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ANTIDIABETIC AND ANTIOXIDANT EFFICACY OF *ANDROGRAPHIS PANICULATA* IN ALLOXANIZED ALBINO RATS

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Abstract: Antidiabetic potential and antioxidant activity of ethanol extract of *Andrographis paniculata* leaves were assessed in alloxan-induced diabetic rats. Results revealed that, diabetic rats showed increase in blood glucose and decrease in plasma insulin ($p<0.01$) levels after 48 hrs of alloxan administration. The oral administration of ethanol extract at a dose of 100 and 200 mg/kg of body weight, for 30 days exhibited a significant reduction in the blood glucose level compared to the standard drug-treated rats and diabetic control. The level of serum triglycerides, phospholipids and total cholesterol were significantly increased in diabetic rats. The concentration of urine, glucose, glycosylated Haemoglobin was significantly increased, whereas haemoglobin, total protein, albumin and liver glycogen were significantly decreased in diabetic animals. Administration of *A. paniculata* leaf extract decreased the diabetic complication. The marker enzymes of liver toxicity such as serum alanine transaminase (ALT), serum aspartate transaminase (AST), serum acid phosphatase (ACP) and serum alkaline phosphatase (ALP) were elevated significantly in diabetic control. The liver glycogen levels also increased significantly in alloxan-induced diabetic rats. Catalase and vitamin C levels were increased *Andrographis paniculata* fed rats. Phytotherapy with the *A. paniculata* ethanol extract showed significant restoration in enzymatic and non enzymatic activities in diabetic animals. The phyto-treatment showed more efficient antihyperglycemic effect than the standard drug glibenclamide.

Keywords: *Andrographis paniculata,* alloxan, antidaiabetic, antioxidant, blood-glucose, insulin

Introduction

Diabetes is a serious metabolic disorder with micro and macro vascular complications that results in significant morbidity and mortality. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to increase the number of diabetes world wide. The current treatment, although provide a good glycemic control but do a little in preventing complications (Vats et *al.*, 2004). There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (Holman *et al.*, 1991). The World Health Organization (WHO) (1980) has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs (Upathaya *et al*., 1991). The pharmaceutical drugs are either too expensive or have undesirable side effects. Treatment with sulphonylureas and biguanides are also associated with side effects (Rang *et al*., 1991). *Andrographis paniculata* (Burm.f) Nees is used extensively in the Indian traditional system of medicine. In the present investigation antidiabetic efficacy of the plant extract of *Andrographis paniculata* on alloxan induced diabetic rats.

Study area:

Materials and Methods

Adult Wistar rats weighing 160-200 g of either sex were maintained in large polypropylene cages in a well-ventilated room temperature with natural day-night cycle and fed with balanced rodent pellet and water *ad libitum* throughout the experimental period. They were quarantined for 1 week prior to the experiments to acclimatize them to laboratory conditions. The study protocol was approved by the IAEC (Institutional Animal Ethics Committee of CPCSEA, New Delhi, Govt. of India). Fresh whole plants of *Andrographis paniculata* were collected from Sivapuram Pudukkottai District, during the months of September-December 2006 and identified by Dr.P. Jayaraman (Director), Plant Anatomy Reasearch centre, Chennai. The leaves were collected and dried in shade for 15 days and made to coarse powder and extracted with ethanol by the method of Harborne, 1973. The extract was preserved in an air tight container, in a refrigerator and used to evaluate the antidiabetic and antioxidant efficacy.

Alloxan was purchased from Loba chemie. All the other chemicals used in the experiment were analytical grade. A total of 36 rats (30 diabetic surviving rats, 6 normal rats) were experimented. The rats were divided into 6 groups of 6 rats each. Rats were orally fed with two doses of ethanol extracts (100 and 200 mg/kg body weight per day) and glibenclamide (600µg/kg). Experimental diabetes was induced by using alloxan monohydrate following the method of Trivadi *et al*., 2004.

