250 and 500 mg/kg of the extract respectively. Saline, leaf extract and the standard drug were administered orally using a mouth gauge.

### Blood collection

Rats were fasted overnight; blood was drawn from the tail by tail vein puncture during the experimental period and from cardiac puncture at the end of the experimental period (after 28 days). Serum was separated by centrifuging the collected blood samples at 10,000 rev/min for 10 min at 4°C and used for the estimation of creatinine and urea.

### Urine collection

Urine from normal control rats, diabetic and diabetic treated rats were collected under a layer of toluene by keeping the rats in metabolic cages for 24 h. Collected urine samples were filtered through filter paper, centrifuged and stored at 4°C until further analysis.

### Preparation of liver tissue homogenate

After 28 days, rats were dissected under anesthesia. Liver was rinsed in ice cold distilled water followed by chilled 0.9% saline. About 1 g of liver tissue was homogenized in 10 ml of 10 mmol/l phosphate buffered saline (pH 7.4) using REMI

homogenizer fitted with a teflon plunger (REMI Laboratory Instruments, Mumbai, India). The homogenate was centrifuged at 10,000 rev/min at 4°C for 15 min and the supernatant was used for the determination of antioxidant liver enzymes *viz.*, superoxide dismutase, catalase and glutathione peroxidase.

### Analytical Methods

#### Blood glucose estimation

Blood samples were collected by tail vein puncture at weekly intervals for a period of 28 days. Fasting blood glucose was measured by glucose oxidase-peroxidase (GOD-POD) method in mg/dl using a digital glucometer (Braun Omnitest<sup>R</sup> EZ, Germany) (Trinder, 1969).

#### Creatinine estimation

Creatinine estimation was carried out in both serum and urine samples using creatinine estimation kit (Excel Diagnostics Pvt. Ltd., Hyderabad, India) according to manufacturer's instruction (Folin and Wu, 1919).

#### Serum urea estimation

Serum urea was estimated using urea estimation kit (Excel Diagnostics Pvt. Ltd., Hyderabad, India) according to manufacturer's instruction (Talke and Schubert, 1965).

#### Estimation of superoxide dismutase, catalase and glutathione peroxidase

The activity of superoxide dismutase was assayed in a Daytona analyser using RANDOX SOD kit (Woolliams *et al.*, 1983). The activity of catalase was assayed by hydrogen peroxide method (Luck, 1971). The activity of glutathione peroxidase was assayed in a Daytona analyzer using RANDOX glutathione peroxidase kit (Paglia and Valentine, 1967).

#### Histopathological studies

At the end of the study, the rats were sacrificed, whole pancreas from each animal was removed, washed in normal saline and fixed in 10% formalin, embedded in paraffin and sections of 3-5  $\mu$ m thickness were cut and routinely stained with basic dye haematoxylin and acidic dye eosin to differentiate the nucleus and cytoplasm. The sections were studied at 10 $\times$  and 40 $\times$  magnifications for the islet cell characteristics using a binocular compound microscope.

### STATISTICAL ANALYSIS

All the values of fasting blood sugar, biochemical estimations, body weight were expressed as mean  $\pm$  standard error of mean (SEM). Statistical difference was evaluated by using one way analysis of variance (ANOVA) followed by Turkey's test. Data were considered statistically significant at  $p$  value  $\leq$  0.05. Statistical analysis was performed using Graph Pad statistical software.

### RESULTS

#### Effect of leaf extracts on blood glucose level

Administration of Streptozotocin produced 68.7% increase in fasting blood glucose levels of diabetic control rats compared to the normal control rats and the increased glucose levels in group D rats was maintained over a period of four weeks. Administration of *A. paniculata* ethanol leaf extract to Streptozotocin induced diabetic rats for four weeks produced a significant blood glucose reduction [Table 1]. Reduction in blood glucose was observed from the first week by both extract and glibenclamide. At the end of 4<sup>th</sup> week, 500 mg/kg of extract produced 29.2% ( $p = 0.000$ ) blood glucose reduction in group G rats. Similar to group G rats, there was a lowering of 25.2% ( $p = 0.000$ ) blood glucose in the rats treated with 250 mg/kg of the extract. Among the two doses of the extracts used, 500 mg/kg of the extract showed greater reduction in blood glucose level which was comparable to glibenclamide.

