ANTIHYPERGLYCEMIC ACTIVITY OF *Exacum wightianum* Arn. AN ENDEMIC MEDICINAL PLANT.

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ABSTRACT

An attempt was made to study the beneficial effects of ethanol extract of *Exacum wightianum* Arn. (Gentianaceae) on its antihyperglycemic activity in streptozotocin induced diabetic rats. The pilot studies were carried after oral administration at doses of ethanolic extract of *E. wightianum* 100, 200, 500 and 1000 mg/kg b.wt. in sub-acute study. In diabetic induced rats fed with *E. wightianum* ethanol extract at 100 and 200 mg/kg body b.wt., the fasting plasma glucose levels were reduced to normal body and liver weight were found to be increased. Where as blood glucose, protein, albumin and creatinine levels were estimated after two weeks. The extract significantly inhibited the induction of albuminuria, proteinemia and uremia. The present study clearly indicated a significant antidiabetic activity with the ethanol extract of *E. wightianum* supports the traditional usage of the plant by Ayurvedic physicians for the control of diabetes. Also the extract is useful in preventing the incidence of long term complications of diabetes mellitus.

Keywords: Albuminuria, *Exacum wightianum*, Proteinemia, Streptozotocin and Uremia.

INTRODUCTION

Diabetes mellitus (DM) currently is a chronic metabolic disorder/syndrome resulting from a variable interaction of hereditary and environmental factors. It is characterized by abnormal insulin secretion or insulin receptor or post receptor events affecting metabolism involving carbohydrates, proteins and fats in addition to damaging liver, kidney and β-cells of pancreas[1]. The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000[2].This chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries[3]. Generally, it may be delayed, lessened or prevented by maintaining blood glucose values close to normal by adhering strict intake of food items.

In modern medicine, no satisfactory effective therapy is still available to cure the diabetes mellitus. Now-a-days insulin therapy is encouraged for the management of diabetes mellitus, but there are several drawbacks like insulin resistance [4], anorexia nervosa, brain atrophy and fatty liver [5] after chronic treatment. Recently, there has been increasing interest in the use of medicinal plants in modern medicine in the world to prevent or to cure diseases and scientific evidence in terms of modern medicine is also lacking in most cases. However, today, it is necessary to provide scientific proof to justify the use of plant or its active principles [6].

The herb *Exacum wightianum* Arn. belongs to the family Gentianaceae. It has a long history of use in Ayurvedic medicine (the traditional medicine of India). Since time immemorial, many traditional plant treatments are available for diabetes. It is extensively used as bitter tonic and febrifuge in the Ayurvedic system of medicine [7]. The extract has long been used in folk medicine for the treatment of hepatitis, cholecystitis, pneumonia, malaria, dysentery and spasm; whereas the recent investigations have shown that some xanthones possess a marked hypoglycemic activity when administered to rats. In the present investigation the effect of ethanolic extract of *E. wightianum* were studied on streptozotocin- induced diabetic male wister albino rats.

MATERIALS AND METHODS

Collection of plant material

All aerial parts and roots of *Exacum wightianum* (Gentianaceae) was collected during blooming season August, 2010 from Naduvattam, Uthagamamdalam, the Nilgiri Hills, Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist, Mr. M. Murugesan, Scientist, SACON, Coimbatore.

Preparation of extract

500 g of shade dried and pulverized whole plant powder of *E. wightianum* was defatted with petroleum ether and the residue was then re-extracted with ethanol by using soxhlet apparatus. This extract was stored at 4ºC and used for further studies.

Experimental Animal

Male Wistar Albino rats weighing 180-250 g were obtained from Agricultural University, Animal house lab, Trissur, Kerala. All the animals were maintained in polycarbonated cages in an animal room with 12 h light/12 h dark cycle at temperature of 22 ±2ºC and humidity of 45-60%. They were fed with commercial pelleted rats chow and free access water during the entire period of experiment. The experiments were performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA).