Blood samples were drawn at weekly intervals till the end of study (i.e., 3 weeks). At the end of 3 weeks, all the rats were killed by decapitation under pentobarbitone sodium (60 mg/kg) anesthesia. Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose and plasma was separated for assay of insulin. Liver and kidney were dissected out, washed in ice cold saline, patted dry and weighed. The following biochemical analyses were carried out: Urine Sugar was detected by reagent based Uristix from Bayer Diagnostics. Total protein, Blood Glucose (Sasaki *et al*., 1972), estimation of haemoglobin (Dacie and Lewis, 1977), glycosylated haemoglobin (Bannon, 1982), liver glycogen (Morales *et al*., 1973), Cholesterol (Zlatkis *et al*., 1953), Triglycerides (Foster and Dunn, 1973), Estimation of phospholipids (Rouser *et al*., 1970), estimation of Protein (Lowry *et al*., 1951), estimation of serum albumin, (Reinhold, 1953), Determination of serum aspartete transaminase (Mohun and Cook, 1957), determination of serum alanine transaminase (Mohun and Cook, 1957), alkaline Phosphatase (King, 1965), acid phosphatase (Gutman and Gutman, 1940), estimation of urea (Natelson, 1957) assay of catalase (Sinha, 1972) and Vit C (Omaye *et al*., 1979). Statistical analysis was performed using the SPSS 11.5 software package. Group mean values were compared with Duncan's Multiple Range Test and analyzed with one way Analysis of Variance (ANOVA).

Results

Urine sugar was significantly increased in diabetic rats when compare to the control rats,after treatment of *Andrographis paniculata* urine sugar level is significantly decreased when compare to the diabetic control rats. A significant increase in blood glucose and significant reduction in body weight were observed in diabetic rats, compared to control rats. Oral administration of *Andrographis paniculata* (100 and 200 mg/kg body weight) for 21

days shows significant reduction in blood glucose compared to untreated diabetic rats. Blood glucose levels were found to be increased significantly by % in diabetic control, whereas the rats treated with 100 mg/kg of *Andrographis paniculata* showed a decrease at % and 200 mg/kg at %. The decrease in blood glucose levels was significantly better than the diabetic rats treated with glibenclamide.Haemoglobin levels were found to be decreased significantly in alloxanized diabetic rats. Liver glycogen,total protein, and albumin were significantly decreased in diabetic rats when compare to the normal control rats.Administration of *Andrographis paniculata* extract significant changes in liver glycogen, total protein and albumin.There was a significant reduction in heamoglobin and serum protein while glycosylated hemoglobin and blood urea significantly increased in diabetic rats when compared with control rats. Oral administration of *Andrographis paniculata* (100 and 200 mg/kg b.wt) significantly restored the value to near normal. Control animals administered with *Andrographis paniculata* 200 mg/kg body weight did not show any significant change in any of the parameters studied. A significant increase in the total cholesterol, triglycerides,urea and phospholipids were found in the liver and kidney of the diabetic rats compared to the tissue of normal control rats. SGOT, SGPT, ACP and ALP were significantly increased in diabetic rats when compared to the normal control rats. Catalase and vitamin C levels were significantly decreased in the serum of the diabetic rats, compared to the normal control rats. Analyzed data are expressed as means with their standard deviations.

Table-1: Antidiabetic efficacy of *Andrographis paniculata.*

Experimental Group	Urine sugar	Blood glucose (mg/dl)	Haemoglobin (g/dl)	Glycosylated haemoglobin (mg/Hb)	Liver glycogen (Mg/g tissue)	Total protein (g/dl)	Albumin (g/dl)	Urea (mg/dl)
Normal control	Nil	96.0±3.23^{a}	14.0±0.54^{c}	1.61±0.03^{a}	3.9±0.16^{d}	6.92±0.45^{d}	4.13±0.24^{d}	28.4±2.10^{a}
Diabetic control	+++	224.10±6.67^{c}	9.93±0.42^{a}	3.2±0.14^{c}	2.4±0.08^{b}	4.93±0.26^{a}	3.1±0.26^{a}	39.2±2.72^{d}
Diabetic +APE (100) mg/kg	+	134.21±5.38^{ab}	11.5±0.32^{b}	2.21±0.08^{bc}	2.9±0.06^{a}	5.26±0.51^{b}	3.8±0.32^{bc}	35.2±3.1^{c}
Diabetic +APE (200mg/kg)	+	124.34±4.9^{b}	13.21±0.52^{bc}	1.91±0.07^{a}	3.4±0.14^{cd}	6.12±0.28^{cd}	3.91±0.36^{c}	29.6±3.2^{b}
Diabetic + glibenglamide	+	126.32±3.8^{b}	13.19±0.63^{bc}	2.1±0.08^{b}	3.1±0.12^{c}	5.93±0.35^{c}	3.73±0.29^{b}	31.2±2.9^{b}

Values are expressed as Mean ± Standard Deviation (SD); Level of significance=0.05; APE= *Andrographis paniculata* extract

Table-2: Effect of *Andrographis paniculata* leaf extract on total cholesterol, triglyceroids and phospholipids.

