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Evaluation of Protective Effects of *Euphorbia thymifolia* Linn against Streptozotocin induced Diabetic Neuropathy in Rats

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ABSTRACT

The term Diabetes mellitus describes a metabolic disorder of multiple etiologies and is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Neuropathy, a common complication of diabetes mellitus, is generally considered to be related to duration and severity of hyperglycemia. An effective and primary aim in the treatment of diabetic neuropathy is to control the blood glucose level to delay the progression of neuropathy and then to control naturopathic pain. In present investigation *Euphorbia thymifolia* Linn methanolic extract was studied for diabetic neuropathy and results shows a significant activity at 400mg/kg dose of ETME in streptozotacin induced diabetic neuropathy with P>0.01.

Keywords: *Euphorbia thymifolia* Linn., Diabetic neuropathy, Streptozotocin

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INTRODUCTION

Diabetic neuropathy has been defined as presence of symptoms and signs of peripheral nerve dysfunction due to diabetes. Neuropathy, a common complication of diabetes mellitus, is generally considered to be related to duration and severity of hyperglycemia [1,2]. Usually more than 50% of patients with duration of diabetes of 25 years or more are affected, making it as one of the most common disease of the nervous system [3]. It has been estimated that the prevalence of diabetes for all age groups worldwide was 2.8% (171 million) in the year 2000. The urban population in developing countries is projected to double between 2000 and 2030 [4]. The prevalence of diabetes has increased steadily throughout the past several decades. The Centers for Disease Control and Prevention estimated that the prevalence of known diabetes in people > 18 years of age has increased from 5.1% in 1997 to 10.1% in 2009 [5].

An effective and primary aim in the treatment of diabetic neuropathy is to control the blood glucose level to delay the progression of neuropathy and then to control neuropathic pain. Several tri-cyclic antidepressants (TCA's) are used in the treatment of diabetic neuropathic pain, but they have some serious side effects. Currently only symptomatic treatment for painful diabetic neuropathy is available but there is no ideal drug, which can cure diabetic neuropathy [6,7]. Drugs with antioxidant property may be useful to treat Diabetic neuropathies (DNs). *Euphorbia thymifolia* Linn. belonging to family Euphorbiaceae, commonly known as Choti Dudhi is an annual herb. The plant has medicinal value like stimulant, laxative, antihelmanthic, antibacterial, expectorant, blood purifier, diuretic and used in amenorhoea, dysmenorrhoea, helminthiasis, intestinal worms and in wound healing [8,9]. The plant also reported to have antimicrobial [10,11],antibacterial [12], antioxidant and antiviral [13], simplex virus-2infection [14], laxative [15] and diabetes [16].

Euphorbia thymifolia Linn is commonly found throughout the India in arid and semi-arid regions abundantly. It is commonly found in waste places, along roads side and in fallow fields. The essential oil of *Euphorbia thymifolia* which contain cymol, carvacrol, limonene, 2 sesqueterpines and salicylic acid is used in medicinal soap for the treatment of erysipelas and its spray is used as mosquito and flies repellent. Phytochemically *Euphorbia thymifolia* are reported to have tannins, steroids, alkaloids, flavonoids, triterpines, saponins and sugars. The stem and leaves contain 5, 7, 4-trihydroxy flavones- 7-glycoside [17,18,19].

MATERIALS AND METHODS

Plant material

The whole plants of *Euphorbia thymifolia* Linn. were collected from the crop fields and barren area from Sriganganagar district of Rajasthan. The plants of *Euphorbia thymifolia* Lin*n.* were collected in the month of July 2009 in the morning time. The plant was authenticated by the botanist in the Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (RUBL20664) has been kept in herbarium in Department of Botany, University of Rajasthan, Jaipur.

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Materials

Streptozotocin(sigma chem.)
Glmiperide (Dr.Reddy's)
Rota rod (Rolex)
Eddy's hot plate (Inco)
Glucometer (Horizontal one touch Jonson and Jonson).