#### Effect of leaf extract on body weight

Prior to Streptozotocin administration, there were no significant differences in the average body weights of all the 7 groups of experimental animals. By the end of the first week after diabetes was experimentally induced, the weights of groups D, E, F, and G were significantly reduced as shown in Table 2.

This weight loss continued for four weeks in diabetic control animals. Administration of *A. paniculata* ethanol leaf extract (250 mg/kg and 500 mg/kg) and glibenclamide significantly increased the body weights in diabetic rats as compared to diabetic control rats. The effect of 500 mg/kg of extract on the body weight of diabetic rats was better than glibenclamide.

#### Effect on kidney parameters

Serum and urinary creatinine levels and serum urea levels of normal and diabetic rats treated with *A. paniculata* ethanol leaf extract is shown in Table 3. Diabetic control rats exhibited higher serum creatinine, urinary creatinine and serum urea levels compared to those of normal rats. The creatinine and urea levels were significantly decreased by glibenclamide and the extract due to 28 days of treatment.

In groups F and G animals there was a reduction in serum creatinine [74.1% and 71.9% ( $p = 0.000$ )], urinary creatinine [63.4% and 60.6% ( $p = 0.000$ )] and serum urea (50.5% and 49.3% ( $p = 0.000$ )) levels as compared to diabetic untreated animals. In the normal control rats fed with the extract (groups B and C) the creatinine and urea levels were similar to the normal control rats. No mortality was observed in the extract treated rats and behavior of the treated rats also appeared normal.

#### Effect on liver antioxidant enzymes

As shown in Table 4, Streptozotocin induced diabetic control rats showed a significant decrease in superoxide dismutase,

**Table 1:** Effect of ethanol extract of *Andrographis paniculata* leaves on glucose levels in streptozotocin induced diabetic rats (n=6).

Group	Treatment	Fasting blood glucose levels (mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
Group A	Normal control rats (0.9% NaCl)	77.0 ± 1.82	78.25 ± 0.95	77.75 ± 2.21	79.50 ± 2.64	79.25 ± 1.70
Group B	Normal control rats with 250 mg/kg b.w. of extract	78.25 ± 2.31	78.0 ± 3.16	79.25 ± 2.88	79.15 ± 2.21	79.50 ± 1.29
Group C	Normal control rats with 500 mg/kg b.w. of extract	78.75 ± 2.62	78.70 ± 2.26	78.50 ± 3.31	79.25 ± 2.98	79.75 ± 2.75
Group D	diabetic control rats	246.25 ± 9.53*	284.0 ± 5.47*	309.50 ± 8.73*	319.75 ± 7.80*	319.50 ± 8.66*
Group E	Diabetic rats with glibenclamide (0.5 mg/kg b.w.)	248.50 ± 13.52	222.2 ± 7.27**	190.0 ± 4.54**	178.25 ± 6.80**	157.75 ± 8.95**
Group F	Diabetic rats with extract (250 mg/kg b.w.)	235.75 ± 10.65	219.50 ± 7.41**	205.50 ± 6.24**	189.75 ± 10.24**	176.25 ± 9.06**
Group G	Diabetic rats with extract (500 mg/kg b.w.)	233.0 ± 12.03	216.75 ± 8.65**	198.50 ± 8.18**	179.75 ± 10.07**	163.25 ± 12.33**

Values are expressed as mean ± S.E.M.

\* P ≤ 0.05, significantly different from normal control group.

\*\* P ≤ 0.05, significantly different from diabetic control group.

**Table 2:** Effect of treatment of *Andrographis paniculata* leaf extract on body weight of Streptozotocin induced diabetic rats (n=6).