Experimental Group	Total cholesterol (mg/dl)			Triglycerides (mg/dl)			Phospolipids (mg/dl)		
	Plasma	Liver	Kidney	Plasma	Liver	Kidney	Plasma	Liver	Kidney
Normal control	4.25± 0.22^{a}	105.16± 5.69^{a}	5.25± 0.27^{a}	5.05± 0.25^{a}	72.13± 3.71^{a}	41.50± 2.06^{a}	94.00± 4.48^{a}	82.00± 4.10^{a}	148.62± 7.49^{a}
Diabetic control	9.50± 0.45^{d}	230.12± 11.25^{d}	9.98± 0.49^{d}	11.01± 0.56^{d}	124.62 ± 6.23^{d}	73.07± 3.80^{d}	158.01± 8.26^{d}	152.01± 6.32^{d}	188.38± 9.57^{d}
Diabetic+APE (100mg/kg)	5.12± 0.23^{c}	154.13± 6.82^{c}	7.23± 0.52^{c}	8.01± 0.43^{c}	110.52± 5.83^{c}	65.91± 3.01^{c}	112.21± 5.44^{c}	113.52± 5.2^{c}	172.31± 7.21^{c}
Diabetic+APE (200mg/kg)	4.51± 0.33^{b}	125.42± 5.42^{b}	5.75± 0.30^{a}	7.32± 0.31^{b}	83.84± 4.64^{b}	52.0± 2.71^{b}	107.43± 4.23^{b}	96.36± 5.28^{b}	161.90± 8.10^{bc}
Diabetic + glibenglamide	5.25± 0.26^{b}	130.12± 6.19^{bc}	6.32± 0.29^{b}	7.90± 0.38^{b}	92.71± 4.48^{bc}	56.50± 2.96^{bc}	108.21± 3.39^{bc}	100.52± 5.87^{b}	154.00± 7.74^{b}

Values are expressed as Mean ± Standard Deviation (SD); Level of significance=0.05;
APE= *Andrographis paniculata* extract

Table-3: Antioxidant efficacy of *Andrographis paniculata* leaves.

Experimental Group	SGOT (IU/L)	SGPT (U/L)	Acid phospatase (IU/L)	Alkaline phospatase (IU/L)	Catalase (µ moles of H_2O_2 utlized/min /mg/protein)	Vitamin C (mg/dL^{-1})
Normal control	78.54±3.88^{a}	67.04±3.43^{a}	5.67±0.29^{a}	9.56±0.48^{a}	24.70±1.18	12.50±0.62
Diabetic control	147.50 ±6.76^{d}	95.78±4.96^{d}	8.50±0.48^{d}	23.85± 1.41^{d}	14.10±0.82	7.50±0.53
Diabetic +APE (100 mg/kg)	110.50±7.21^{c}	82.13±3.56^{c}	7.21±0.52^{c}	14.81±0.51^{c}	18.82±1.06	8.93±0.62
Diabetic +APE (200mg/kg)	97.70±5.21^{bc}	78.54±4.23^{b}	6.02±0.33^{b}	11.68±0.65^{b}	21.06± 1.01	11.02±0.61
Diabetic + glibenglamide	90.03±4.53^{b}	82.37±4.13^{c}	7.08±0.41^{bc}	12.75±0.72^{bc}	17.87±0.93	10.21±0.67

Values are expressed as Mean ± Standard Deviation (SD); Level of significance=0.05;
APE= *Andrographis paniculata* extract

Discussion

Blood sugar increased in alloxan-injected animals, since alloxan causes a massive reduction in insulin release, by the destruction of the beta-cells of the Islets of Langerhans and inducing hyperglycemia (Goldner and Gomori, 1943). Oral administration of (100 and 200 mg/kg *Andrographis paniculata* body wt.) resulted in a significant reduction in the blood glucose. Protein synthesis is decreased in all tissues due to absolute or relative deficiency of insulin in alloxan-induced diabetic rats (Jorda et al.,1982). Present result protein level in diabetic rats significantly decreased when compares to the control rats. . A significant elevation in serum creatinine and urea levels indicate an impaired renal function of diabetic animals.(Shinde and Goyal,2003). In the present study urea level was significantly increased in diabetic rats.