Preparation for plant powder

The whole plants collected were washed with plain water and then with distilled water and were allowed to dry in the shade. Dried material was ground in a stainless steel grinder to powder. The powder was sieved through 40 mesh sieve. This powder was used for extraction purpose during the investigation [20, 21].

Preparation of plant extract

The whole plant was shade dried, powdered and subjected to successive solvent extractions with petroleum ether (40-60%), chloroform and methanol in soxhlet extractor and cold maceration of the successive marc in water.

The marc left after petroleum ether and methanol extraction was dried completely in hot-air oven below 50°C. The dried material was packed in a percolator. The aqueous extract was obtained by cold maceration of the successive marc in water. The marc was dip in distilled water for three days. The extract was then collected through tape of the percolator. The excess water was evaporated on water bath. The resultant extract was distilled in vacuum under reduced pressure in order to remove the aqueous solvent completely. It was dried and kept in a desiccators till experimentation.

Animals

Adult male or female Wistar albino rats weighing 150-200 g were used in the experiment. After procurement of the animals they were allowed to acclimatize to the environment, for a period of seven days. Thereafter, the animals were housed in individual ventilated cages at a room temperature of 22 ± 1°C in a 12 hour light/dark cycle. The experimental activities were performed during the light phase. Food and water were made available *ad libitum*. The experiment was reviewed and approved by the IAEC, Gyan Vihar School of Pharmacy Reg. No. 1234/a.08/ CPCSEA, Suresh Gyan Vihar University, Jaipur.

Experimental Procedure

Streptozotocin induced diabetes

Healthy Wistar strain albino rats of either sex weighing about 150-200 grams were taken. Animals were deprived to food for 16 hours but allowed free access to water after that blood sample was collected from tail of rats and measure blood glucose level by using digital display glucometer . Then they were injected with streptozotocin dissolved in 0.1M sodium citrate and citric acid at a dose of 60 mg/kg body weight intraperitoneally [22]. Then animals were kept for 21 days during which food and water was allowed. After 7 and 21days of streptozotocin administration blood glucose level, body weight, grip strength and pain sensation measurements were taken. The animals showed fasting blood glucose level above 250 mg/dl considered diabetic after that they were divided into seven groups in which each group contain six animals. After that the administered control, standard and test drug orally. The blood glucose level, body weight, grip strength and pain sensation measurements of each rat were taken weekly after oral administration of drug/extracts. The mean values of these parameters were taken for each group and compared with the value of standard drug.

Body weight : Diabetic animals show reduction in body weight hence body weight of all the animals measured every week till the completion of study [23].

Grip strength : Grip strength used for evaluation of muscle strength during Diabetic Neuropathy. The test was being used to assess muscular strength or neuromuscular function in rodents with the help of rota rod apparatus as per the procedure given by [24].

Pain threshold : The evaluation of pain threshold was done to evaluate sensory functions. The hot plate test was carried out according to the method of Eddy's *et al*. Animals were placed on the hot plate maintained at 55±1^{o}C and the reaction time was recorded as response latency. The response latencies were measured before treatment and after treatment. The cut off time for **hot plate** latency was set at 10 seconds (24).

Biochemical Estimations: The blood samples were collected from rat-tail vein on day before injecting the streptozotocin and on subsequent 7and 21 days after injection of streptozotocin for estimation of blood glucose level. The blood glucose estimation was done weekly after administration of test compounds, with the help of glucometer.

Statistical analysis

Statistical analysis was carried out as per standard method. All results were expressed as mean ± S.D. Groups of data were compared with the analysis of variance (ANOVA) followed by Dunnett's t-test values for statistical significance.P>0.01 was considered significant.

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RESULTS

Effect of Streptozotocin on body weight and blood glucose level

The body weight decreased rapidly in STZ treated diabetic rats and the blood glucose levels increased rapidly after STZ injection. The present study evaluates Diabetic Neuropathy by muscle grip strength and pain threshold ,body weight parameters over a period of fourteen days after treatment with plant extract.

