Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDAEMIC AND ANTIOXIDANT ACTIVITY OF *CYNOGLOSSUM ZEYLANICUM* (VAHL EX HORNEM) THURNB EX LEHRN IN ALLOXAN INDUCED DIABETIC RATS

M.ANITHA[1], K. RAJALAKSHMI[2], S. MUTHUKUMARASAMY[3] AND V.R.MOHAN[2*]

[1]Department of Botany, Sri Parasakthi College for Women, Courtallum, Tamil Nadu, [2]Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu, [3]Department of Botany, Sri K.G.S. Arts College, Srivaikuntam, Tamil Nadu. Email: vrmohanvoc@gmail.com

Received: 26 Sep, 2012, Revised and Accepted: 30 Oct, 2012

ABSTRACT

The ethanol extract of *Cynoglossum zeylanicum* whole plant (ECZW) (Family: Boraginaceae) was investigated for its antioxidant, antihyperlipidaemic and antidiabetic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The ethanol extracts of *C. zeylanicum* at a dose of 150 and 300mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *C. zeylanicum* whole plant (ECZW) extract on blood glucose, plasma insulin, creatinine, glycosylated haemoglobin, urea serum lipid profile [total cholesterol (TR), triglycerides (TG), low density lipoprotein – cholesterol (LDL-C), very low density lipoprotein – cholesterol (VLDL-C), high density lipoprotein – cholesterol (HDL-C) and phospholipid (PL) serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases] (SGPT), and serum glutamate oxaloacetate transaminases (SGOT), and alkaline phosphatase (ALP)], lipoprotein peroxidation (LPO) antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx) were measured in the diabetic rats. The ethanol extract of *Cynoglossum zeylanicum* whole plant (ECZW) elicited significant reductions of blood glucose (p<0.05), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant enzymes. The extracts also caused significant increase in plasma insulin (p<0.05) in the diabetic rats. From the above results, it is concluded that ethanol extract of *Cynoglossum zeylanicum* possesses significant antidiabetic, antihyperlipidaemic and antioxidant effects in alloxan induced diabetic rats.

Keywords: Antioxidant, Antihyperlipidaemic, Antidiabetic, *C. zeylanicum*, Alloxan.

INTRODUCTION

Diabetes mellitus is a syndrome, initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins[1]. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and postprandial blood sugar levels. The peroxidation of cellular membrane lipids can lead to cell necrosis and is purportedly associated with various chronic disorders including carcinogenesis and hyperglycemia[2].This pathological condition occurs as a result of the loss of insulin-producing pancreatic beta-cells by an environmentally triggered autoimmune reaction. Currently available oral antidiabetic agents have a number of serious adverse effects. Treatment with sulphonylureas and biguanides are also associated with side effects[3]. Hence, search for a new drug with low cost, more potential, without adverse effects is being pursued in several laboratories all around the world. Since time immemorial, patients with non-insulin requiring diabetes have been created orally in folk medicine with a variety of plant extracts[4].Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one[5]. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidaemic remedies. The antihyperglycemic effect of these plants are of their ability to restore the function of pancreatic tissues by increasing insulin output or inhibit the intestinal absorption of glucose or to be the facilitation of metabolites in insulin dependent processes[6].

More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternate and safe effects on diabetes mellitus.

Cynoglossum zeylanicum (Vahl ex Hornem) Thurnb ex Lehrn belongs to Boraginaceae family. It is commonly known as "Jathakkai". Decoction prepared from the whole plant is used to arrest vomiting by Badaga community in Nilgiri Biosphere Reserve, Tamil Nadu. However, inspite of traditional use, pharmacology of its whole plant has not yet been explored scientifically. So far no reports are available in antidiabetic activity of this plant. The present investigation was carried out to evaluate the antidiabetic, antihyperlipidaemic and antioxidant activity of the ethanol extracts of whole plant of *Cynoglossum zeylanicum* against alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *Cynoglossum zeylanicum* were freshly collected from the well grown healthy plants inhabiting the natural forests of Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. The plant were identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The *C. zeylanicum* whole plant (ECZW) was shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *C. zeylanicum* whole plant (ECZW) was packed in a Soxhlet apparatus and extracted with ethanol The extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures[7, 8, 9].The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar Albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2º 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*.

