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Research Article

EVALUATION OF *IN VIVO* ANTIDIABETIC ACTIVITY OF *ANDROGRAPHIS ECHIOIDES* (L.) NEES

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ABSTRACT

The aim of this study was to investigate the effect of ethanol extract of the whole plant of *Andrographis echioides* as antihyperglycemic, antihyperlipidemic and antioxidant effect in alloxan induced diabetic rats. Diabetes was induced in Wistar albino rats by administration of alloxan monohydrate (150mg/kg). The *A. echioides* at a dose of 100, 200 and 400mg/kg of body weight was administrated at single dose per day to diabetes induced rats for a period of 14 days. The effect of *A. echioides* on blood glucose, insulin, urea, creatinine, HbA_1C, serum protein, albumin, globulin, serum enzymes [Serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT), alkaline phosphatase (ALP)], serum lipid profile, [total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and lipidperoxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X) and reduced glutathione (GSH) were measured in the diabetic rats. The *A. echioides* elicited significant reduction of blood glucose, lipid parameters except HDL-C, serum enzymes and LPO and significantly reduced insulin, HDL-C, SOD, CAT, GP_X and GSH at the dose of 400mg/kg which was compared with the diabetic control. From the above results, it is concluded that ethanol extract of *A. echioides* whole plant possesses significant antihyperglycemic, antihyperlipidemic and antioxidant effects in alloxan induced diabetic rats.

Keywords: *Andrographis echioides*, Alloxan, Insulin, HbA_1C, HDL-C, LPO, SOD, GSH.

INTRODUCTION

The number of people living with diabetes is estimated as 382 million people worldwide as of 2014 and this number is expected to increase to over 592 million people in less than 25 years[1]. In 2012, 1.5 million deaths were reported to be directly caused by diabetes[2]. The disease has several pathogenic processes ranging from autoimmune destruction of pancreatic β-cell resulting in absolute insulin deficiency (Type I) to multiple abnormalities leading to the resistance to insulin action by body cells (Type II)[3]. Symptoms of diabetes mellitus include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger (polyphagia), weight loss, vision changes and fatigue[2]. Chronic hyperglycemia of diabetes leads to long term microvascular and macrovascular complications[4]. These complications include retinopathy, nephropathy, neuropathy and cardiovascular disorders[3]. Normal fasting plasma glucose levels range between 3.5-6.7 mmol/l (63-120.6 mg/dl). After a meal the blood glucose level rises to approximately 8 mmol/l and rarely exceeds this level. Repeated fasting blood glucose levels ≥ 7.0 mmol/l (126 mg/dl) or 2-hour postprandial glucose values ≥ 11.1 mmol/l (200 mg/dl) is considered to be diagnostic criteria for diabetes and correlates with HbA1c threshold of 6.5%[5]. Type I diabetes patients always require exogenous insulin. For type II patients therapy options start with life style modifications but as disease progresses oral hypoglycemic drugs or insulin or both are required to obtain glycemic target for diabetes management[6]. Although insulin is an essential drug for diabetes management its continuous access is a major problem in many developing countries[7]. Due to the many side effects associated with conventional antidiabetic drugs there is a growing interest in the herbal sources[8].

According to World Health Organization (WHO), upto 80% of the world's population in developing countries relies on traditional medicine practices for their primary health care needs[9]. Plants contain a great diversity of bioactive compounds which makes them a possible source for different types of drugs[10]. For example the widely used hypoglycemic drug Metformin is originally derived from the medicinal plant *Galega officinalis*[11]. More than 400 traditional plants have been reported to have antidiabetic effect[12]. Some of these herbs are proven to provide symptomatic relief and assist in the prevention of the secondary complication of the disease, while others were reported to help in regeneration of β-cells and in overcoming insulin resistance[13].

The genus *Andrographis* is native of India contains 28 species of small annual shrubs essentially distributed in tropical Asia. Some of them are medicinally important. *Andrographis echioides* which is commonly known as 'false water willow' is an herb commonly found throughout India. The plant *Andrographis echioides* are used to treat goiter, liver diseases[14], fever, fertility problems, bacterial[15], malarial, helmintic, fungal, diarrhea and larvicidal disorders[16,17]. Leaf juice boiled with coconut oil is used to control falling and graying of hair[18].