Measurements of body weight of the rats of all experimental groups are shown in table 1 and figure 1. The body weight increased normally in control rats, while STZ induced diabetic rats (Diabetic control) showed a significant decrease in body weight as soon as one week post-STZ injection (Pre: 179.5 ± 5.54 to 158.83 ± 5.84, $P<0.01$). A progressive loss of body weight was noted after 21 days (pre: 179.5 ± 5.54 to 140± 7.74, $P<0.001$). The maximum decrease in body weight was observed after 5 weeks STZ-injection (pre: 179.5 ± 5.54 to 127.16 ± 7.08, $P<0.001$). The weight of the animals of other groups was also decreased significantly till day 21 as compared to control group (Table 1). On the day 21 the animals treated with Glimiperide drug (1 mg/kg), ETAE-200mg/kg, ETAE-400mg/kg, ETME-200mg/kg and ETME-400mg/kg were observed with significant increase in their body weight as compared to Diabetic control group of animals.

The blood glucose level of all experimental groups, except control group was increased significantly after the STZ injection till day 21 (Table 2 and Figure 2). On the day 28 and 35 of diabetes induction,. In the diabetic group (Diabetic control) the blood glucose levels increased to the maximum measurable value of 325.5 ± 6.56 mg/dl on day 35 and found to be significant increased ($P<0.001$) compared to the value of day1 was 82.66 ± 5.20 mg/dl. In contrast, control animals remained normoglycaemic during the entire testing period of 14 days (Table 2). The animals treated with glimiperide drug (1 mg/kg), ETAE-200mg/kg, ETAE-400mg/kg, ETME-200mg/kg and ETME-400mg/kg were observed with significant decrease ($P<0.001$) in blood glucose level compared to Diabetic control group on the day 28 and 35. The blood glucose level of Glimiperide treated group on the day 28 and 35 and ETME-400mg/kg on day 35 was found to be comparable with control group.

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Table 1: The effect of *Euphorbia thymifolia* on body weight of SZT-induced diabetic rats.

Groups	Change in body weight on different days				
	Day-1	Day-7	Day-21	Day-28	Day-35
Control	180.33±7.65	190.33±11.69	201.66±13.66	208.33±10.80	214.16±10.20
Diabetics control	179.5±5.54	158.83±5.84***	140.00±7.74***	134.50±7.84***	127.16±7.08***
Glimiperide drug(1 mg/kg)	178.16±7.27	155.83±7.35***	138.83±10.30***	147.00±7.74***	159.50±7.58***,a
ETAE-200	180.83±8.61	164.5±3.93***	141.66±11.69***	147.66±10.83***	156.66±10.87***,a
ETAE-400	179.16±9.70	157.5±5.00***	142.16±5.11***	151.33±4.54***	160.16±6.96***,a
ETME-200	178.83±3.76	163.33±8.75***	139.66±9.93***	148.00±9.69***	158.16±9.08***,a
ETME-400	183.5±6.89	159.16±6.64***	137.16±9.28***	147.83±9.06***	157.33±6.86***,a

Values are mean ± SD, n=6, where *** is P<0.001 when compared to control group, and a= P<0.001 when compared to diabetic control group