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 [10]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/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, and 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) [11]. 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 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *C. zeylanicum* whole plant (100mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *C. zeylanicum* whole plant (150mg/kg body weight)

Group V: Diabetic rats given standard drug glibenclamide (600µg/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 minutes. Serum glucose was measured by the O-toluidine method[12]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit[13]. Urea estimation was carried out by the method of Varley[14]; serum creatinine was estimated by the method of Owen *et al*[15]. Glycosylated haemoglobin (HBA_1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan[16]. Serum total cholesterol (TC) [17], total triglycerides (TG) [18], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C[19], high density lipoprotein cholesterol (HDL-C) [20] and phospholipids[21] were analyzed. Serum protein[22] and serum albumins was determined by quantitative colorimetrically 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[23]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong[24]. Catalase (CAT)[25], superoxide dismutase (SOD)[26], lipid peroxidation (LPO)[27], reduced glutathione (GSH)[28] and glutathione peroxidase (GPx)[29] were analyzed in the normal, diabetic induced and drug treated rats.

Statistical Analysis

The data were analyzed using student's t-test statistical methods. For the statistical Tests a p values of less than 0.01 and 0.05 was taken as significant.

RESULTS AND DISCUSSION

The phytochemical screening of ethanol extract of *C. zeylanicum* whole plant (ECZW) revealed the presence of alkaloid, anthraquinone, catechin, flavonoid, phenol, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein. Acute toxicity study revealed that non-toxic nature of the ethanol extract of *C. zeylanicum* whole plant.

The fasting blood glucose (FBG) levels of normal, diabetic and drug treated rats are summarized in Table 1. Alloxan at a dose of 150mg/kg produced marked hyperglycemia as evident from significant (p<0.001) elevation in FBG level in alloxan induced group as compared to normal control group. The administration of ethanol extract of *Cynoglossum zeylanicum* whole plant (ECZW) in alloxan induced diabetic rats at doses of 100 and 150mg/kg body weight produced significant (p<0.01) and dose dependent fall in blood glucose levels when compared with the alloxan induced group. The FBG reducing effect of *C. zeylanicum* at a dose 150µg/kg was found to be comparable to that of the reference drug glibenclamide (600µg/kg body weight). Moreover, the 4 week treatment with more effective dose (150mg/kg body weight) of the *C. zeylanicum* whole plant extract decreases FBG significantly from 209.16mg/dl to 104.33mg/dl. The sharp fall of fasting plasma glucose levels was a clear evidence of significant antidiabetic effect of *C. zeylanicum* whole plant (ECZW) extract.

Table 1: Effect of ethanol extract of *Cynoglossum zeylanicum* whole plant on blood glucose level of normal, diabetic induced, and drug treated rats at different time intervals.

Groups	Blood glucose level in mgs/dl				
	0 day	1 week	2 week	3week	4 week
Group I	69.33±2.17	78.23±0.67	88.17±1.24	78.27±1.94	73.17±2.16
Group II	204.32±5.27***	213.16±6.17***	236.36±3.84***	209.17±5.31***	216.16±5.32 ***
Group III	216.74±6.17***	209.17±5.54***	184.27±5.54ns	162.16±4.84**[a]	142.23±4.84*[a]
Group IV	209.16±7.26***	184.21±5.23***	148.19±3.92[a]	121.32±4.08[a]	104.33±3.98[aa]
Group V	219.32±6.34***	131.16±6.21*[a]	124.16±4.08*[a]	99.32±1.61	91.68±1.36[aa]

Each Value is SEM of 6 animals (* 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 shows the levels of blood glucose, plasma insulin, urea, creatinine and glycosylated haemoglobin of normal, diabetic control and drug treated rats. There was a significant (p<0.001) in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of whole plant extract of *C. zeylanicum* (Group III & IV) and glibenclamide (Group V) tends to bring the parameters significantly (p<0.05, p<0.01) towards the normal. It has been reported that using medicinal plant extract to treat alloxan induced diabetic rats result in activation of β-cells and insulinogenic effects[30]. *C. zeylanicum* (ECZW) may also have brought about hypoglycemic action through stimulation of surviving β-cells is islets of langerhans to release more insulin. This was clearly evidenced by the increased levels of plasma insulin in diabetic rats treated with *C. zeylanicum* (ECZW). A number of other plants have also been observed to exert hypoglycemic activity through insulin release stimulatory effects [31, 32, 33, 34,35,36].