Groups	Treatment	Average body weight (g)				
		Day 1	Day 7	Day 14	Day 21	Day 28
Group A	Normal control rats (0.9% NaCl)	142.50 ± 5.0	146.25 ± 2.50	152.50 ± 5.0	160.0 ± 4.08	166.25 ± 4.78
Group B	Normal control rats with 250 mg/kg b.w. of extract	141.0 ± 2.25	145.25 ± 4.25	153.75 ± 4.78	159.0 ± 4.50	164.75 ± 4.71
Group C	Normal control rats with 500 mg/kg b.w. of extract	140.0 ± 0.0	146.25 ± 4.08	153.50 ± 5.25	161.25 ± 5.70	167.50 ± 6.20
Group D	diabetic control rats	135.50 ± 5.77	115.0 ± 5.77*	105.0 ± 5.77*	95.0 ± 4.08*	86.25 ± 4.78*
Group E	Diabetic rats with glibenclamide (0.5 mg/kg b.w.)	132.50 ± 5.0	120.0 ± 8.16**	125.0 ± 5.77**	123.75 ± 4.78**	126.25 ± 2.50**
Group F	Diabetic rats with extract (250 mg/kg b.w.)	120.0 ± 0.00	117.0 ± 2.44	118.75 ± 2.50**	122.50 ± 2.88**	123.75 ± 4.78**
Group G	Diabetic rats with extract (500 mg/kg b.w.)	125.0 ± 5.77	118.75 ± 2.50	121.25 ± 2.50**	125.0 ± 4.08**	125.50 ± 5.25**

Values are expressed as mean ± S.E.M.

\* P ≤ 0.05, significantly different from normal control group.

\*\* P ≤ 0.05, significantly different from diabetic control group.

**Table 3:** Effect of treatment of *Andrographis paniculata* leaf extract on serum creatinine, urinary creatinine and serum urea levels in normal and streptozotocin induced diabetic rats (n=6).

Groups	Treatment	Serum creatinine (mg/dl)	Urinary creatinine (mg/day)	Serum urea (mg/dl)
A	Normal control rats (0.9% NaCl)	0.47 ± 0.04	18.72 ± 1.83	23.74 ± 0.73
B	Normal rats with extract (250 mg/kg b.w.)	0.44 ± 0.04	18.42 ± 0.75	23.79 ± 0.26
C	Normal rats with extract (500 mg/kg b.w.)	0.43 ± 0.08	18.25 ± 0.34	23.72 ± 0.43
D	Diabetic control rats	1.82 ± 0.17	51.52 ± 3.76	61.94 ± 1.54
E	Diabetic rats with glibenclamide (0.5 mg/kg b.w.)	0.60 ± 0.07*	23.61 ± 2.38*	29.73 ± 0.59*
F	Diabetic rats with extract (250 mg/kg b.w.)	0.51 ± 0.01*	20.25 ± 0.91*	31.40 ± 0.62*
G	Diabetic rats with extract (500 mg/kg b.w.)	0.47 ± 0.04*	18.85 ± 0.71*	30.63 ± 0.46*

Values are expressed as mean ± S.E.M.