Induction of experimental diabetes

Diabetes was induced by administering intraperitonial injection of a freshly prepared solution of Streptozotocin monohydrate dissolved in 0.1 M cold citrate buffer (pH 4.5) to the overnight fasted rats [8] and the STZ solution is made cold using cold citrate buffer immediately before administration. The drug induced hypoglycemia in all rats was controlled by treating 5% glucose solution. The blood glucose above 250 mg/dl on the third day after injection was considered as diabetic rats. At this time the treatment was started on the fifth day after injection and considered as first day of treatment.

Experimental design

The streptozotocin induced rats were divided into five groups each contains five numbers of rats. Group I served as normal rats without any treatment, Group II served as diabetic control rats. Group III diabetic rats given glibenclamide (600 µg/kg b.wt.).Group IV served as diabetic rats given ethanolic extract of *E. wightianum* (100 mg/kg b.wt.).Group V served as diabetic rats given ethanolic extract of *E. wightianum* (200 mg/kg b.wt.). At the end of the experiment all the animals were deprived of food overnight, anesthetized and sacrificed by cervical dislocation. Blood was collected in heparinised tubes and used for the further estimation of biochemical studies.

Toxicity study

E. wightianum ethanolic extract was orally administrated at a concentration of 250,500,750 and 1000 mg/kg body weight/ day for a period of 14 days. The toxic effects were measured by body weight and morphological changes.

Estimation of Insulin, Blood glucose, Urea and Creatinine

The blood glucose level was estimated by the method of O-toludine by Sasaki [9], Insulin was estimated by radio immuno assay kit purchased from stat Diagnostics, Mumbai, India [10]. Urea level was assayed according to the method of Varley [11] and Creatinine level was estimated by Owen et al., [12].

Statistical analysis

All data were expressed as means ± S.E. Significant differences among the groups were determined by one-way analysis of variance using the DMRT statistical analysis program. Statistical significance was considered at $p<0.05$.

RESULTS

The ethanol extract of *E. wightianum* was administrated orally to rats at the doses of 100, 200, 500 and 1000 mg/kg b.wt. and the mean death rats were observed. The results showed that numbers of deaths of rats were observed at the different dose levels. There was no morphological change like respiratory distress, hair loss, restlessness, convulsions, laxative, coma, weight loss etc. there was no lethality or any toxic reactions found at any of the doses selected till the end of treatment period (Table1).

Table 2 shows the effect of *E. wightianum* extract on body weight and organ weight in normal and streptozotocin induced diabetic rats. A significant weight loss was observed in the diabetic control group (Group II). The body weight and organ weight were increased in the *E. wightianum* extract treated groups IV and V at two concentrations 100 and 200 mg/kg b.wt. A significant improvement was observed in the group III treated with the standard drug, glibenclamide (Fig.1 a, b).

The table 3 shows the effects of *E. wightianum* ethanol extract administered on streptozotocin induced diabetes for 14 days drug treatment. *E. wightianum* ethanol extract at the doses of 100 and 200 mg/kg b.wt. treatment significantly decreased the blood glucose level in streptozotocin induced diabetes rats (Group II). There was a significant elevation in blood glucose level with significant decrease in serum insulin levels in streptozotocin diabetic rats, compared with normal rats. Administration of *E. wightianum* extracts 100 and 200 mg/kg b.wt. and glibenclamide treated group III bring blood glucose and serum insulin towards normal levels. The effect of *E. wightianum* extracts 200 mg/kg b.wt. was significantly better than 100 mg/kg b.wt. The administration of *E. wightianum* extract and glibemclamide showed a significant effect in lowering blood glucose and increasing serum insulin. In the diabetic control group II, the levels of serum creatinine was found to be increased in comparison with control. Treatment with *E. wightianum* extract significantly prevented the streptozotocin induced creatinine level. The diabetic rats administrated with *E. wightianum* extract at 100 and

200 mg/Kg b.wt. and glibenclamide at the dose of 600µg/Kg b.wt., daily orally for 14 days consequently orally by 1GC altered the values of insulin, glucose and creatinine when compare to control. The levels of the urea in streptozotocin diabetic rats were significantly higher than the control. When these diabetic rats treated with two concentrations of extracts (100 and 200 mg/kg b.wt.) decreased the levels when compare to group II (Fig.2-5).