Table 2: Effect of ethanol extract of *Cynoglossum zeylanicum* whole plant on the Insulin, blood glucose, urea, creatinine and HbA_1C level of normal, diabatic induced and drugs treated rats.

Parameter	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	HbA_1C (%)
Group I	18.56±0.72	82.16±1.16	16.32±0.74	0.73±0.17	3.94±0.11
Group II	8.91±0.26**	202.14±9.16***	36.16±1.19**	1.84±0.84*	12.22±0.34**
Group III	11.56±0.91*	131.23±6.71*[a]	31.68±1.24*	1.68±0.17ns	8.21±0.26[a]
Group IV	16.31±0.54[a]	118.17±5.27[a]	33.94±1.84*	1.74±0.24*	10.54±0.17*
Group V	19.26±1.64[aa]	81.16±5.11[aa]	17.33±0.91[a]	0.70±o.21[aa]	5.27±0.84[a]

Each Value is SEM of 6 animals (* $p < 0.05$; ** $p < 0.01$ *** $p<0.001$ 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).

A significant ($p<0.01$, $p<0.05$) elevation in urea and creatinine were observed in alloxan induced diabetic rats (Group II), when compared to control rats. The *C. zeylanicum* (ECZW) were administrated orally (Group III & IV) to rats for thirty days, reversed the urea and creatinine level to near normal.

The level of total protein, albumin, globulin and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats are presented in the Table 3. A significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (group II), when compared to control (Group I) and glibenclamide treated rats (Group V). On administration of ethanol extract *C. zeylanicum* (ECZW) whole plant to the diabetic rats, protein, albumin and globulin levels were found to be restored in normal. These results were in accordance with the effect of *Eugenia floccosa* leaf in diabetic rats[32]. The increased level of serum protein, albumin and globulin in alloxan induced diabetic rats are presumed and gluconeogenesis during diabetes[37]. The animals treated with alloxan developed hepatic damage which was evident from the increase in the enzyme activities. Pretreatment with ethanol extract of *C. zeylanicum* whole plant (ECZW) and glibenclamide resulted in a decrease of transaminase activities in alloxan treated rats. The SGPT and SGOT levels increases as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes[38].

The elevated levels of SGPT and SGOT in alloxan induced diabetic rats may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan[39]. Elevation of serum biomarker enzymes such as SGPT, SGOT and ALP was observed in diabetic rats indicating impaired liver function, which is obviously due to hepatocellular necrosis. In this study, the ethanol extract of *C. zeylanicum* whole plant (ECZW) regulated the activity of SGPT, SGOT and ALP in liver of rats intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study[40].

Table 3: Effect of ethanol extract of *Cynoglossum zeylanicum* whole plant on the protein, albumin, globulin, SGOT, SGPT and ALP level of normal, diabetic induced, and drug treated rats.

Groups	Parameters Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	8.64±0.84	4.72±0.11	3.92±0.15	21.22±3.54	19.33±1.74	154.21±1.68
Group II	6.04±0.13*	3.74±0.18	2.30±0.17*	169.24±5.39***	183.74±2.84***	208.63±5.23**
Group III	6.84±0.21*	3.94±0.11	2.90±0.21*	119.86±3.16**	161.17±1.57**	165.22±5.34[aa]
Group IV	7.27±0.17	4.08±0.13	3.19±0.14	57.91±2.64*[aa]	43.87±2.17[aa]	186.33±2.81*
Group V	8.49±0.36[a]	4.58±0.21	3.91±0.16[a]	24.13±3.14[aa]	20.18±1.94[aa]	142.16±1.84[aa]

Each Value is SEM of 6 animals (* $p < 0.05$; ** $p < 0.01$ *** $p<0.001$ 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).