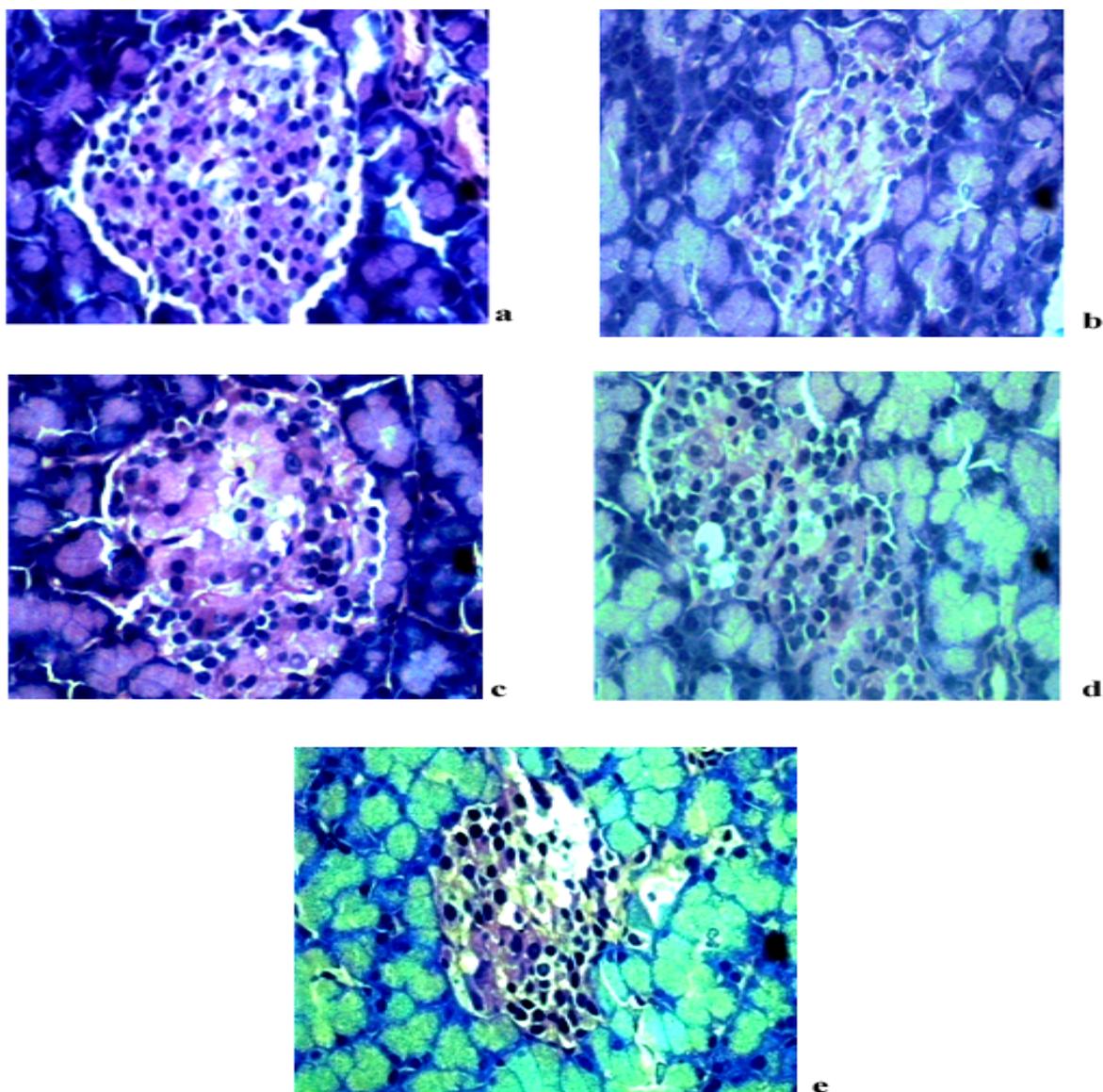
\* P ≤ 0.05, significantly different from diabetic control group.

**Table 4:** Effect of ethanol extract of *Andrographis paniculata* leaves on liver antioxidant enzymes in normal and streptozotocin induced diabetic rats (n=6)

Groups	Treatment	Superoxide dismutase (U/mg protein)	Catalase (U min <sup>-1</sup> mg <sup>-1</sup> protein)	Glutathione peroxidase (U/mg protein)
A	Normal control rats (0.9% NaCl)	10.50 ± 0.54	10.70 ± 1.13	5.73 ± 0.28
B	Normal rats with extract (250 mg/kg b.w.)	10.00 ± 0.49	10.60 ± 0.66	5.70 ± 0.14
C	Normal rats with extract (500 mg/kg b.w.)	10.32 ± 0.44	10.97 ± 0.81	5.82 ± 0.16
D	STZ induced diabetic control	3.23 ± 0.30	6.37 ± 0.48	3.45 ± 0.36
E	STZ+ glibenclamide (0.5 mg/kg b.w.)	10.02 ± 0.33*	8.82 ± 0.63*	5.26 ± 0.27*
F	STZ+extract (250 mg/kg b.w.)	9.78 ± 0.43*	8.05 ± 0.20*	4.82 ± 0.21*
G	STZ+extract (500 mg/kg b.w.)	10.29 ± 0.40*	8.57 ± 0.29*	5.31 ± 0.26*

Values are expressed as mean ± S.E.M.

\* P ≤ 0.05, significantly different from diabetic control group.



**Fig. 1;** Photomicrographs of rat pancreas stained by H and E stain of normal (a) and diabetic (b) rats and effects of glibenclamide (c), 250 mg/kg b.w. ethanol extract (d) and 500 mg/kg b.w. ethanol extract (e) of *Andrographis paniculata*. Microscope magnification (40 $\times$ )

catalase and glutathione peroxidase levels as compared to the normal rats. Treatment with *A. paniculata* ethanol leaf extract for 28 days produced a significant increase in the activities of the antioxidant enzymes comparable to that of glibenclamide treated group.

Treatment with 500 mg/kg of extract produced more pronounced effect with an increase in the activities of superoxide dismutase (10.29 U/mg protein), catalase (8.57 U/min/mgprotein) and glutathione peroxidase (5.31 U/mg protein). The activities of the enzymes in the non diabetic rats treated with extracts (groups B and C) were similar to the normal control rats.