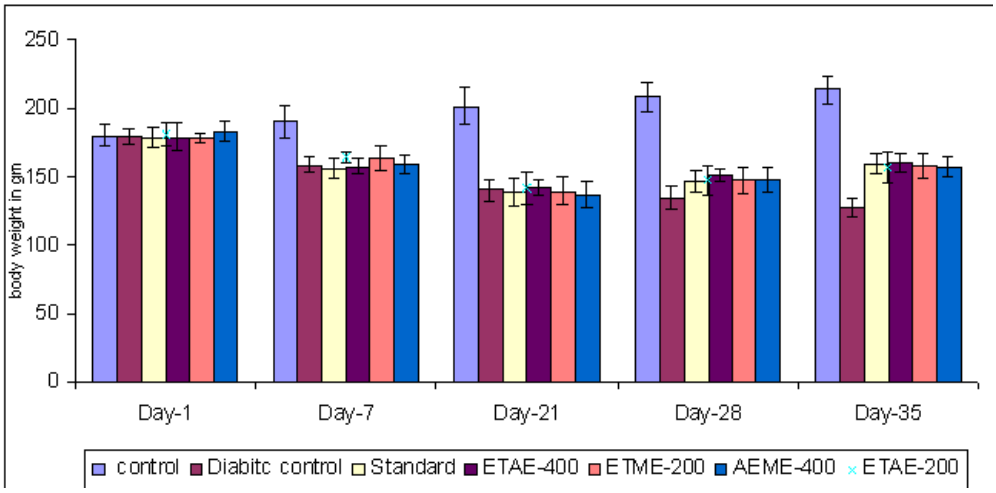

Fig. 1: The effect of *Euphorbia thymifolia* on body weight of SZT-induced diabetic rats.

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Table 2: The effect of *Euphorbia thymifolia* on Blood glucose level of SZT-induced diabetic rats.

Groups	Change in blood glucose levels on different days				
	Day-1	Day-7	Day-21	Day-28	Day-35
Control	83.33±5.50	80.16±4.35	82±6.13	82.16±3.18	83.83±7.98
Diabetic control	82.66±5.20	303.33±10.69***	312±6.32***	319.16±5.98***	325.5±6.56***
Glimiperide drug(1 mg/kg)	82.5±7.36	308±8.22***	316.66±8.38***	81.83±5.34b,a***	82±5.47b,a***
ETAE-200	82±5.96	308±13.74***	318.33±7.22***	133.66±17.96***,a***	129.16±8.08***,a***
ETAE-400	82±5.01	302.33±11.91***	313.5±6.65***	123.66±11.69***,a****	115.33±9.62***,a***
ETME-200	83.33±6.18	305.83±13.79***	318.16±9.19***	121±9.20***,a***	101.66±6.97**,a***
ETME-400	81.83±4.11	310.33±12.11***	319.66±10.96***	108.5±10.69***,a***	90±7.01b,a***,c

Values are mean ± SD, n=6, ** = P<0.01, *** = P<0.001 when compared to control group, b= ns when compared to control group, a*= P<0.001 when compared to diabetic control group, c= ns when compared to standard group.**

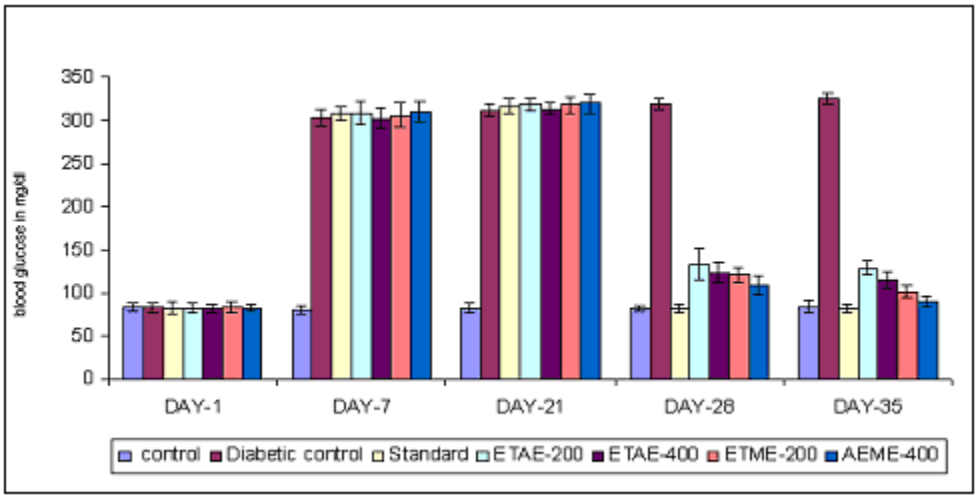

Fig. 2: The effect of *Euphorbia thymifolia* on Blood glucose level of SZT-induced diabetic rats.