Table 4 shows the levels of TC, TG, LDL-C, VLDL-C, HDL-C and PL in the serum of diabetic rats showed significantly ($p<0.01$; $p<0.001$) increased serum lipid profiles except HDL-C when compared with normal rats. The ethanol extract of *C. zeylanicum* whole plant (ECZW) treated rats showed a significant ($p<0.05$) decrease in the content of lipid profiles, when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared with normal rats. Administration of ethanol extract of *C. zeylanicum* whole plant (ECZW) and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents a risk factor for coronary heart diseases[41].

Table 4: Effect of ethanol extract of *Cynoglossum zeylanicum* whole plant on the TC, TG, LDL-C and PL in the plasma of normal, diabetic induced, and drug treated rats

Groups	Parameter TC (mg/dl)	TG(mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	PL (mg/dl)
Group I	126.27±3.84	76.91±2.14	54.16±2.04	56.73±1.98	15.38±0.76	180.38±3.56
Group II	192.16±4.93***	134.60±4.16***	26.38±1.94**	138.86±3.72**	26.92±1.21*	239.02±4.37**
Group III	153.28±3.17*	86.27±2.11[a]	45.18±2.04*[a]	90.56±2.43*[a]	17.54±0.94ns	204.41±5.88*
Group IV	132.16±1.84 [a]	81.33±1.93[a]	48.91±1.94*[a]	66.59±2.74[a]	16.66±0.32[a]	185.62±3.55 [a]
Group V	118.59±2.21[aa]	75.24±1.29[aa]	51.63±1.86[aa]	51.92±2.12[aa]	15.04±0.58[a]	173.54±4.27[aa]

Each Value is SEM of 6 animals (* $p < 0.05$; ** $p < 0.01$ *** $p<0.001$ 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).

During diabetes, enhanced activity of the enzyme, increased lipolysis and release more fatty acids into the circulation[42]. The increased fatty acid concentration also increases the β-oxidation of fatty acids, producing more acetyl Co-A and cholesterol during diabetes. In normal condition, insulin increases receptor-mediator removal of LDL-cholesterol and decreased activity of insulin,

during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglycedemia have been reported to occur in diabetic rats[41]. The increased concentration of free fatty acid may be due to lipid break-down and this may cause increased generation of NADPH-dependent microsomal lipid peroxidation. Phospholipids were increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core[43]. Increased phospholipids levels in tissues were reported by Venkateswaran[44]; Pari and Satheesh[45] in streptozotocin diabetic rats. Administration of ethanol extract of *Senna auriculata* leaf and glibenclamide decreased the levels of phospholipids.

The activities of LPO, SOD, CAT, GPx and GSH in the alloxan induced diabetic rats were presented in Table 5. In the present study, the alloxan induced diabetic rats had shown increased activities of LPO. The levels of SOD, CAT, GPx and GSH in the serum were significantly (p<0.01; p<0.001) reduced in alloxan induced rats. Treatment with *C. zeylanicum* whole plant (ECZW) extract exhibited reversal of all these parameters to near normal levels. Hyperglycemia results in free radical formation through various biochemical reactions. Free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radical produced may react with poly unsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn results in elevated production of free radicals[46]. Lipid peroxide mediated tissue damage has been observed in the development of both type I and type II diabetes. It has been observed that insulin secretion is closely associated with lipoxygenase-derived peroxides[47].

In the present study, LPO was significantly lower in the *C. zeylanicum* whole plant (ECZW) extract treated groups compared to the diabetic control group. The above results suggest that the ethanol extract of *C. zeylanicum* may exert antioxidant activities and protect the tissues from lipid peroxidation.

Table 5: Effect of ethanol extract of *Cynoglossum zeylanicum* whole plant on serum LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats.

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	1.84±0.027	614.23±17.36	32.94±1.94	468.13±11.32	73.22±1.84
II	5.61±0.031**	324.56±16.24***	15.14±0.86**	234.59±16.32***	41.91±1.23***
III	3.86±0.034*[a]	389.54±15.29**	19.66±1.04*	263.11±15.27*[a]	53.74±1.81*
IV	3.28±0.054*[a]	421.59±15.29*[a]	23.76±1.84*	314.19±13.92 *[aa]	60.08±1.24 [a]
V	2.04±0.024[a]	594.68±18.21[aa]	34.91±1.59[aa]	447.27±11.46[aa]	88.14±1.94[aa]

Each Value is SEM of 6 animals (* 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).