#### Histopathological studies

Figure 1(a) is the photomicrograph of the pancreas of a normal rat showing normal architecture and normal islets of

langerhans. Figure 1(b) is the photomicrograph of the pancreas of diabetic untreated rat. There were lymphocytic infiltrations. Atrophy and destruction of beta cells were marked. Islet cells were small and shrunken. Figure 1(c) is photomicrograph of the pancreas of diabetic rat treated with glibenclamide.

There was an increase in the number of islet cells. Hematoxylin and eosin sections of the pancreas of diabetic rats treated with ethanol leaf extract of *A. paniculata* are shown in figures 1(d) and 1(e). In animals treated with 250 mg/kg and 500 mg/kg of extract there were regenerative changes in tissue architecture of islet cells of pancreas. In animals treated with 500 mg/kg b.w. of extract, there was a marked increase in the number and size of islet cells especially in the beta cell region compared to animals treated with 250 mg/kg of extract.

## DISCUSSION

Streptozotocin (N-[methylnitrocarbonyl]-D-glucosamine) is well known for its selective pancreatic islet beta cell cytotoxicity and in many animal species and it induces diabetes that resembles human hyperglycemic non-ketotic diabetes mellitus (Papaccio *et al.*, 2000). Further, rats treated with Streptozotocin displays many of the features seen in human subjects with uncontrolled diabetes and are invaluable when studying the mechanism by which hyperglycemia may contribute to complications such as nephropathy, retinopathy and neuropathy (Obrosova *et al.*, 2005). In the present study, Streptozotocin induced diabetes produced a significant increase in blood glucose levels in the diabetic rats. In diabetic rats treated with *A. paniculata* leaf extract, there was a greater reduction in the blood glucose level at a concentration of 500 mg/kg when compared to 250 mg/kg. This shows that the effect of ethanol extract was dose dependent. Moreover, the results on leaf extract also indicate a prolonged duration of antidiabetic action in a chronic study which could be due to multiple sites of action possessed by the active principle of *A. paniculata*. The antidiabetic effect of the extract may be due to the presence of one or more than one antihyperglycemic principle and their synergistic properties as *A. paniculata* contains polyphenols, flavonoids and diterpenoids as the major bioactive components (Mukherjee *et al.*, 2006). The possible mode of action may be the extra-pancreatic action such as increased glucose uptake or  $\alpha$ -glucosidase inhibition or mediation of  $\beta$ -endomorphin (Rao, 2006).

Streptozotocin induced diabetes is characterized by a severe loss in body weight (Kalaiarasi and Pugalendi, 2009). The decrease in the body weight of diabetic rats in this study was due to the loss or degradation of structural proteins to provide amino acids for gluconeogenesis during insulin deficiency resulting in muscle wasting and weight loss. Due to insulin deficiency, protein content is decreased in muscular tissue by proteolysis (Babu *et al.*, 2007). In the present study, diabetic control rats showed marked reduction in their body weights when compared to normal rats. The weight loss was reverted by administration of *A. paniculata* extract to the diabetic rats for a period of 28 days. The ability of the extract to protect body weight loss in diabetic rats seems to be the result of their ability to reduce hyperglycemia.

Diabetic nephropathy is the leading cause of diabetes related morbidity and mortality. The pathogenesis of diabetic nephropathy is related to chronic hyperglycemia and hemodynamic alterations in renal microcirculation and structural changes in glomerulus as evident by the significant elevation in creatinine and urea levels (Cryer, 2001). Measurement of creatinine and urea levels reflects the function of kidneys. Streptozotocin induced diabetes is associated with generation of free radicals and oxidant tissue damage, increasing the risk of renal complications. The reactive oxygen species produced affect the renal functions by promoting varieties of vasoactive mediators such as thromboxane, which in turn causes renal vasoconstriction and alter the glomerular filtration rate (Craven *et al.*, 1992). Hence

measurement of these two parameters enables the study of toxic effects of the drug on the kidney. The increased creatinine and urea levels in the diabetic rats indicates renal damage due to abnormal glucose regulation, including elevated glucose and glycosylated protein tissue levels, hemodynamic changes within the kidney tissue and increased oxidative stress (Veeramani *et al.*, 2008). Treatment with ethanol leaf extract of *A. paniculata* caused a reduction in the creatinine and urea levels thereby enhancing the renal function that is generally impaired in diabetic rats. The normal levels of creatinine and urea in the normal healthy rats fed with the extract of *A. paniculata* at a concentration of 250 mg/kg and 500 mg/kg for a period of 28 days revealed the non-toxic nature of the ethanol extract and also showed the normal kidney function.