DISCUSSION

Streptozotocin induces "chemical diabetes" in a wide variety of animal species by damaging the insulin secreting pancreatic β -cell, resulting in a decrease in endogenous insulin release [13, 14]. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in streptozotocin-induced diabetic animals [15, 16]. In the present study, the ethanol extract of *E. wightianum* effectively decreased the blood glucose in streptozotocin-induced diabetic rats, which is even better than glibenclamide.

The results of the present study indicate that *E. wightianum* extract brought back the body weight, liver weight, glucose, insulin, protein and antioxidant. Aqueous extract of *Punica granatum* has brought decreased body weight of diabetic rats to normal [17]. The ability of this extract to prevent the body weight loss seems to be due to its anti-diabetic activity. Prakasam *et al.*,[18] have reported that a reduction in body weight was observed in STZ diabetic animals, but when animals were treated with *Casearia esculenta* root extracts, the decrease in the body weight was minimized to almost nil. A significant weight loss was observed in allaxon induced diabetic rats than normal rats, when treated with aqueous extract of *Laportea ovalifolia* and Tolbultamide in streptozotocin induced diabetic rats. The body weight was improved when compared with the untreated diabetic rats [19]. Valentao et al.,[20] have reported that *Hypericum androsacmum* infusion restored the hepatic disorder induced by t-BHP.

Ethanolic extract of *E. wightianum* was found to be inducing insulin release from pancreatic cells of diabetic rats resulted in hypoglycemic activity. Ahmed et al.,[21] have fed ethanol extract of *Pterocarpus marsupium*, which significantly lowered blood sugar level with corresponding increase in blood insulin level in streptozotocin induced diabetic rats. It is evident from this study that there was an increase in insulin level in diabetic rats treated with *E. wightianum* extract. Many plants have been investigated for their hypoglycemic and insulin release stimulatory effects (Al-hader et al., 1994). Hypoglycemic effect of *Ziziphus jujuba* on normoglycemic rats was reported by Aydin et al.,[23].This study substantiates the anti-diabetic effects of *Z. jujuba* which is comparable to that of glibenclamide. The administration of ethanol extract of rhizome of *Nelumbo nucifera* markedly reduced the blood sugar level of diabetic induced rats [24].Generally streptozotocin has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of streptozotocin-induced free radical damage [25].

The administration of Glibenclamide also decreased the levels of urea and creatinine to some extent [26]. The changes in urine composition that follow the consumption of *Hibiscus sabdariffa* extracts at different concentrations and for various periods of time resulted in significant decrease in the urinary concentration of creatinine, uric acid and potassium[27]. Stabilization of serum creatinine and urea levels through administration of the extract *E. wightianum* is further a clear indication of the improvement of the functional status of the liver cells.

Our results thus clearly demonstrated that the ethanolic extract of *E. wightianum* has potent antihyperglycemic in STZ induced diabetic rats. Further studies are warranted to isolate and characterize the bioactive antidiabetic principles from this plant, which can therefore be used as an alternative remedy for the treatment of diabetes mellitus and oxidative stress associated diabetic complications.

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Table1. Toxicity studies with *E. wightianum* extract by the method of Karbar adapted by Aliu and Nwude (1982).

Group (Dose)*	**No of Rats**	**Death**	**Dose difference**	**Mean death**	**Dose different X Death**
Group I (Normal saline)	5	0	0	0	0
Group II (100mg/kg BW)	5	0	100	NM	NM
Group III (200 mg/kg BW)	5	0	200	NM	NM
Group IV (500 mg/kg BW)	5	0	500	NM	NM
Group V (1000 mg/kg BW)	5	0	1000	NM	NM

* All treatment given one dose only; NM- No Mortality
Group I : Control rats given normal saline orally by using an intragastric catheter tube (IGC).
Group II : Diabetic rats given *E. wightianum* drug at the dose of 100 mg/ Kg body weight orally by IGC
Group III : Diabetic rats given *E. wightianum* drug at the dose of 200 mg/ Kg body weight , orally by IGC
Group IV: Diabetic rats given *E. wightianum* drug at the dose of 500 mg/ Kg body weight orally by IGC
Group V: Diabetic rats given *E. wightianum* drug at the dose of 1000 mg/ Kg body weight orally by IGC

Table 2. Effect of treatment for 14 days with extract of *E. wightianum* on body and liver weight of normal, diabatic induced and drug treated adult albino rats.