Numerous studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes mellitus[48]. In the present study, the SOD, CAT, GSH and GPx activities were significantly reduced in the serum of diabetic rats. These observations emphasize 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 during diabetes mellitus may be due to the production of reactive oxygen free-radical that can themselves reduce the activity of these enzymes.

Reduced glutathione is a potent free radical scavenges GSH within the islet of β cell and is an important factor against the progressive destruction of the β cell following partial pancreatectomy[49]. Depletion of GSH results in enhanced lipid peroxidation. This can cause increased GSH consumption and can be correlated to the increase in the level of oxidized glutathione (GSSG). Administration of *C. zeylanicum* 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 [50]. SOD, CAT and GPX are enzymes that destroy the peroxides and play a significant role in providing antioxidant defenses to an organism. GPx and CAT are involved in the elimination of H_2O_2. SOD acts to dismutate superoxide radical to H_2O_2, which is then acted upon by GPx. The functions of all three enzymes are interconnected and a lowering of their activities results in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats[51]. Administration of *C. zeylanicum* extract increased its activity of these enzymes and thus may help to avoid the free radicals generated during diabetes mellitus. These results reveal the protective role of plant extract in decreasing lipid peroxidation and by normalizing antioxidant system.

In the present study, the administration of *Cynoglossum zeylanicum* whole plant (ECZW) extracts to alloxan induced hyperglycemic rats demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profile including lipid content, as compared to alloxan control rats. Also ECZW treatment resulted in significant modulation of lipid peroxidation, endogenous non enzymatic and enzymatic antioxidant and detoxification status. The phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, terpenoids, glycosides, steroids, saponin and phenols. Several authors reported that flavonoids, steroids/terpenoids, phenolic acids are known to be bioactive antidiabetic principles[52,53]. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues[54, 55]. In the present study, the phytochemical analysis of ethanol extract of *C. zeylanicum* whole plant clearly prints out the presence of above said active principles. The preliminary investigation on the antihyperglycemic, antihyperlipidaemic and antioxidant efficacy of ethanol extract of *Cynoglossum zeylanicum* whole plant (ECZW) will be significant to proceed further in this path for the isolation of active principles responsible for the antidiabetic activity.

ACKNOWLEDGEMENT

The Authors wishes to thank Dr. R. Sampatharaj, Honorary Advisor, Samsun Clinical Research Laboratory, Tirupur, for their assistance in animal studies.

REFERENCES

1. Scheen JA. Drug treatement of non-insulin dependent diabetes mellitus in the 1990s. Achi Fut Develop Drug 1997; 54: 355-368.
2. Bavarva JH, Narasimhacharya AV. *Leucas cephalotes* regulates carbohydrate and lipid metabolism and improves antioxidant status in IDDM and NIDDM rats. J Ethnopharmacol 2010; 127: 98-102.
3. Rang HP, Dale MM. The Endocrine system pharmacology. 2nd ed. Harlow: UK: Longman; 1991. p. 504.
4. Shruthi A, Latha KP, Vagdevi HM, Pushpa B, Shwetha C. Antidiabetic activity of the leaves extracts of *Wrightia tinctoria* an alloxan induced diabetic rats. J Chem Pharmaceu Res 2012; 4: 3125-3128.
5. Bailey CJ, Day C. Traditional treatments for diabetes. Diabe care 1989; 12: 553-564.

6. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2012; 2: 320-330.
7. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies on *Merugan kizhangu*. Bull. Med Ethnobot Res 1981; 3: 84-96.
8. Anonymous. Phytochemical investigation of certain medicinal plants used in Ayurveda. Central Council for Research in Ayurveda and Siddha, New Delhi;1990.
9. Lala PK. Lab manuals of Pharmacognosy CSI Publishers and Distributers, Kolkata; 1993.
10. OECD. Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method. OECD. Paris; 2002.
11. Nagappa AN, Thakurdesai PA, Venkat Rao N, Sing J. Antidiabetic activity of *Terminalia catappa* Linn. fruits. J Ethnopharmacol 2003; 88: 45-50.
12. Sasaki T, Masty S, Sonae A. Effect of acetic acid concentration on the colour reaction in the Otoluidine boric acid method for blood glucose estimation. Rinshbo Kagaku 1972; 1: 346-353.
13. Anderson L, Dinesen B, Jorgonsen PN, Poulsen F, Roder ME. Enzyme immune assay for intact human insulin in serum or plasma. Clin Chem 1993; 39: 578-582.
14. Varley H. Practical clinical biochemistry, Arnold Heinemann Publication Pvt. Ltd., 1976. p. 452.
15. Owen JA, Iggo JB, Scangrett FJ, Steward IP. Determination of creatinine in plasma serum, a critical examination. J Biochem 1954; 58: 426-437.
16. Karunanayake EH ,Chandrasekharan NV. An evaluation of a colorimetric procedure for the estimation of glycosylated haemoglobin and establishment of reference values for Sri Lanka. J Nat Sci Coun Sri Lanka 1985; 13: 235-258.
17. Parekh AC, Jung DH. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. Anal Chem 1970; 112: 1423-1427.
18. Rice EW. Triglycerides in Serum In: Standard Methods. Clinical Chemistry. 9ed Roderick MP, New York: Academic press; 1970. p. 215-222.
19. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultra centrifuge. Clin Chem 1972; 18: 499-502.
20. Warnick GR, Nguyan T, Albers AA. Comparison of improved precipitation methods for quantification of high density lipoprotein cholesterol. Clin Chem 1985; 31: 217.
21. Takayama M, Itoh S, Nagasaki T, Tanimizu I. A new enzymatic method for determination of serum phospholipids. Clin Chem Acta 1977; 79: 93 – 98.
22. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin's phenol reagent. J Bio Chem 1951; 193: 265-275.
23. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer J Clin Path 1957; 28: 56-63.
24. King EJ and Armstrong AR . Determination of serum and bile phosphatase activity. Cannad Med Assoc J 1934; 31: 56-63.
25. Bergmayer HU. UV method of catalase assay. In Methods of Enzymatic Analysis, Weiheim Deer field Beach, Florida, Bansal. 1983; 3: 273.
26. Madesh M, Balasubramanian KA. Microtitre plate assay for superoxide dismutase using MTT reduction by superoxide. Ind J Biochem Biophys 1998; 35: 184-188.
27. Rehman S. Lead – induced regional lipid peroxidation in brain. Toxicol Lett 1984; 21: 333-337.
28. Prins HK, Loos JA. In Glutathione; Biochemical methods in red cell genetics, edited by J.J Yunis. New York: Academic Press; 1969. p. 127-129.
29. Pagila DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-169.
30. Padmini K, Chakrabarti CH. Effects of Bittergourd (*Momordica charantia*) seed and glibenclamide in streptozotocin induced diabetes mellitus. Ind J Exp Biol 1982; 20: 232-235.
31. Shanmugasundaram R, Kalpana Devi V, Tresina Soris P, Maruthupandian A, Mohan VR Antidiabetic, antihyperlipidaemic and antioxidant activity of *Senna auriculata* (L.) Roxb leaves in alloxan induced diabetic rats. Int J PharmTech Res 2011; 3: 747-756.
32. Kala SMJ, Tresina PS, Mohan VR. Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia singampattina* Bedd leaves in Alloxan induced diabetic rats. Int J Pharma Pharmace Sci 2012a; 4: 412-416.
33. Kala SMJ, Tresina PS, Mohan VR. Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia floccosa* Bedd leaves in Alloxan induced diabetic rats. J Basic Clin Pharmacy 2012b; 3: 235-240.