Effect of Streptozotocin on muscle grip strength and on thermal sensation

Measurement of muscle grip strength was used to diagnose the diabetic neuropathy after 21 days of induction of STZ. The muscle grip strength reduced significantly in all STZ treated groups that showed the induction of diabetic neuropathy. In the normal control group the muscle grip strength was normal (28.50 ± 3.83 to 29.50 ± 2.42 min), so there was not a statistically significant difference found in control group (P<0.001) but in the diabetes induced group there was significant difference was found in the muscle grip strength (28.83 ± 3.76 to

2.83 ± 0.75 min, (P<0.001). The grip strength of Glimiperide control and all test group animals were increased significantly (P<0.001) compared to the Diabetic control group on the day 28 and 35 (Table 3 and Figure 3). The grip strength of glimiperide drug (1 mg/kg) and ETME-400mg/kg treated animals were found to be comparable with control group.

In rats, a single systemic injection of streptozotocin induced a hyperalgesic reaction observed twenty one days after the onset of diabetes as reported by Eddy's *et al*. In the present study, hyperalgesic reaction was evaluated for a period of twenty one days post-STZ treatment. The paw jumping response was measured by Eddy' hot plate. There were significant difference was found in paw jumping response after 21 days in the diabetes induce rates. There was no significant difference was found in the control group in which diabetes was not induced (2.33±0.51 to 2.83±0.75). In diabetic induce rats (Diabetic control) there was significant increase found in the paw jumping response (2.5±0.54 to 6.66±1.03). The paw jumping response of all standard and test groups on the day 28 and 35 were reduced significantly compared to the Diabetic control group (Table 4 and Figure 4). The paw jumping response of all the test groups and glimiperide drug (1 mg/kg) on day 28 and 35 were found to be comparable with Diabetic group and control group.

Table 3: The effect of *Euphorbia thymifolia* on muscle grip strength of SZT-induced diabetic rats

Groups	Change in muscle grip strength on different days				
	Day-1	Day-7	Day-21	Day-28	Day-35
Control	28.50±3.83	28.16±3.97	29.66±2.58	28.66±2.94	29.50±2.42
Diabetic control	28.83±3.76	18.66±2.58***	5.16±0.75***	3.66±0.51***	2.83±0.75***
Glimiperide Drug (1 mg/kg)	29.16±2.78	19.50±2.88***	5.50±1.04***	18.50±2.88***,a***	25.50±3.50b,a***
ETAE-200	30.00±4.14	18.50±3.27***	5.50±1.04***	8.16±1.94***,a**	11.33±1.75***,a***
ETAE-400	28.83±3.48	19.66±1.86***	5.50±0.83***	9.83±1.60***,a***	15.83±2.40***,a***
ETME-200	29.83±3.25	19.00±3.09***	5.66±1.03***	9.50±1.87***,a***	12.33±2.16***,a***
ETME-400	28.83±2.78	19.16±3.06***	5.33±1.03***	15.83±1.47***,a***,c	25.00±4.09b,a***,c

Values are mean ± SD, n=6, where ***= P<0.001 when compared to control group, b= ns when compared to control group, a***= P<0.001 when compared to diabetic control group, c= ns when compared to standard group.