Parameter	Body weight (gm)	Liver weight (gm)
Group I	207 ± 8.21	7.56 ± 0.15
Group II	158.53 ± 6.50	4.52 ± 0.31
Group III	190.71± 11.63	6.73 ± 0.41
Group IV	192.13 ± 10.92	7.13 ± 0.53
Group V	197.53 ± 10.47	5.76 ± 0.39

Each Value is * SEM of 5 animals * P < 0.05
Group I: Rats given only saline (by using an intragastric catheter tube (IGC).
Group II: Streptozotocin induced diabetic rats (drug at the dose of 200 mg/ Kg b.wt.)
Group III: Streptozotocin induced diabetic rats treated with glibenclamide at the dose of 60 mg/ Kg b.wt.
Group IV: Streptozotocin induced diabetic rats treated with crude plant extract of *E.wightianum* at the dose of 100 mg/ Kg b.wt. orally for 14 days.
Group V: Streptozotocin induced Diabetic rats treated with crude plant extract of *E.wightianum* at the dose of 200 mg/ Kg b.wt. orally for 14 days.

Table 3: Effect of treatment for 14 days with extract of *E.wightianum* on the insulin, blood glucose, urea, creatinine levels of normal, diabatic induced and drug treated adult albino rats

Parameter	Insulin (MIu/ml)	Bloodglucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Group I	19.41±1.41	94.21 ± 5.10	15.31±1.3	0.51±0.01
Group II	07.41±0.09	272.11± 1.42	41.61±2.4	1.73±0.07
Group III	10.47±1.08	110.34 ± 8.3	21.02±1.9	0.95±0.02
Group IV	13.63±1.03	105.41 ± 6.4	16.63±1.6	0.74±0.5
Group V	18.32±1.45	95.32 ± 9.4	18.31±1.7	0.69±0.4

Each Value is * SEM of 5 animals * P < 0.05
Group I: Rats given only saline (by using an intragastric catheter tube (IGC).
Group II: Streptozotocin induced diabetic rats (drug at the dose of 200 mg/ Kg body weight)
Group III: : Streptozotocin induced diabetic rats treated with glibenclamide at the dose of 60 mg/ Kg b.wt.
Group IV: Streptozotocin induced diabetic rats treated with crude plant extract of *E.wightianum* at the dose of 100 mg/ Kg b.wt. orally for 14 days.
Group V: Streptozotocin induced diabetic rats treated with crude plant extract of *E.wightianum* at the dose of 200 mg/ Kg b.wt. orally for 14 days.

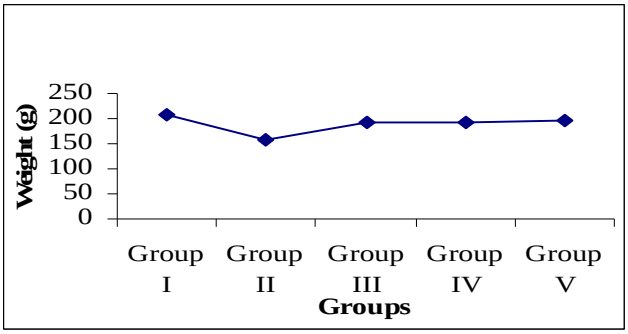

Fig. 1a. Effect of *E.wightianum* on the body weight of normal, diabetic and drug treated rats.

