Journal of Pharmaceutical Research Vol. 9, No. 3, July 2010 : 104-108.

POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *TYLOPHORA INDICA* LEAVES FOR ANTIDIABETIC PROPERTY IN RAT

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Received on : 29.10.2009 Revised : 28.05.10 Accepted : 07.06.10

ABSTRACT

The present study was carried out to ascertain the role of hydroalcoholic extract of *Tylophora indica* leaves for its possible antidiabetic potentials in streptozotocin (STZ) induced diabetic Wistar female rats. A single intraperitoneal injection of STZ (55 mg/kg) elevated the glucose level >230 mg/dl after 3 days. Oral administration of the hydroalcoholic extract of *Tylophora indica* leaves at 100 and 250 mg/kg resulted in significant reduction (P <0.001) in blood glucose level. Body weight was significantly reduced (P <0.001) in STZ induced diabetic rats when compared to normal rats, while in diabetic rats *Tylophora indica* leaves extract (P<0.001) prevented significantly the decrease in body weight in a dose dependant manner. Total cholesterol, triglyceride, LDL-c and HDL-c levels were altered in STZ induced diabetic rats, which were considerably restored to near normal in animals treated with *Tylophora indica* extract. The administration of hydroalcoholic extract of *Tylophora indica* resulted in significant (P<0.001) increase in the levels of superoxide dismutase, catalase, glutahione peroxidase, glutathione-S-transferase and reduced glutathione, thus resulting in reduced free radical formation in liver tissues of the diabetic rat. These observations demonstrated the dose dependent antidiabetic effect of hydroalcoholic extract of *Tylophora indica* leaves in STZ-induced diabetic rat.

Keywords: *Tylophora indica; Streptozotocin; Antidiabetic; Antioxidants.*

INTRODUCTION

Diabetes mellitus is a global health problem characterized by abnormal insulin secretion and derangement in carbohydrate and lipid metabolism[1,2]. The currently suggested mechanism underlying diabetes and diabetic complications is oxidative stress[3,4]. Enzymes such as superoxide dismutase, catalase, glutathione peroxidase, etc. are involved in this detoxification process[5,6]. Thus oxidative stress has been shown to have a role in the causation of diabetes type I and II and as such antioxidants may have role in the alleviation of diabetes and related problems [7-9].

Currently, there is growing interest in herbal remedies due to the side effects associated with the therapeutic agents (oral antidiabetic agents and insulin) for the treatment of diabetes mellitus[10]. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes[11]. The leaves of *Tylophora indica* are traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma[12], myocardial dysfunction[13] and hypertension[14, 15]. As the leaves of *Tylophora indica* is known to possess strong antioxidant potential, it was our interest to explore whether this herbal scavenger of free radical is able to combat the diabetic conditions in animals. Hence the present investigation was undertaken to demonstrate the protective effect of different doses of hydroalcoholic extract of *Tylophora indica* leaves (HETI) in streptozotocin (STZ) induced diabetic rat.

MATERIALS AND METHODS

Chemical

Streptozotocin (STZ) and glyburide were obtained from Sigma Chemical Co (St Louis, MO-USA). Bio-chemical kits and all other chemicals utilized were of analytical grade.

Plant extraction and selection of dose

The leaves of *Tylophora indica* was purchased from medicinal garden Danvantrivana, Bangalore University, in the month of June 2007. The plant material (Voucher Specimen No.-RRCBI 0691) was authenticated by Regional Research Institute (Ay.), Bangalore. The leaves were shade dried and powdered (moderately coarse). The extraction was carried out with 70% of methanol in soxhlet for about 72 hrs. The obtained syrupy mass was concentrated and dried in hot air oven. The oral dose was selected assuming that leaves are very safe at a dose of 5 g/kg, *p.o* as per limit tests of OECD guidelines[16], 1/50th and 1/20th of the safe dose corresponding to 100 mg/kg and 250 mg/kg were used for oral administration.

Animals

Inbred female Wistar rats weighing 190±10 g were used for the present investigation. The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) and water *ad libitum*. The

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experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines. Animals were housed in stainless steel cages and kept in a room where a 12 h light/dark cycle was maintained. Rats had free access to water and standard feed throughout the period of the experiment.

Induction of Diabetes in Rats

After one week of grouping, the rats were subjected to overnight fasting. Diabetes was induced with a single intraperitoneal injection of STZ at a dose of 55 mg/kg body weight. The STZ was freshly dissolved in citrate buffer (0.01M, pH 4.5)[17]. The injection volume was prepared to contain 1.0 ml/kg[18]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 3 days, blood glucose levels were measured and the animals with a glucose concentration of more than 230 g/dL were classified as diabetic [19] and taken for the experiment. Administration of the hydroalcoholic extract of *Tylophora indica* was started on 4th day after STZ injection and this was considered to be the 1st day of treatment, which was continued for 15 days.