The present study deals with antidiabetic effect of ethanol extract of the whole plant of *Andrographis echioides* on alloxan induced diabetic rats and also evaluate protein metabolite, liver enzyme level changes, lipid profile and antioxidant potential in alloxan induced diabetic rats. The effect produced by this drug on different parameters was compared with those of glibenclamide, a reference drug.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *Andrographis echioides* (L.) Nees (AE) were collected from Surandai, Tirunelveli District, Tamil Nadu. The plant samples were identified with the help of local flora and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of collected plants was deposited in the Ethnopharmacological Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu, India.

Preparation of Plant Extract for Anticancer Activity

The whole plant of *A. echioides* were cut into small pieces, washed and dried at room temperature; the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered whole plant was separately packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract of the whole plant was used for preliminary phytochemical screening[19] and antidiabetic activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were housed under standard environmental conditions at temperature (25±2 °C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*. The antidiabetic study was carried out as per IAEC approval No. 1012/C06/CPSEA-Corres-2008-2009.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD–423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study[20]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5 mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, upto 2000 mg/kg body weight.

Induction of Experimental Diabetes

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg)[21]. Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

In the present investigation, a total of 36 rats (30 diabetic surviving rats and 6 normal rats) were taken and divided into six groups of 6 rats each. Group I: Normal untreated rats Group II: Diabetic control rats Group III: Diabetic rats given ethanol extract of *A. echioides* whole plant (100 mg/kg body weight) Group IV: Diabetic rats given ethanol extract of *A. echioides* whole plant (200 mg/kg body weight) Group V: Diabetic rats given ethanol extract of *A. echioides* whole plant (400 mg/kg body weight) Group VI: Diabetic rats given standard drug glibenclamide (600 µg/kg body weight).

Biochemical Analysis

The animals were sacrificed at the end of experimental period of 30 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 min. Serum glucose was measured by the O-toluidine method[22]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit[23] Urea estimation was carried out by the method of Varley[24]; serum creatinine was estimated by the method of Owen *et al.*[25]. Glycosylated haemoglobin (HBA_1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan[26]. Serum total cholesterol (TC)[27], total triglycerides (TG)[28], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C)[29], high density lipoprotein cholesterol (HDL-C)[30] and phospholipids[31] were analyzed. Serum protein[32] and serum albumins was determined by quantitative colorimetric method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel[33]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong[34]. Lipid peroxidation (LPO)[35], Glutathione peroxidase (GPx)[36], reduced glutathione (GSH)[37], superoxide dismutase (SOD)[38] and catalase (CAT)[39] in serum, liver and kidney were analysed in the normal, diabetic induced and drug treated rats.

Statistical Analysis

The data was analyzed using student's t-test statistical methods. For the statistical tests *p* values of less than 0.001, 0.01 and 0.05 was taken as significant.

RESULTS

Phytochemical Screening and Acute Toxicity Studies

The preliminary phytochemical screening of ethanol extract of the whole plant of *A. echioides* revealed the presence of alkaloid anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of the whole plant of *A. echioides*.

Effect of Whole Plant Extract of *Andrographis echioides* on Serum Profile

The effect of whole plant extract of *A. echioides* on the serum insulin, glucose, urea, creatinine and glycosylated haemoglobin of normal and diabetic treated rats are shown in table 1. The results revealed that the insulin level was significantly ($p<0.001$) reduced in diabetic rats (Group II) compared to normal rats (Group I) but the other biochemical parameters like glucose, urea, creatinine and glycosylated haemoglobin were significantly ($p<0.01$; $p<0.001$) increased in diabetic rats than control rats. Administration with the whole plant ethanol extract of *A. echioides*, at 400mg/kg body weight dose (Group V) and glibenclamide (Group VI) tends to bring the above said parameters significantly towards normal.

Effect of Whole Plant Extract of *A. echioides* on Protein and Liver Marker Enzymes

The levels of total protein, albumin, globulin and liver marker enzymes such as SGPT, SGOT, and ALP in the serum of diabetic rats are presented in table 2. When compared with normal control rats (Group I), the diabetic control rats (Group II) had decreased levels of serum total protein, albumin, globulin and elevated levels liver marker enzymes such as SGPT, SGOT, and ALP. After treatment with the whole plant ethanol extract of *A. echioides* at 200 and 400 mg/kg body weight doses (Group IV and V) and glibenclamide (Group VI), the total protein, albumin, globulin and liver marker enzymes were brought back to near normal levels.