There is increasing evidence in both experimental and clinical studies which suggests that diabetes is associated with oxidative stress, leading to an increased production of reactive oxygen species, including superoxide radical, hydrogen peroxide and hydroxyl radical (Gokce and Haznedaroglu, 2008).

Recently, much attention has been focussed on the role of oxidative stress and it has been suggested that oxidative stress may constitute the key and common events in the pathogenesis of different diabetic complications. Streptozotocin induced hyperglycemia induces free radical generation which thereby leads to DNA damage, protein degradation, lipid peroxidation and finally culminating into damage to various organs of the body like liver, kidney, brain and eyes (Yazdanparast *et al.*, 2007). An imbalance of oxidant/antioxidant defence systems result in alterations in the activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. In the present study, the activity of superoxide dismutase, catalase and glutathione peroxidase were decreased in the liver homogenate of diabetic control rats compared to normal rats, indicating dysfunction in antioxidant defensive system which could be due to free radical induced inactivation or glycation of the enzyme in diabetic state (Al-Azzawie and Alhamdani, 2006). Treatment with *A. paniculata* ethanol leaf extract for a period of 28 days reversed the activities of these enzymatic antioxidants, which could be due to the upregulation mRNAs of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase and which may in turn counteract the oxidative stress. It has been shown that polyphenols present in majority of plants are responsible for this upregulation (Jagetia, 2007). Normal antioxidant enzyme levels in non diabetic control rats treated with *A. paniculata* extract indicates the normal functioning of the liver and also the non-toxic nature of the extracts.

Pancreatic beta cells have been proposed to be selectively sensitive to Streptozotocin because the enzyme N-acetylglucosamine transferase is expressed at higher levels in the beta cell than in any other cell (Hanover *et al.*, 1999). The decrease in the cellularity, small and shrunken islets and destruction of beta cells within islets of langerhans observed in diabetic rats in the present study reflects the cytotoxicity of streptozotocin. The beta cells in some islets of diabetic pancreas were found to be fusiform.

The change in shape of cells can be attributed to the partial damage by Streptozotocin. *Andrographis paniculata* ethanol extract significantly increased the number and size of islet cells especially in the beta cell region. The protective effect of *A. paniculata* ethanol leaf extract may be due to the andrographolide or other diterpenoids, flavonoids or polyphenols present in the plant (Rao *et al.*, 2004).

Theoretically, hypoglycemic plants act through a variety of mechanisms such as improving insulin sensitivity, augmenting glucose dependent insulin secretion and stimulating the regeneration of islets of langerhans in pancreas of Streptozotocin induced diabetic rats (Sezik *et al.*, 2005). Moreover, the role of antioxidant compounds in both protection and therapy of diabetics have been considered in various scientific researches. Treatment of Streptozotocin injected diabetic animals with N-acetyl-L-Cysteine, a well known antioxidant, prevented hyperglycemia through reduced oxidative stress and restoring beta cell function (Takatori *et al.*, 2004). In this regard, it is anticipated that the ethanol leaf extract of *A. paniculata* acts by decreasing the oxidative damage to pancreatic tissue. However, by this speculation, we do not exclude the possibilities of other mechanisms by which the ethanol leaf extract exerts its effects.

In conclusion, our data suggests that the ethanol leaf extract of *A. paniculata* possess significant antidiabetic potential as seen by the lowering of blood glucose levels in diabetic rats. The ethanol extract was effective in lowering the creatinine and urea levels in diabetic rats thus improving the kidney functions. In addition to its antihyperglycemic activity the extract also showed significant antioxidant activity as seen by the increase in the antioxidant status in diabetic rats. Moreover, the ethanol extract was not found to exert any toxic side effect on kidneys and liver in extract fed normal rats. The results from the present study proves the significant antihyperglycemic, antioxidant, islet cell regenerative and non toxic nature of *A. paniculata* leaves which could be used in the management of diabetes. This study justifies the use of *A. paniculata* in Ayurvedic formulations.

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