Experimental Design

Thirty female Wistar rats were used in this study. The rats were randomized and divided into five groups of six animals each. Group I: Control rats, received citrate buffer (0.01M, H 4.5); Group II: Diabetic controls, received STZ (55 mg/kg body weight, i.p.) once; Group III, IV and V: Diabetic rats, receiving 100 mg/kg of hydroalcoholic extract of *Tylophora indica,* 250 mg/kg of hydroalcoholic extract of *Tylophora indica* and 5 mg/kg of Glyburide orally, respectively, for 15 days.

At the end of the experiment, all the rats were decapitated after fasting for 16 hours. Blood was collected without anticoagulant to separate serum for various biochemical estimations. The liver was dissected out and cleared off blood. This was immediately transferred to ice-cold containers containing 0.9% NaCl and homogenized in 0.1N Tris-HCl buffer (pH 7.4). The tissue homogenates were used for estimating the following: reduced glutathione (GSH) by the method of Ellman [20]. Protein was estimated by the method of Lowry et al [21]. The activity of superoxide dismutase (SOD) was assayed by the method of Misra et al [22]. The activity of glutathione peroxidase (GPx) was assayed according to the method of Rotruck et al [23]. Catalase (CAT) activity was assayed by the method of Sinha [24]. Glutathione-S-transferase (GST) was estimated by the method of Habig et al [25].

Measurement of body weight & blood glucose level

The body weight and blood glucose level were measured at every 5 days interval. Blood samples were obtained by tail vein puncture of both the normal and STZ induced diabetic rats. Blood glucose level was measured by single touch glucometer.

Serum Total Cholesterol

Serum total cholesterol (TC) was quantified by specrophotometric method [26] by the addition of enzyme present in reagent kit. The absorbance of red quinoneimine complex was determined at 505 nm. The value of TC present in serum was expressed in mg/dL.

Serum Lipoprotein Cholesterol

Serum LDL-c was measured according to protocol of Friendswald et al[27]. Serum HDL-c was measured y the method of Waenic et al[28].

Serum Triglyceride

Serum triglyceride was measured by using kit. The absorbance was noted at 520 nm. The value was expressed in the unit of mg/dL [29].

Statistical analysis

Statistical evaluation of data was performed by using One-way analysis of variance (ANOVA) followed by Dunnet's t-test [30]. *P*-values < 0.05 were considered as significant.

RESULTS

Blood Glucose Level and Body Weight Changes

A significant (*P*<0.001) fall in body weight of diabetic rats was seen by fifth day of the experimental period compared to normal control. With treatment of animals with HETI 100 and 250 mg/kg as well as glyburide, significant (*P*<0.001) recovery was observed in body weight by 15th day of the experiment compared to diabetic control. High dose of HETI (250 mg/kg) and glyburide were found to be equally effective in reversing the changes in the body weight. The results were shown in (Table 1).

Table 1. *Effect of hydroalcoholic extract of Tylophora indica on body weight in STZ induced diabetic rats.*

Treatment	Body weight (g)			
	Initial	5th day	10th day	15th day
Normal control	173.33 ± 2.2	181.33 ± 1.8	182.22 ± 1.3	183.13 ± 4.5
Diabetic control	174.54 ± 2.1	139.11 ± 3.1***	134.32 ± 2.3***	124.24 ± 3.2***
Diabetic + 100 mg/kg HETI	175.22 ± 3.5	151.33 ± 3.4***a	159.21 ± 4.2***	165.44 ± 3.3*
Diabetic + 250 mg/kg HETI	175.11 ± 2.8	156.22 ± 2.2***a	168.31 ± 5.1*aa	177.19 ± 8.5 aaa
Diabetic + 5mg/kg glyburide	178.18 ± 3.1	162.13 ± 1.3*a	169.24 ± 1.2*aa	175.33 ± 4.3 aaa

*All values are mean ± SEM, n=6; *P<0.05, **P<0.01, ***P< 0.001 when compared to Normal control; aP<0.05, aaP<0.01, aaaP< 0.001 when compared to diabetic control.*

The results of blood glucose level changes in normal, STZ induced diabetic rats and HETI/glyburide treated diabetic rats were shown in (Table 2). There was significant (*P*<0.001) increase in blood glucose levels in STZ induced diabetic rats when compared with normal control. Administration of HETI at a dose of 100 and 250 mg/kg significantly (*P*<0.001) decreased blood glucose level in STZ induced diabetic rats. The results are found to be in a dose dependent manner, comparable with that of standard glyburide.