Effect of Whole Plant Extract of *A. echioides* on Serum Lipid Profile

Table 3 illustrates the effect of whole plant ethanol extract of *A. echioides* on the levels of total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, VLDL-C and PL in the serum of experimentally induced diabetic rats. It is evident from the results that the serum levels of total cholesterol (TC), triglycerides (TG), very low density lipoproteins (VLDL-C) low density lipoproteins (LDL-C) and phospholipids (PL) ($p<0.001$; $p<0.01$) increased whereas, serum high density lipoproteins (HDL C) level was significantly reduced ($p<0.01$) in diabetic rats when compared to normal control group. It was further evident that whole plant extract treated group significantly reduced the levels of TC, TG, VLDL-C, LDL-C and PL whereas significantly ($p<0.05$) increased HDL-C respectively in a dose dependent manner.

Effect of Whole Plant Extract of *Andrographis echioides* on Antioxidant Enzymes

In the present study, the alloxan induced diabetic rats were found to have increased LPO, GP_X, GSH, SOD and CAT in the serum, liver and kidney when compared with control. Administration of whole plant extract to the diabetic rats resulted in significant ($p<0.05$; $p<0.01$; $p<0.001$) decrease in the activity of LPO and increased activity of GP_X, GSH, SOD and CAT (Table 4-6).

Table 1: Effect of ethanol extract of whole plant of *A. echioides* (AE) on serum insulin, blood glucose, urea, creatinine and HbA_1C level of normal, diabetic induced and drugs treated rats

Groups	Dose (mg/kg body weight)	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	HbA_1C (%)
I	Normal Control	15.32±0.94	73.92±1.31	19.31±0.36	0.73±0.14	4.01±0.36
II	Diabetic Control	7.54±0.27***	201.35±4.84***	32.08±0.95**	2.14±0.11**	10.46±0.34**
III	AE(100)	8.21±0.54**	189.56±3.16**	26.63±0.31*	1.92±0.36*	9.16±0.39*
IV	AE(200)	10.53±0.92ns [a]	126.34±4.62*	19.14±0.54ns	0.84±0.15ns	7.32±0.11ns
V	AE(400)	14.63±0.24 [aa]	102.65±3.18 [aa]	16.93±0.16[a]	0.76±0.26ns	5.11±0.86ns
VI	Glibenclamide (600 µg/kg)	16.32±0.14 [aa]	84.65±2.11[aa]	15.83±0.54[a]	0.63±0.15[a]	4.84±0.36[a]

Values are expressed as mean ± SEM, n= 6 in each group
* $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control *Vs* diabetic induced control , drug treated group [a] $p < 0.05$;[aa] $p < 0.01$ Significance between diabetic induced control control *Vs* drug treated group. NS: Not significant

Table 2 : Effect of ethanol extract of whole plant of *A. echioides* (AE) on protein, albumin, globulin, SGOT, SGPT and ALP level of normal, diabetic induced and drug treated rats

Groups	Dose (mg/kg body weight)	Parameters					
		Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
I	Normal Control	8.11±0.36	4.54±0.11	3.57±0.21	19.67±1.08	23.16±1.31	156.36±3.19
II	Diabetic Control	6.21±0.15*	3.86±0.16*	2.35±0.32*	106.39±2.11***	126.15±1.84**	218.65±2.67**
III	AE(100)	6.83±0.14*	3.98±0.27	2.85±0.11	93.16±1.84**	113.16±1.67*	184.15±1.92**
IV	AE(200)	7.38±0.27ns	4.07±0.11	3.31±0.52	67.22±1.34**	84.65±1.86*	172.86±2.08*
V	AE(400)	7.76±0.16ns	4.18±0.24	3.58±0.27[a]	34.16±1.56[a]	38.11±2.16[a]	169.31±1.68[a]
VI	Glibenclamide (600 µg/kg)	7.98±0.22ns	4.36±0.12[a]	3.62±0.17[a]	26.13±0.98[a]	34.26±1.92[a]	146.26±1.26[aa]

Values are expressed as mean ± SEM, n= 6 in each group
* $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control *Vs* diabetic induced control , drug treated group [a] $p < 0.05$;[aa] $p < 0.01$ Significance between diabetic induced control control *Vs* drug treated group. NS: Not significant.

