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Immunostimulant, cerebroprotective & nootropic activities of *Andrographis paniculata* leaves extract in normal & type 2 diabetic rats

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***Background & objectives*: A large number of plants have been recognized to be effective in the treatment of diabetes mellitus. Persistent hyperglycaemia is associated with decreased function of immune system and cerebral ischaemia mainly due to increased oxidative stress and inflammatory response. *Andrographis paniculata* is a medicinal plant widely used in folk medicine for various purposes. In this study the effect of chronic administration (7 days) of methanolic extract of *A. paniculata* leaves was studied in rats with experimentally induced diabetes, nootropic and immunostimulant activities were evaluated. The effect of acute administration of methanolic extract of *A. paniculata* leaves was also studied for cerebroprotective activity.**

***Methods*: Type 2 diabetes was induced in rats by streptozotocin (STZ) (65 mg/kg) + nicotinamide (150 mg/kg). Various biochemical parameters were estimated using standard methods.**

***Results*: A significant ($P<0.05$) increase in cognitive function was observed in both normal and type 2 diabetic rats. Nootropic activity in terms of per cent reduction in latency period was more in type 2 diabetic rats. A significant increase in blood lymphocyte count, splenic lymphocyte count and peritoneal macrophage count was observed in both normal and type 2 diabetic rats. Immunostimulant activity was observed more in type 2 diabetic rats. The per cent decrease in cerebral infarction was more in type 2 diabetic rats when compared to normal rats. The per cent increase in superoxide dismutase (SOD) levels was more in type 2 diabetic rats.**

***Interpretation & conclusions*: The antioxidant activity of the methanolic extract of *A. paniculata* leaves was evident by decreased tissue malondialdehyde (MDA) levels and increased SOD levels. These properties may be responsible for the observed cerebroprotective activity. The methanolic leaf extract of *A. paniculata* showed significant immunostimulant, cerebroprotective and nootropic activities in normal and type 2 diabetic rats.**

Key words *Andrographis paniculata* - cerebroprotective - diabetes - immunostimulant - nootropic - rats - streptozotocin

Type 2 diabetes mellitus is the most common form of diabetes, which causes a growing concern all over the world, predominantly because of the consequent chronic complications[1]. Hyperglycaemia is associated with decreased function of immune system[2], and, therefore, the patients with diabetes are more prone

to infections. Decreased blood supply, along with the inadequate immune response, delays the wound healing in those affected. These patients have a 2-6 fold increased risk of thrombo embolic strokes when compared to the non diabetic and are vulnerable to stroke related mortality and morbidity. Population based studies have shown that patients with type 2 diabetes have an increased risk of cognitive impairment, dementia and neurodegeneration[3].

With strict glycaemic control, diabetic complications can be delayed, but practically, it is not possible and many patients with chronic diabetes eventually develop complications. The mechanisms involved include oxidative stress, inflammatory mediators and glycation end products.

Currently used antidiabetic drugs aimed to control hyperglycaemia, are not reported to have antioxidant and anti-inflammatory properties. Hence, compounds having multiple activities like antihyperglycaemic, antioxidant and anti-inflammatory may be more useful in treating diabetes as well as complications arising out of diabetes. Many herbal drugs and extracts have been tried for this purpose. *Andrographis paniculata* commonly known as "King of Bitters," is a member of the plant family, Acanthaceae. *A. paniculata* is reported to have antihyperglycaemic, antioxidant and other biological activities like antibacterial, anti-human immunodeficiency virus, immunostimulatory, antipyretic, antidiarrhoeal, antivenom, antihepatotoxic, anti-inflammatory, and antimalarial activities[4].

Hence the present study was carried out to evaluate the effect of methanolic extract of *A. paniculata* leaves for its immunostimulant, cerebroprotective and nootropic activities in normal rats and those with experimentally induced type 2 diabetes.

Material & Methods

Plant material: *Andrographis paniculata* Nees (Acanthaceae) (10 kg) was collected in July 2006 from Mamundur forest, Mallimadugu village, Tirupati (rural), Chittor District, Andhra Pradesh, India. Leaves were dried in shade and powdered. The authentication of the plant was done by Dr K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India, and the voucher specimen (No. 0054/AP) was deposited in the Herbarium of the Department of Botany, Andhra University, Visakhapatnam, India.