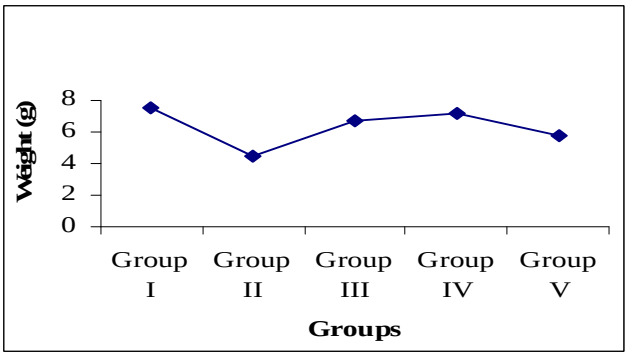

Fig. 1b. Effect of *E.wightianum* on the liver weight of normal, diabetic and drug treated rats.

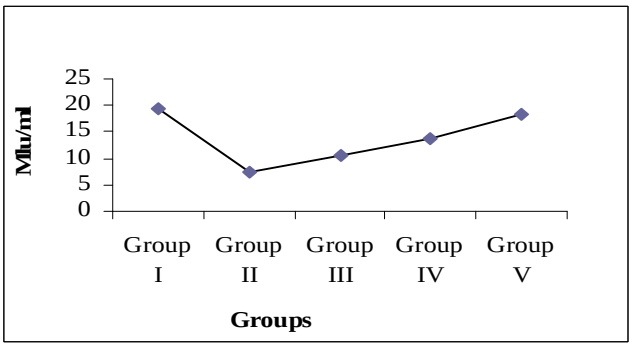

Fig. 2. Effect of *E.wightianum* extracts on the serum insulin level of normal, diabetic and drug treated rats.

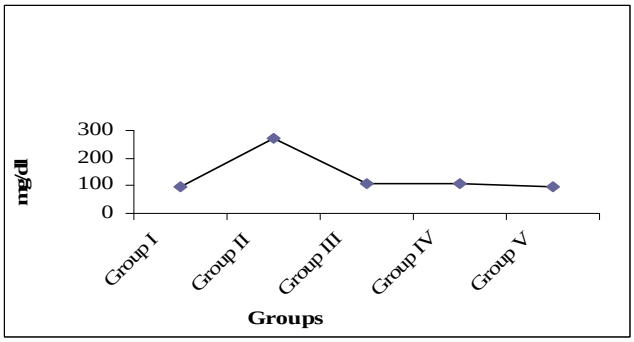

Fig. 3. Effect of *E.wightianum* extracts on the serum blood glucose level of normal, diabetic and drug treated rats.

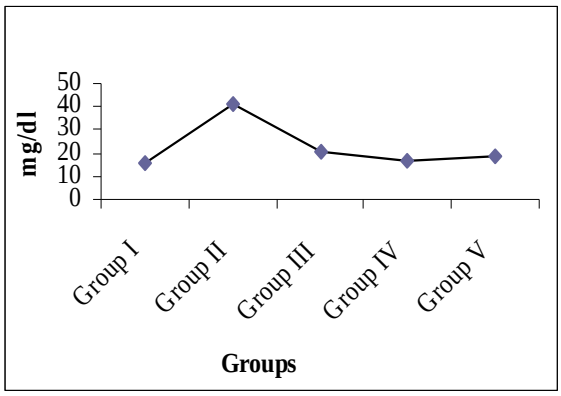

Fig. 4. Effect of *E.wightianum* extracts on the urea level of normal, diabetic and drug treated rats.

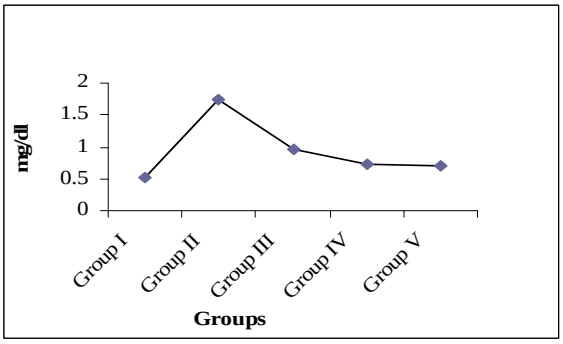

Fig. 5. Effect of *E.wightianum* extracts on the serum Creatinine level of normal, diabetic and drug treated rats.