Table 3: Effect of ethanol extract of whole plant of *A. echioides* (AE) on TC, TG, LDL-C and PL in the plasma of normal, diabetic induced, and drug treated rats

Groups	Dose (mg/kg body weight)	Parameter					
		TC (mg/dl)	TG(mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	PL (mg/dl)
I	Normal Control	118.54±2.63	106.31±1.67	38.16±1.31	59.12±1.45	21.26±1.03	173.50±2.18
II	Diabetic Control	206.16±1.98***	163.66±2.39***	19.54±1.08**	153.89±3.29***	32.73±0.95**	251.48±3.29***
III	AE(100)	191.31±1.36***	151.54±2.94**	22.36±1.16*	137.64±2.75***	30.31±0.32*	238.27±2.64**
IV	AE(200)	168.16±1.92**	134.88±1.36*	26.92±1.84*	114.26±1.47*[a]	26.98±0.38ns	217.66±2.49 ns [a]
V	AE(400)	131.64±1.82ns [a]	126.16±1.08[a]	32.88±1.36[a]	73.53±1.04ns [a]	25.23±0.58ns	185.16±2.18 [aa]
VI	Glibenclamide (600 µg/kg)	144.16±1.94ns [a]	119.31±1.36[aa]	35.96±1.84[a]	84.34±1.26ns [aa]	23.86±0.91ns	196.30±2.42[aa]

Values are expressed as mean ± SEM, n= 6 in each group
* $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control *Vs* diabetic induced control , drug treated group [a] $p < 0.05$;[aa] $p < 0.01$ Significance between diabetic induced control control *Vs* drug treated group. NS: Not significant.

Table 4: Effect of ethanol extract of whole plant of *A. echioides* (AE) extract on serum LPO, GP_X, GSH, SOD and CAT in the normal, diabetic and drug treated rats

Groups	Dose (mg/kg body weight)	Parameters				
		LPO (nanomol/mg protein)	GP^X (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	Normal Control	2.36±0.18	512.16±1.12	42.67±0.98	511.67±5.63	76.19±1.13
II	Diabetic Control	5.16±0.24***	304.15±1.84***	16.22±0.34***	362.22±4.16***	29.13±0.92***
III	AE(100)	4.82±0.31***	348.26±2.16***	21.46±0.84**	416.16±3.84***	36.26±1.08***
IV	AE(200)	3.78±0.48**	396.15±1.84**	29.66±1.53ns	486.26±1.84*[a]	62.84±2.05ns [a]
V	AE(400)	3.03±0.92ns [a]	426.34±2.06*[a]	34.13±1.26ns [a]	538.13±2.16[aa]	83.56±1.98ns [aa]
VI	Glibenclamide (600 µg/kg)	2.86±0.14ns [aa]	442.36±3.61[aa]	31.84±1.17[aa]	511.67±3.15[aa]	79.68±1.67ns [aa]

Values are expressed as mean ± SEM, n= 6 in each group
* $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control *Vs* diabetic induced control, drug treated group;
[a] $p < 0.05$; [aa] $p < 0.01$ Significance between diabetic induced control control *Vs* drug treated group. NS: Not significant

Table 5: Effect of ethanol extract of whole plant of *A. echioides* (AE) extract on liver LPO, GP_X, GSH, SOD and CAT in the normal, diabetic and drug treated rats

Groups	Dose (mg/kg body weight)	Parameters				
		LPO (nanomol/mg protein)	GP^X (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	Normal Control	0.101±0.036	56.92±1.16	38.46±1.65	23.42±1.11	12.16±0.13
II	Diabetic Control	0.516±0.015***	22.63±1.84***	19.34±0.96**	13.84±0.83**	3.08±0.11**
III	AE(100)	0.386±0.039***	31.48±1.39**	24.16±0.81*	16.16±0.74ns	5.84±0.48*
IV	AE(200)	0.224±0.074**[aa]	40.86±1.26ns [a]	29.84±1.16ns [a]	20.31±1.08ns [a]	8.16±0.68ns
V	AE(400)	0.188±0.036*[aaa]	48.65±1.16ns [a]	33.16±1.48 [aa]	26.65±1.13[aa]	11.84±0.93ns [aa]
VI	Glibenclamide (600 µg/kg)	0.136±0.048ns [aaa]	59.16±1.92ns [aa]	46.92±1.43[aaa]	27.36±1.64[aa]	14.16±0.76ns [aa]

Values are expressed as mean ± SEM, n= 6 in each group
* $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control *Vs* diabetic induced control, drug treated group;
[a] $p < 0.05$;[aa] $p < 0.01$ Significance between diabetic induced control control *Vs* drug treated group. NS: Not significant

Table 6: Effect of ethanol extract of whole plant of *A. echioides* (AE) extract on liver LPO, GP_X, GSH, SOD and CAT in the normal, diabetic and drug treated rats