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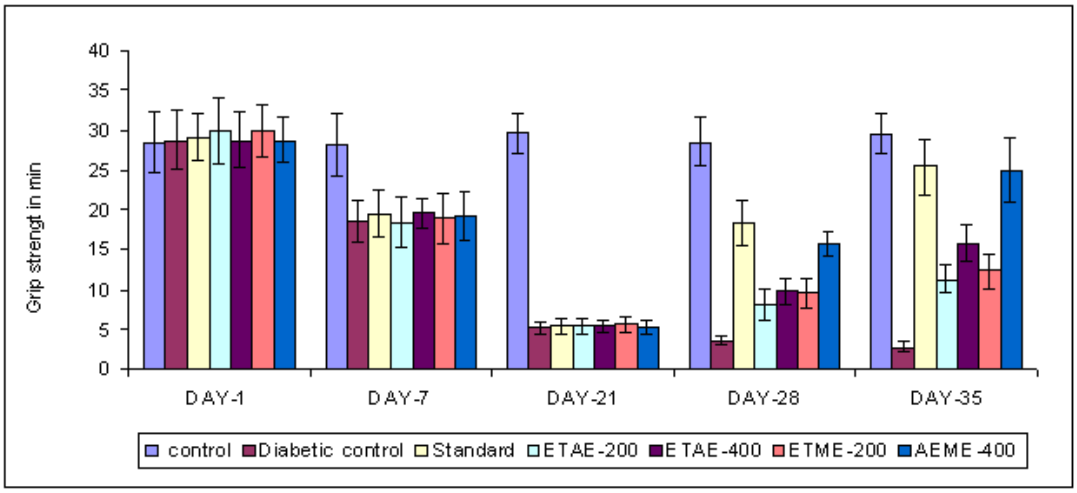

Figure 3: The effect of *Euphorbia thymifolia* on muscle grip strength of SZT-induced diabetic rats

Table 4: The effect of *Euphorbia thymifolia* on hot plate induced thermal pain in SZT-induced diabetic rats

Groups	Change in thermal pain sensation on different days				
	Day-1	Day-7	Day-21	Day-28	Day-35
Control	2.33±0.51	2.5±0.54	2.5±0.54	2.66±0.51	2.83±0.75
Diabetic control	2.5±0.54	3.5±0.54	5.66±1.03***	6.5±0.83***	6.66±1.03***
Glimiperide drug(1 mg/kg)	2.33±0.51	3.5±0.83	5.33±1.03***	3.16±0.75^{a***,b}	3±0.98$^{b,\ a***}$
ETAE-200	2.66±0.51	3.66±0.51*	5.33±0.81***	4.5±1.22^{**,a**,c}	4.33±0.51^{*,a***,c}
ETAE-400	2.5±0.54	3.33±0.51	5.66±0.81***	4.16±0.75^{*,a***c}	4±0.63^{b,a***,c}
ETME-200	2.5±0.54	3.5±0.54	5.83±0.75***	3.83±0.75^{a***,b,c}	3.33±0.81^{b,a***,c}
ETME-400	2.33±0.51	3.66±0.81*	5.16±0.75***	3.66±0.51^{a***,b,c}	3.16±0.75^{b,a***,c}

Values are mean ± SD, n=6, where *= P<0.05, **= P<0.01, ***= P<0.001 when compared to normal control group, b= ns when compared to control group, a**= P<0.01, a***= P<0.001 when compared to diabetic control group, c= ns when compared to standard group.

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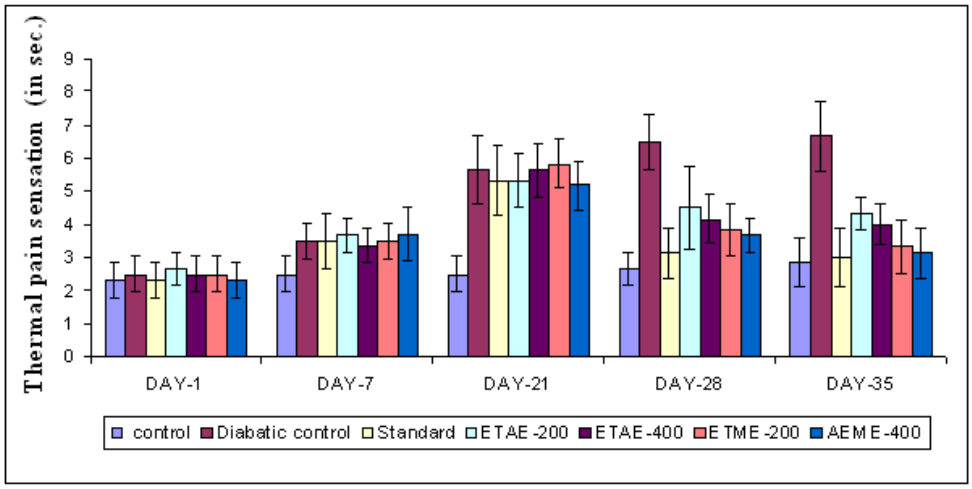

Figure 4: The effect of *Euphorbia thymifolia* on hot plate induced thermal pain in SZT-induced diabetic rats.