Table 2. *Effect of hydroalcoholic extract of Tylophora indica on blood glucose levels in STZ induced diabetic rats.*

Treatment	Blood glucose level (mg/dL)				
	Initial day	1st day	5th day	10th day	15th day
Normal control	83.11 ± 3.1	84.22 ± 1.4	85.21 ± 1.4	85.21 ± 1.2	85.32 ± 2.8
Diabetic control	87.21 ± 1.3	231.21 ± 2.4***	246.21 ± 7.2***	264.21 ± 1.2***	271.53 ± 3.4***
Diabetic + 100 mg/kg HETI	85.21 ± 1.3	233.21 ± 3.2***	196.13 ± 3.1***^{aaa}	162.13 ± 4.2***^{aaa}	134.62 ± 3.3***^{aaa}
Diabetic + 250 mg/kg HETI	85.22 ± 1.3	234.33 ± 3.1***	176.11 ± 3.3***^{aaa}	137.32 ± 1.8***^{aaa}	116.46 ± 2.1***^{aaa}
Diabetic + 5mg/kg glyburide	84.22 ± 3.2	232.24 ± 3.1***	151.19 ± 4.2***^{aaa}	125.33 ± 5.4***^{aaa}	108.38 ± 3.1*^{aaa}

*All values are mean ± SEM, n=6; *P<0.05, **P<0.01, ***P< 0.001 when compared to Normal control; ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P< 0.001 when compared to diabetic control.*

Serum Lipids

The changes in the level of serum lipids on 15th day in control and experimental rats are illustrated in (Table 3). The total-cholesterol, LDL-Cholesterol and triglyceride significantly increased and HDL-Cholesterol significantly decreased in STZ induced diabetic rats (*P*<0.001) when compared with the normal rats. The HETI (100 mg/kg and 250 mg/kg) offered significant protection against alteration in the serum lipids of diabetic rats. The results are also dose dependent. The results are comparable with that of standard glyburide.

Table 3. Effect of hydroalcoholic extract of *Tylophora indica* on serum lipid profile in STZ induced diabetic rats.

Treatment	Serum total cholesterol (mg/dL)	Serum HDL (mg/dL)	Serum LDL (mg/dL)	Serum Triglyceride (mg/dL)
Normal control	118.22 ± 1.8	43.21 ± 1.1	56.43 ± 2.2	69.93 ± 3.1
Diabetic control	211.33 ± 2.2***	29.81 ± 1.3***	111.21 ± 3.4***	176.21± 3.2***
Diabetic + 100 mg/kg HETI	143.98 ± 3.1*^{a}	35.33 ± 2.4*^{aaa}	73.22 ± 1.2*^{aaa}	121.16 ± 3.5***^{aa}
Diabetic + 250 mg/kg HETI	130.32 ± 3.5^{aaa}	42.11 ± 3.5^{aaa}	59.11 ± 2.8^{aaa}	72.11 ± 5.7***^{aaa}
Diabetic + 5mg/kg glyburide	125.22 ± 3.6^{aaa}	43.31 ± 1.5^{aaa}	56.21 ± 6.2^{aaa}	75 11 ± 3.2***^{aaa}

*All values are mean ± SEM, n=6; *P<0.05, **P<0.01, ***P< 0.001 when compared to Normal control; ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P< 0.001 when compared to diabetic control.*

Antioxidant enzymes

A significant decrease (*P*<0.001) in the activities of antioxidant enzymes such as SOD and CAT were observed in the liver of STZ induced diabetic rats when compared with that of normal rats. Upon administration of 100 and 250 mg/kg body weight of HETI, the activities of both SOD and CAT were significantly reversed to near normal. The levels of Reduced glutathione, Glutathione peroxidase, Glutathione-S-transferase were significantly depleted in STZ induced diabetic rats. Treatment with HETI significantly increased the levels of these antioxidant enzymes in diabetic rats. The results are shown in (Table 4).

Table 4. Effect of hydroalcoholic extract of *Tylophora indica* on antioxidant enzymes in STZ induced diabetic rats.