The present study also indicates that *Andrographis paniculata* can inhibit alloxan renal toxicity as evident from the blood urea level. In the present study, the glycosylated hemoglobin level was high showing that the diabetic animals had high blood glucose level. The values decreased significantly in *Andrographis paniculata* administered animals showing the influence of the leaf extracts on sugar reduction. Insulin influences the intracellular utilization of glucose in a number of ways. Increase in glycogen in liver can be brought about by an increase in glycogenesis and/or a decrease in glycogenolysis. So the *Andrographis paniculata* extract could have stimulated glycogenesis and/or inhibited glycogenolysis in the diabetic rat liver. Insulin and sulfonylurea drugs (*e.g.*, glibenclamide) cause hypoglycemia when taken in excessive doses and overt hypoglycemia is the most worrisome effect of these drugs (Chakrabarti and Rajagopalan, 2002). The significant increase in the level of triglycerides in liver and kidney of diabetic control rats may be due to the lack of insulin. Since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis triglycerides (Frayn, 1993). *Andrographis paniculata* reduces triglycerides, cholesterol and phospolipids in tissues of alloxan-induced diabetic rats. Further more, the present study reveals, higher levels of triglycerides, cholesterol and phospholipids in diabetic rats (Khosla, 1995).The decrease in protein and albumin may be due to microproteinuria and albuminuria, which are important clinical markers of diabetic

nephropathy (Mauer *et al.*, 1981) and/or may be due to increased protein catabolism (Almdal and Vilstrup, 1988). The results of the present study demonstrated that the treatment of diabetic rats with the aqueous extract of *Andrographis paniculata* caused a noticeable elevation in the plasma total protein and albumin levels as compared with their normal levels. Such improvement of serum protein and albumin was previously observed after the oral administration of *Balanites aegyptiaca* to experimentally diabetic rats (Mansour and Newairy, 2000). A significant elevation in serum urea levels indicates an impaired renal function of diabetic animals (Shinde and Goyal, 2003). Thus, it would appear that the *Andrographis paniculata* leaves supplement lowered the plasma urea levels by enhancing the renal function that is generally impaired in diabetic rats. These results are similar to other previous studies on the mesocarp extract of *B. aegyptiaca* (Saeed *et al*., 1995) and herbal formulation D-400 (Dubey *et al*., 1994). The serum AST and ALT levels increased as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes (Chalasani *et al*., 2004). Similarly in the present study, it was observed that the levels of serum AST and ALT in alloxan induced diabetic rats were elevated. It may be due to leaking of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan (Stanely, 1999). AST and ALT were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats (Hye *et al*., 2005). In this study, the administration of ethanol extract to alloxan-induced diabetic rats reduces AST and ALT levels.

The serum AST and ALT levels increase as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes.44 Similarly in the present study, it was observed that the levels of serum AST and ALT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan.45 AST and ALT were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats.46 In this study, the administration of water soluble fraction of ethanol extract to alloxan-induced diabetic rats reduces AST and ALT levels efficiently than aqueous extract treated rats. In addition to the assessment of AST and ALT levels during diabetes, the measurement of enzymatic activities of phosphatases such as acid

phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants.47 In our study, serum ACP and ALP increased considerably in alloxan induced diabetic rats. Elevated level of these enzymes in diabetes may be due to extensive damage to liver in the experimental animals by alloxan. Treatment with water soluble fraction of ethanol extract in alloxan-induced diabetic rats produces a more significant decline in these levels than the aqueous extract treated rats. From the present observation, it was evident that water soluble fraction of ethanol extract protects the adverse effects of lipid peroxide mediated tissue damage in alloxan induced diabetic rats.

In addition to the assessment of AST and ALT levels during diabetes, the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants (Som Nath Singh, *et al.,* 2001). In Present study, serum ACP and ALP increased considerably in alloxan induced diabetic rats. Elevated level of these enzymes in diabetes may be due to extensive damage to liver in the experimental animals by alloxan.

Catalase causes reduction of H_2O_2 whereas GPx reduces H_2O_2 and lipid peroxides (Shull and Marsh, 1991). Several studies have demonstrated lowered non-enzymatic antioxidant levels and enzymatic antioxidant activities in streptozotocin induced diabetic rats (Ananthan *et al*., 2004; Venkateswaran, 2002). In the present study *A. paniculata* leaf extract is found to increase serum concentrations of catalase (CAT). Vitamin C, a major extra cellular non-enzymatic antioxidant, has crucial role in scavenging several reactive oxygen species. It functions as a free-radical scavenger and successfully prevents detectable oxidative damage under all types of oxidative stress. Reduction in tissue ascorbic acid was observed in STZ-diabetic rats. The decrease could have been due to increased utilization of ascorbic acid as an antioxidant defense against increased reactive oxygen species or to a decrease in the GSH level, since GSH is required for the recycling of ascorbic acid (Hunt, 1996). The present study reveals that considerable changes in all parameters after the treatment of *A. paniculata* leaf extract.

Conclusion: Therefore, *A. Paniculata* an indigenous herb shows potential antioxidant and antidiabetic properties, which could be due to the presence of potent antihyperglycaemic factors. Further study is underway in our laboratory to isolate the active principles.

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