Preparation of methanolic extract of A. paniculata leaves: Shade-dried and powdered leaves (890 g) were subjected to extraction using methanol (CH_3OH) exhaustively for a minimum of eight times for every three to four days by successive cold and hot extraction processes. The extract was concentrated to dryness *in vacuo.* The methanolic extract of the plant leaves (15 g) was tested for immunostimulant, cerebroprotective and nootropic activities in June 2010 in the laboratories of Pharmacology Division, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Chemicals used: Streptozotocin and nicotinamide were purchased from Sigma Chemicals, USA. All other chemicals (sodium carbonate, sodium hydroxide, copper sulphate, sodium potassium tartarate, bovine serum albumin, Folin - Ciocalteau's phenol, nitroblue tetrazolium, nicotinamide adenine dinucleotide hydride (NADH)) used were of analytical grade.

Animals: Wistar albino rats of either sex weighing 150-200 g procured from Mahaveer Enterprises, Hyderabad, India, were used in the study. The animals were maintained on a 12 h light - 12 h dark cycle. They were fed with standard pellet diet (Rayans Biotechnologies Pvt. Ltd., Hyderabad) and water *ad libitum*. Animals were fasted for 16 h prior to drug administration allowing access only to water and were deprived of both food and water during the experiment.

Induction of type I diabetes mellitus: To induce type I diabetes, albino rats of either sex were fasted over night before injecting with streptozotocin (STZ). STZ was dissolved in citrate buffer *p*H 4.5 at a dose of 40 mg/kg immediately before use and injected into the tail vein of rats which were lightly anaesthetized with ether.

Induction of type 2 diabetes mellitus: To induce type 2 diabetes, 65 mg/kg dose of STZ was given after the administration of 150 mg/kg dose of nicotinamide[5]. Blood glucose levels were estimated after 48 h for the confirmation of diabetes induction.

Dose response studies were conducted for glucose reduction in both type I and type 2 diabetes rats using 100, 200 and 400 mg per kg doses of leaf extract. The present dose *i.e.* 100 mg/kg (oral) was selected for chronic administration and 50 mg/kg (i.p.) was selected for acute administration.

Evaluation of effects of chronic administration (7 days): Twenty four rats were taken and divided into four groups. Group I served as vehicle treated control, Group II served as rats treated with the extract (100 mg/kg), Group III served as type 2 diabetic vehicle

treated control and Group IV served as type 2 diabetic rats treated with the extract (100 mg/kg). The fasting blood samples were taken before the administration of the extract. The extract was given orally daily once for seven days. At the end of seven days again the blood samples were collected[6] and analyzed. The blood glucose was estimated by glucose oxidase-peroxidase (GOD-POD) method[7].

Evaluation of immunostimulant activity: Blood lymphocyte count was carried out using Leishman stain. To determine splenic lymphocyte count, spleen was dissected, macerated and washed with 10 ml balanced salt solution (BSS *p*H 7.2) and pellets were resuspended in 2 ml of BSS and counting was done with haemocytometer. Peritoneal macrophages were collected at different days of treatment by washing peritoneal cavity with chilled BSS. The peritoneal fluid was incubated at 37 ºC for 60 min in glass petridish. Cold ethylenediaminetetraacetic acid (EDTA, 2%) was added to the petridish and flushed gently and kept at 4 ºC for 30 min. Suspension was centrifuged and suspended in 1 ml of BSS. Counting was done with haemocytometer in the presence of neutral red.

Evaluation of nootropic activity: Twenty four rats were taken and divided into four groups as described earlier. Spatial memory was evaluated by using Morris water maze[8] before and after the administration of the extract.

Evaluation of effects of acute administration: The rats (n=30) were divided equally into 5 groups. Group I served as normal control, animals were treated with 0.2 ml saline i.p., group II served as vehicle control, animals were treated with 0.2 ml of 99 per cent dimethyl sulphoxide (DMSO) i.p., group III served as *per se* control, animals were treated with methanolic extract of *A. paniculata* 50 mg/kg i.p., group IV served as type 2 diabetic control, animals were treated with 0.2 ml of 99 per cent DMSO i.p., and group V served as type 2 diabetic, animals were treated with methanolic extract of *A. paniculata* leaf extract 50 mg/kg i.p. The rats were anaesthetized by giving thiopentone sodium (35 mg/kg) i.p.