Treatment	SOD	CAT	GPx	GSH-Rase	GST	TBARS
Normal control	6.22 ± 0.12	34.32 ± 2.2	6.76 ± 0.43	0.88± 0.07	8.44 ± 0.71	0.85 ± 0.14

*All values are mean ± SEM, n=6; *P<0.05, **P<0.01, ***P< 0.001 when compared to Normal control; ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P< 0.001 when compared to diabetic control.*

DISCUSSION

The research envisaged was designed to evaluate antidiabetic property of hydroalcoholic extract of *Tylophora indica* leaves (HETI) in STZ induced diabetic rats by virtue of their antioxidant potential. The result of the study demonstrated the benefits of HETI on par with standard hypoglycemic drug by scavenging oxidative free radicals.

Streptozotocin (STZ) is toxic to â-cells of pancreas and widely used for induction of experimental diabetes mellitus in animals, resulting in the generation of reactive oxygen species[31]. STZ causes a significant increase in the level of blood glucose in animals. The ameliorative potential of hydroalcoholic extract of *Tylophora indica* significantly decreased the blood glucose level in these animals suggesting that it has antidiabetic properties. The decrease in body weight in diabetic rats is due to excessive breakdown of tissue proteins [32]. Treatment with *Tylophora indica* improved body weight significantly in a dose dependent manner, indicating prevention of muscle wasting due to hyperglycemic condition.

Diabetes is also known to be associated with an increase in the synthesis of cholesterol, which may be due to the increased activity of HMG CoA reductase [33]. Increased LDL-cholesterol may arise from glycosylation of the lysyl residues of apoprotein B as well as from decreasing affinity for the LDL receptor and hence, decreased metabolism [34]. A number of observations indicate that plasma HDL cholesterol is low in untreated insulin-deficient diabetics [35], which was associated with a decline in HDL-turnover rate. Further the HDL-cholesterol levels correlate with lipoprotein lipase (LPL) levels in IDDM patients [36]. Hyper-triglyceridemia is a common finding in patients with diabetes mellitus and is responsible for vascular complications [37]. It has been reported that deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes[38]. However, oral administration of hydroalcoholic extract of *Tylophora indica* exhibited hypocholesterolemic and hypotriglyceridemic effects while at the same time increasing HDL-c, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different issues.

The decreased activities of SOD and CAT in liver during diabetes may be due to increased production of reactive oxygen radicals that can themselves reduce the activity of these enzymes [39]. SOD is an important defense enzyme, which converts superoxide radicals to hydrogen peroxide [40]. Increase in SOD activity could be due to its induction by increased production of superoxide, which has been implicated in cell dysfunction. Hydrogen peroxide has been reported to act as an inducer of tissue SOD [41]. CAT is a hemeprotein, which decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [42]. Any compound, natural or synthetic, with antioxidant properties, might contribute towards the partial or total alleviation of this damage[43]. The result of SOD and CAT activity clearly shows that the *Tylophora indica* contains a free radical-scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of hydrogen peroxide and hydroxyl radicals.

Reduced glutathione (GSH) is major endogenous antioxidant which counterbalances free radical mediated damage and it is well known that GSH is involved in the protection of normal cells structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions [44]. GSH is also known to protect the cellular system against the toxic effects of lipid peroxidation [45]. The decrease in GSH level in liver during diabetes is probably due to its increased utilization by the hepatic cells that could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes [46]. A relative depletion of NADPH due to aldose reductase activation and secondary to reduced production through the pentose cycle impairs GSH regeneration and leads to depletion of this free radical scavenger [47]. GSH reacts with free radicals and is a crucial substrate for GPx and GST, which takes part in the cellular defense mechanisms against intermediate oxygenated products of metabolism [48]. GPx metabolizes hydrogen peroxide to water by using GSH as a hydrogen donor [49]. The activities of GPx and GST were observed to decrease significantly in diabetic rats. Treatment with *Tylophora indica* significantly recovered he GSH content and increases in the levels of GSH dependent enzymes GPx, GST, which clearly indicates the protective effect of *Tylophora indica* as antioxidants.

Many of the complications of diabetes including retinopathy and atherosclerotic vascular disease, the leading cause of mortality in diabetes have been associated with oxidative stress and antioxidants have been considered as combating source [50,51]. Flavonoids, tannins, anthocyanins, ß-carotene and other phenolic constituents present in leaves of plant origin are potential antioxidants and may play a beneficial role in the prevention of several chronic disorders [52,53]. Thus the regimen of *Tylophora indica* may be considered as a potential source of natural antioxidant and antihyperglycemic activity that helps in reducing oxidative stress in diabetic condition, which may have beneficial role in the management of diabetes.

Acknowledgement

The authors are grateful to Prof. Suresh Nagpal, Chairman, Krupanidhi Educational Trust and Dr. Amit Kumar Das, Principal, Krupanidhi College of Pharmacy for providing facilities to carry out the work.

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