Groups	Dose (mg/kg Body weight)	Parameters				
		LPO (nanomol/mg protein)	GP_X (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	Normal Control	0.057±0.016	8.14±0.84	34.16±1.16	26.13±1.16	31.84±0.92
II	Diabetic Control	0.226±0.034***	3.84±0.13***	14.81±0.92**	10.16±0.92*	14.16±0.54**
III	AE(100)	0.106±0.092**	5.16±0.26*	19.16±0.24*	13.24±0.23*	19.26±0.34ns
IV	AE(200)	0.165±0.031* [a]	5.84±0.92ns [a]	26.84±0.16ns [a]	17.36±0.11ns	23.96±0.18ns
V	AE(400)	0.101±0.016[aa]	6.16±0.16ns [aa]	31.86±0.21 [aa]	21.65±0.36ns	26.33±0.36[a]
VI	Glibenclamide (600 µg/kg)	0.092±0.011[aa]	6.94±0.24[aa]	40.11±0.67[aa]	24.98±0.94[a]	34.84±0.96[aa]

Values are expressed as mean ± SEM, n= 6 in each group
* $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control *Vs* diabetic induced control, drug treated group;
[a] $p < 0.05$;[aa] $p < 0.01$ Significance between diabetic induced control control *Vs* drug treated group. NS: Not significant

DISCUSSION

Diabetes is a disorder of carbohydrate, fat and protein metabolism caused due to insufficient production of insulin or due to its inhibitory action, which can be considered as a major cause of high economic loss which can in turn impede the development of nations[40]. Diabetes mellitus causes disturbances in the intake of glucose as well as glucose metabolism. Alloxan induced hyperglycemia has been described as a useful experimental model to study the activity of hygoglycemic agents. It is postulated to induce diabetes by degeneration and necrosis of β- cells of the islets of Langerhans of pancreas, which leads to reduction in insulin release[41]. In the present study, *A.echioides* may also have brought about hypoglycemic action through stimulation of surviving β- cells of islets of Langerhans to release more insulin. This was clearly evidenced by the increased levels of plasma insulin in diabetic rats treated with *A.echioides* whole plant.

The whole plant ethanol extract of *A.echioides* was treated on alloxan induced diabetic rats. The results based on biochemical parameters, were compared with normal control, diabetic control and the positive control rats treated with glibenclamide, after thirty days of treatment. The result of the present study showed significant changes in biochemical parameters of the experimentally induced diabetes. Blood glucose, serum insulin, urea and creatinine levels of ethanol extracts and glibenclamide treated rats were compared with the control. The glucose level was significantly (p<0.001) high in alloxan induced diabetic control rats compared with normal control. Administration with the whole plant extract of *A.echioides* (400 mg/kg body weight) decreased the blood glucose level. The orally administered *A.echioides* whole plant extract to alloxan induced diabetic rats elicited a significant antidiabetic activity and significantly (p<0.01) increased the plasma insulin levels. Ethanol extract of whole plant of *A.echioides* treated (Group V 400 mg/kg body

weight) rats showed a significant (p<0. 01) increase in plasma insulin level when compared with alloxan induced diabetic control. Alloxan, β - cytotoxin, induces chemical diabetes in a wide variety of animal species by damaging the insulin secreting β - cells of the pancreas. Alloxan causes time and concentration-dependent degenerative lesions of the pancreatic β – cells[42,43]. The mechanism of action of increase in plasma insulin concentration could be due to long- lasting stimulant effect on β - cells of pancreatic islets or to pancreatic β - cells regeneration.

The hypoglycemic activity of ethanol extract of *Butea monosperma* leaves was found to induce insulin release from pancreatic cells of diabetic rats[44]. Hakkim[45] administered the aqueous and ethanol extract of *Cassia auriculata* flower, which significantly lowered blood glucose level with corresponding increase in insulin level in alloxan induced diabetic rats. It is evident from this study that, there is an increase in insulin level in diabetic rats treated with plant extracts. Many plants have been studied for their hypoglycemic and insulin increase stimulatory effects [46-48].

A significant elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic rats, when compared to control rats. The whole plant ethanol extract of *A. echioides* was administered orally to rats for fourteen days and this reversed the levels of urea and creatinine to near normal. Administration with glibenclamide, the standard antidiabetic drug, also decreased the levels of urea and creatinine to some extent.

Glycosylated haemoglobin is an indicator of the progression of diabetes. Therefore glycosylated haemoglobin was measured in the control and diabetic rats. The increase of glycosylated haemoglobin serves as a marker to know the induction of diabetes. The treatment of ethanol extract of *A. echioides* whole plant in diabetic rats maintained the original levels of glycosylated haemoglobin equal to that of control ones.