DISCUSSION

The present work has been carried out to detect the ant diabetic effect and antioxidant effect of *Euphorbia thymifolia Linn* in STZ induced diabetes and Diabetic Neuropathy. STZ injection caused diabetes mellitus probably due to destruction of the β cells of the islets of langerhans of the pancreas, over-production of glucose and decreased utilization by the tissues is responsible for induction of diabetes. Hyperglycemia accompanied by weight loss were seen in adult rats treated with STZ which were stable for 5 weeks, which indicates the irreversible destruction of β cells of the islets of langerhans of pancreas.

Diabetic neuropathy is a long-term complication of diabetes that develops early in the course of the disease and is observed in 60–70% of all diabetic patients. It is known that diabetic neuropathy is a nerve degenerative disease characterized by axonal degeneration, nerve fiber demyelination, and a reduction in the number of medium to large diameter nerve fibers, particularly in peripheral nerves. Diabetic neuropathy is triggered by hyperglycemia, which leads to a persistent accelerated flux of glucose through the polyol pathway. The rate limiting enzyme in this pathway is aldose reductase. The increased flux through the polyol pathway is followed by abnormal protein kinase C metabolism, oxidative stress, accelerated glycation, and decreased endoneural capillary perfusion, leading eventually to nerve degeneration.

The hypoglycemic effect was observed with the treatment of *Euphorbia thymifolia* Linn. in STZ induced hyperglycemic rats, with the maximum effects seen in ETME-400mg/kg group, which may be due to its antioxidant property and due to active constituents reported in the both extracts.

Induction of diabetes with STZ is also associated with a characteristic loss of body weight, which is due to increased muscle wasting, and loss of tissue proteins. Diabetic rats

treated with *Euphorbia thymifolia* Linn. showed an increase in body weight as compared to the diabetic control, which may be due to its effect in controlling muscle wasting.

Presence and severity of diabetic neuropathy has been shown to be associated with decrease muscle strength in both type-I and II diabetes. In the present study, significant improvement in motor behavior, in particular grip strength after treatment of diabetic animals with *Euphorbia thymifolia Linn.* has been observed. Treatment of *Euphorbia thymifolia* Linn. showed significant increase in grip strength when compared with diabetic control group, where significant increase of grip strength was observed in standard (glimiperide drug (1 mg/kg) drug treated diabetic animal.

Hyperalgesia is a constant feature of sensory dysfunction in spontaneously and experimental model of diabetic neuropathy, we observed hyperglycia and a significant improvement in hot plate response that is pain-threshold of diabetic animals with *Euphorbia thymifolia Linn.* treatment. The response with dose of ETME-400 mg/kg was found to be better than ETME-200mg/kg and ETAE-200mg/kg and 400 mg/kg doses. The analgesic action was found to be near normal. Significant increase in pain threshold was observed in diabetic animals treated only with STZ.

Although the present study did not explore the exact mechanisms for improvement of nerve function for maintenance of grip strength and pain sensitivity by *Euphorbia thymifolia* Linn., it could be due to the presence of flavonoids, reported in the phytochemical investigation of plant extract.

CONCLUSION

The significant antidiabetic and antioxidant activity of *Euphorbia thymifolia* Linn. observed in the present investigation could be the result of synergistic/potentiative action of its constituents, since they contain a diverse array of active principles which are able to target multiple mechanisms involved in the pathophysiology of diabetes. *Euphorbia thymifolia* Linn. has shown significant increase in body weight, increase in grip strength and pain sensitivity. This indicates its protective role against damage to the neurons. Therefore, it can be concluded that *Euphorbia thymifolia* Linn. has significant anti-diabetic and neuroprotective effects in experimental animals.

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