Carotid artery ligation: Surgical technique for the induction of cerebral ischaemia was adapted from earlier studies[9]. Under anaesthesia, a midline incision in neck was given. Common carotid arteries were identified and isolated carefully from vagosympathetic nerve. Rats were made ischaemic by occluding bicommon carotid arteries[9] (BCCA) with a silk thread for 30 min and reperfusion was allowed for 4 h by removing the thread. Body temperature was maintained at about 37 ºC during the period with the help of a heating lamp. The rats were anaesthetized by giving thiopentone sodium (35 mg/kg) i.p.

Quantification of infarct size: Infarct size was measured by using triphenyltetrazolium chloride (TTC) stain[10]. At the end of the experiment, rats were sacrificed by giving high doses of anaesthesia, later decapitated and brains were isolated and thoroughly rinsed with ice chilled 0.9 per cent NaCl. The brain was weighed, and sliced to 0.1cm thick sections and incubated in 2 per cent solution of TTC prepared in *p*H 7.4 phosphate buffer for 60 min at 37 ºC. In viable brain tissue TTC is converted by dehydrogenase enzymes to a red formazan pigment that stains tissue dark red. The infarcted brain tissue that does not take TTC stain.

The tissue malondialdehyde (MDA) levels were measured by the method of Ohkawa *et al*[11]. Superoxide dismutase (SOD) activity was determined by the method developed by Kakkar *et al*[12].

Statistical analysis: Differences between means were tested using One-way ANOVA. Individual groups were compared using, Dunnett's multiple comparison test. $P<0.05$ was considered as significant.

Results

Evaluation of effects of chronic administration: A significant ($P<0.05$) increase in cognitive function was observed in both normal and type 2 diabetic rats. A significant ($P<0.05$) increase in blood lymphocyte count was observed in normal and type 2 diabetic rats treated with the methanolic extract. The per cent increase was more in diabetic rats. Splenic lymphocyte count and peritoneal macrophage count were significantly increased ($P<0.05$) in normal and type 2 diabetic rats. Our results exhibited significant immunostimulant activity in both normal and type 2 diabetic rats. The per cent increase in blood lymphocyte, splenic lymphocyte, peritoneal macrophages was calculated and the per cent variation in cognitive function are given in Table I.

Evaluation of effects of acute administration: The per cent infarction was significantly ($P<0.05$) more in type 2 diabetic rats when compared to normal rats. There was a consistent association between high blood glucose levels and greater infarct size. Elevated blood glucose levels are associated with an increased hypoperfused tissue progressing to infarction[13]. The per

Table I. The effect of chronic administration (7 days) of methanolic extract of *A. paniculata* leaves (100 mg/kg, oral) on blood glucose, immunostimulant and nootropic activities in normal and type 2 diabetic rats

Parameters	Group-I		Group-II		Group-III		Group-IV	
	Before	After	Before	After	Before	After	Before	After
Blood glucose	102 ± 7.3	105 ± 5.5	102.33 ± 5.6	80.83 ± 4.75	318.66 ± 5.7	321.16 ± 8.3	311.33 ± 6.6	171.6 ± 8.3
% change (mg/dl)		+ 2.96		- 21.01*		+ 0.78		- 44.86*
Blood Lymphocytes (%)	55.33 ± 1.60	58.00 ± 1.52	53.83 ± 2.50	65.66 ± 2.61	39.00 ± 2.11	36.83 ± 2.16	38.50 ±2 .47	63.00 ± 2.73
% change		+4.28		+21.97*		-5.56		+63.63*
Splenic Lymphocytes (cells/unit area)	61.76 ± 2.75	62.67 ± 2.18	61.83 ± 2.77	79.66 ± 1.80	49.33 ± 1.68	47.33 ± 1.83	48.17 ± 1.32	62.67 ± 1.74
% change		+1.62		+28.97*		-4.05		+30.1*
Peritoneal macrophages (cells/unit area)	22.67 ± 1.52	24.00 ± 1.86	22.66 ± 2.20	33.83 ± 2.08	15 ± 1.12	13.33 ± 0.98	14.17 ± 0.98	19.67 ± 1.20
% change		+5.86		+49.29*		-11.13		+38.81*
Latency period (sec)	13.51 ± 1.2	13.47 ± 1.1	14.77 ± 1.1	11.05 ± 0.69	22.03 ± 2.2	21.07 ± 1.9	21.7 ± 1.3	14.31 ± 0.89
% change		- 0.29		- 25.18*		- 4.35		- 34.05*

*P<0.05 compared to normal; Values are represented as mean ± SEM; (n=6)

Group I: Vehicle treated normal rats; Group II: Normal rats treated with the extract (100 mg/kg); Group III: Type 2 diabetic vehicle treated rats; Group IV: Type 2 diabetic rats treated with the extract (100 mg/kg)

cent infarction was significantly (P<0.05) reduced in the extract treated normal as well as in type 2 diabetic rats. The degree of cerebroprotective activity was more in type 2 diabetic rats. The per cent reduction in infarction, decrease in MDA and increase in SOD were calculated and are given in Table II.