It is very clear from the results, a significant reduction in serum protein, albumin and globulin was observed in alloxan induced diabetic control rats when compared to normal and glibenclamide treated rats. The administration of whole plant ethanol extract of *A.echioides* to the diabetic rats restored the protein, albumin and globulin levels to normal. These results were in accordance with the effect of *Eugenia singampatiana* and *Polygala rosmarinifolia* in diabetic rats[49,50]. The increased levels of serum protein, albumin and globulin, in alloxan induced diabetic rats presumed to be due to increased protein catabolism and gluconeogenesis during diabetes[51].

Alloxan had a profound effect on the activity of hepatic marker enzymes. The rats treated with alloxan developed hepatic damage which is evident from the increase in the enzyme activities. Serum enzyme SGOT, SGPT and ALP levels were increased significantly (p<0.01; p <0.001) in alloxan induced diabetic rats in comparison with normal animals. The ethanol extract of whole plant of *A.echioides* significantly (p<0.05) decreased the elevated SGOT, SGPT and ALP levels in treated rats. Increased activity of transaminases, which are active in the absence of insulin because of increased availability of amino acids in diabetes, are believed to be responsible for the increased gluconeogenesis and ketogenesis observed in the disease[52]. SGOT, SGPT and ALP levels in serum were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats[53].

In alloxan induced diabetic rats, there was a significant (p<0.001) increase of total cholesterol, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol and phospolipids (PL) and significant (p<0.01) decrease in high density lipoprotein (HDL) cholesterol in serum compared with normal control rats. The ethanol extract of whole plant of *A.echioides* significantly (p<0.05) decreased the level of total cholesterol, triglycerides, LDL, and VLDL cholesterol and significantly (p<0.05) increased HDL cholesterol. This indicates that the whole plant extract had favourable effects, on lipid metabolism of diabetic rats. Derangement of glucose, fat and protein metabolism in diabetes results in the development of hypolipidemia[54-56]. Significant lowering of total cholesterol and rise in HDL cholesterol is a very desirable biochemical state for the prevention of atherosclerosis and ischemic conditions[57].

The results of the present study showed increased lipid peroxidation (LPO) on serum, liver and kidney of alloxan induced diabetic rats, which indicates an increase in the generation of free radicals. Increased lipid peroxidation in diabetes mellitus can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. The present study indicates significantly increased peroxidation of rats exposed to alloxan and its attenuation by *A.echioides* whole plant treatment. This suggests that the protective role of *A.echioides* whole plant extracts could be due to the antioxidative effect of flavonoids present in the whole plant, which in turn act as strong superoxide radicals and singlet oxygen quenchers.

Numerous studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes mellitus[58]. In the current study, the SOD, CAT, GP_X and GSH activities were significantly reduced in the serum, liver and kidney of diabetic rats. These observations emphasise the critical importance of maintaining the antioxidant potential of the pancreatic β - cell in order to ensure both its survival and insulin secretion capacity during times of increased oxidative stress. The decreased activities of SOD and CAT in serum, liver and kidney during diabetes mellitus may be due to the production of reactive oxygen free radical that can themselves reduce the activity of their enzymes.

Reduced glutathione is a potent free radical scavenger GSH within the islet of β- cell and is an important factor against the progressive destruction of the β- cell following partial pancreatectomy[59]. Depletion of GSH resulted in enhanced lipid peroxidation.

This can cause increased GSH consumption and can be correlated to the increased level of oxidised glutathione (GSSG). Treatment of *A. echioides* whole plant resulted in the elevation of GSH levels, which protect the cell membrane against oxidative damage by regulating the redox status of protein in the membrane[60]. SOD, CAT and GP_X are enzymes that destroy the peroxides and play a significant role in providing antioxidant defenses to an organism. GP_X and CAT are involved in the elimination of H_2O_2. SOD acts as dismutase superoxide radical to H_2O_2, which is then acted upon by GP_X. The functions of all three enzymes are interconnected and lowering of their activities resulted in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats. Treatment of ethanol extract of *A.echioides* whole plant increased the activity of these enzymes and thus may help to avoid the free radicals generated during diabetes mellitus.

The *A. echioides* whole plant extract is beneficial in controlling the blood glucose level, improves the lipid metabolism and prevents diabetic complications from lipid peroxidation and

antioxidant systems in experimental diabetic rats. This could be useful for prevention or early treatment of diabetic disorders. Further studies are in progress to isolate, identify and characterize the active principles.

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