34. Shajeela PS, Kalpanadevi V, Mohan VR. Potential antidiabetic, hypolipidaemic and antioxidant effects of *Nymphaea pubercens* extract in Alloxan induced diabetic rats. J Appl Pharmacu Sci 2012; 2: 83-88.
35. Maharana L, Kar DM, Pattnaik S. Antidiabetic evaluation of aqueous extract of aerial parts of *Mollugo pentaphylla* L. International Journal of Pharmacy and Pharmaceutical Sciences 2012;4:269-275.
36. Daisy P, Jeevakani FG. Evaluation of antidiabetic activity of various extracts of *Cassia auriculata* Linn. bark on streptozotocin- induced diabetic wistar rats. International Journal of Pharmacy and Pharmaceutical Sciences 2012;4:312-318.
37. Palanivel R, Thangavel M, Selvendran K, Sakthisekaran D. Insulinomimetric effect of ammonium paratungstate on protein metabolism in streptozotocin induced diabetic rats. Biomed 2001; 21: 23-30.
38. Chalasani N, Aljadhey H, Kesterson J, Murray M.D, Hall S.D. Patients with elevated liver enzymes are not act high risk for station hepatotoxicity, Gastroenterology 2004; 126: 1287-1292.
39. Stanely P, Prince M, Menon V. Hypoglycemic and other related actions of *Tinospora cordifolia* roots in alloxan induced diabetic rats. J Ethnopharmacol 1999; 70: 9-15.
40. Preethi KC, Kuttan R. Hepato and reno protective action of *Calendula officinalis* L. flower extract. Ind J Exp Biol 2009; 47: 163-168.
41. Mironova MA, Klein RL, Virella GL, Lopes-Virella MF. Anti-modified LDL antibodies, LD-containing immune complexes and susceptibility of LDL to *in vitro* oxidation in patients with type 2 diabetes. Diabetes 2000; 49: 1033-1049.
42. Agardh CD, Bjorgell P, Nilson EP. The effect of tolbutamide on lipoproteins and lipoproteinlipase and hormone sensitive lipase. Diabe Res Clin Pract 1999; 46: 99-108.
43. Cohn RM, Roth KS. Lipid and lipoprotein metabolism In: *Biochemistry and Diseases*, Williams and Willkins publishers, Baltimore: 1996. p. 280.
44. Venkateswaran S, Pari L, Saravanan G. Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in Streptozotocin-induced diabetic rats. J Med Food 2002; 5: 97-104.
45. Pari L, Satheesh A.M. Antidiabetic effect of *Boerhavia diffusa*: effect on serum and tissue lipids in experimental diabetes. J Med Food 2004; 7: 472-476.
46. Levy V, Zaltzberg H, Ben-Amotz A, Kanter Y, Aviram M. β-carotene affects antioxidant status in non-insulin dependent diabetes mellitus. Pathophysiol 1999; 6: 157-161.
47. Sathishsekar S, Subramanian S. Antioxidant properties of *Momordica charantia* (bitter gourd) seeds on streptozotocin induced diabetic rats. Asian Pac J Clin Nutri 2005; 14: 153-158.
48. Giugliano D, Ceriello A, Paolisso G. oxidative stress and diabetic vascular complications. Diabe Care 1996; 19: 257-267.
49. Mc Lennan SV, Heffernan S, Wright L *et al.* Changes in hepatic glutathione metabolism in diabetes. Diabetes 1991: 40: 344-348.
50. Inove M, Saito Y, Hirato E *et al*. Regulation of redox status of plasma proteins by mechanism and transport of glutathione and related compounds. J Protein Chem 1987; 36: 169-173.
51. Pattabiraman K, Muthukumaran P. Antidiabetic and antioxidant activity of Morinda tinctoria Roxb fruits extract in streptozotocin-induced diabetic rats. Asian J Pharm Tech 2011; 1: 34-39.

52. Oliver - Bever B. Medicinal plants in tropical West Africa, Cambridge University press, London. 1986; 245-267.
53. Rhemann AV, Zaman K. Medicinal plants with hypoglycemic activity, J Ethnopharmacol 1989; 26: 1-55.
54. Chakravarth BK, Gupta S, Gambir SS, Gode KD. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium* Roxb. Ind J Pharmacol 1980; 12: 123-127.
55. Geetha BS, Mathew BC, Augusti KT. Hypoglycemic effects of leucodelphinidin derivative isolated from Ficus bengalensis Linn. Ind J Physiol Pharmacol 1994; 38: 220-222.