Discussion

The methanolic extract of *A. paniculata* leaves was found to possess significant immunostimulant activity. Many Indian medicinal plants like *Withania somnifera* and *Mangifera indica* have been reported to have immunomodulatory activities[14,15]. Puri *et al*[16] reported that andrographolides *of A. paniculata* induced significant stimulation of antibody and delayed type hypersensitivity (DTH) response to sheep red blood cells (SRBC) in mice[16]. Immunostimulant property of *A. paniculata* was studied on human cell line cultures and it was observed that methanolic extract augmented the proliferation of human peripheral blood lymphocytes (HPBLs)[17].

A significant increase in cognitive function was observed in type 2 diabetic rats treated with the methanolic extract in the present study. There were no earlier reports on the effect of *A. paniculata* on cognitive function. Many population based studies have found an association between type 2 diabetes and an increased risk of developing dementia[3,18-23]. There are many mechanisms through which diabetes could increase risk of dementia including hyperglycaemia, insulin resistance, oxidative stress, advanced glycation end products, inflammatory cytokines, and microvascular and macrovascular disease[3]. *A. paniculata* is reported to have antidiabetic, antioxidant and anti-inflammatory properties. These properties may be responsible for the observed nootropic activity.

A. paniculata showed significant cerebroprotective activity in terms of per cent reduction in infarct size against ischaemia-reperfusion injury in both normal and diabetic rats. A significant reduction in the tissue MDA levels and increase in SOD levels were observed in normal and type 2 diabetic rats treated with *A. paniculata* methanolic extract. Griesmacher *et al*[24] have reported that enhanced production of free radicals was observed in both type 1 and type 2 diabetes, significantly higher levels

Table II. The effect of acute administration of methanolic extract of *A. paniculata* leaves (50 mg/kg, i.p.) on cerebroprotective activity in normal and type 2 diabetic rats

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
% infarction	40.86 ± 2.20	34.88 ± 1.78	30.67 ± 2.68	49.21 ± 3.83	25.15 ± 1.38
(% change)			- 12.06*		- 48.89*
MDA (nmol/g tissue)	96.5 ± 2.18	92.83 ± 0.98	68.16 ± 6.67	96.66 ± 2.29	63.5 ± 1.96
(% change)			- 26.57*		- 34.30*
SOD (Units mg/protein)	21.3 ± 1.52	22.66 ± 1.11	32.83 ± 2.63	18.66 ± 1.52	35.16 ± 1.42
(% change)			+ 45.12*		+ 88.42*

*$P<0.05$ compared to normal; Values are represented as mean ± SEM; (n=6)

Group I: Normal saline treated rats; Group II: Vehicle treated normal rats; Group III: Normal rats treated with the extract (50 mg/kg); Group IV: Type 2 diabetic vehicle treated rats; Group V: Type 2 diabetic rats treated with the extract (50 mg/kg)

were observed in type 2 diabetes. Increased oxidative stress resulting from hyperglycaemia is believed to contribute to the excess cerebral damage. Free radical production is increased during hyperglycaemic stroke in rodents[25]. Hyperglycaemia and hyperglycaemia-induced oxidative stress may be responsible for increased cerebral infarction in diabetes. *A. paniculata* is reported to have antidiabetic, antioxidant and anti-inflammatory properties. In the present study also, antioxidant activity of the methanolic extract of *A. paniculata* leaves was evident by decreased tissue MDA levels and increased SOD levels. These properties may be responsible for the observed cerebroprotective activity.

Chan *et al*[26] reported that andrographolide exhibited neuroprotective effects, with accompanying suppression of nuclear factor kappa B (NF-κB) and microglial activation, and reduction in the production of cytokines including tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), and pro-inflammatory factors such as prostaglandin E_2 (PGE_2).

In conclusion, the methanolic extract of *A. paniculata* leaves was found to possess significant immunostimulant, cerebroprotective and nootropic activities in normal and type 2 diabetic rats. Further studies need to be done to isolate and characterize the active constituent(s).

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