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Potential application of *Euphorbia thymifolia* linn. in diabetic neuropathy

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Abstract

Objective: The present study shows the anti-diabetic activity of ethanolic extract of *Euphorbia thymifolia* Linn leaves in streptozocin (STZ) induced diabetic Wistar rat. This drug is never used for the treatment of diabetic neuropathy therefore; we propose to test this plant in the diabetic neuropathic condition.

Methods: The study was performed on Wistar rat by administration of STZ (65 mg/kg IP) and nicotinamide (110 mg/kg IP). Elevation in blood glucose level shows the induction of diabetes. Parameters like body weight, thermal pain sensitivity, muscle grip strength, blood and urine protein level and creatinine level were used to evaluate the effect of ethanolic extract of *Euphorbia thymifolia* in rat.

Results: The ethanolic extract of *Euphorbia thymifolia* Linn. (red and green) leaves show synergistic effect in thermal pain sensitivity and muscle relaxant. The extract (red and green) showed no weight gain, decreased paw jumping response, increased muscle grip strength, decrease in blood glucose level, increase in blood protein, decrease in urinary protein and decrease serum creatinine level on 35th day of treatment as compared with negative control..

Conclusion: *Euphorbia thymifolia* Linn showed the maximum response in diabetic neuropathy at dose of 200 mg/kg.

Keywords: *Euphorbia thymifolia*, Anti-diabetic activity, Diabetic Neuropathy

Introduction

Diabetes mellitus is a common endocrine disorder caused due to either a deficiency in insulin production or due to the ineffectiveness of the insulin produced [1, 2]. Diabetes mellitus is a syndrome, associated with hyperglycemia, hyperlipidemia, oxidative stress, polyurea, polyphagia, polydypsia, ketosis, nephropathy, neuropathy and cardiovascular disorders [3]. Diabetic Neuropathy is a demonstrable disorder, either subclinical or clinically evident, that occurs in both peripheral and the autonomic nervous systems; caused by diabetes. The symptoms of diabetic neuropathy are often slight at first, but can occasionally flare up suddenly and affect specific nerves so that an affected individual will develop double vision, drooping eyelids, or weakness and atrophy of the thigh muscles [4, 5]. The main risk factor for diabetic neuropathy is hyperglycemia. It is important to note that people with diabetes are more likely to develop symptoms relating to peripheral neuropathy as the excess glucose in the blood results in a condition known as Glucojasinogen [6]. Typical features of neuropathic pain, regardless of the causal injury, include shooting / laminating pain, burning pain, par aesthesia / dysaesthesia, numbness and allodynia (pain produced by a normally non-painful stimulus) [7].

Euphorbia thymifolia Linn (ETL) is small branched, pubescent, prostate annual herb with opposite oblong leaves, commonly known as laghududhika or choti-dudhi [8]. ETL grows very rapidly and completes its life cycle in 3-4 mon; it can be found flowering and fruiting throughout the year in warm tropical conditions. Pollination is effected by insects [9]. ETL is a common weed of cultivated and waste ground, often on sandy or gravelly soils, up to 1650 m altitude [10]. ETL is one of the important multipurpose species of desert and arid regions of the Indian subcontinent. It provides vegetative cover in dry, hot, sandy desert areas where little else grows and is an extremely hardy species. Altitude range: 300-1200 mm, Mean annual rainfall: 100-750 mm, Mean annual temperature: 25-31°C, Soil, it prefers alkaline, sandy and gravelly soils, thriving on shallow, hard soils and rocky outcrops [11]. It grows abundantly in the plains and lower hills throughout India and mainly found in warmer parts of India from Punjab to Kanyakumari ascending to 1660 m in Kashmir. It occurs throughout India in plains and low hills, ascending to 1660 m in Kashmir. Leaves are green-purplish red in colour, opposite, 2 to 5 mm long and 2 to 4 mm broad, oblong, apex mucronate, base obliquely truncate, pubescent, petiole very minute, stipules fabricate with a pointed tip, inflorescence cyathium, involucres sub solitary, very short, axillaries especially in the crowded terminal branch lets, lobes

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short, 4 ciliate, glands minute, stipulate with minute limb, ovary very small, tricarpellary, profusely hairy; stigma bifid; capsules erect, obtusely keeled, pubescent.

Euphorbia comprises about 2000 species and has a worldwide distribution, with at least 750 species occurring in continental Africa and about 150 species in Madagascar and the Indian Ocean islands. Several other *Euphorbia* spp. belonging to this section are medicinally used. *Euphorbia glanduligera* Pax occurs in Namibia, Botswana and South Africa. In Namibia fresh or sun-dried leaves are pounded and rubbed into scarifications in the chest to increase milk flow in lactating women. *Euphorbia polycnemoides* [12]. In Nigeria the medicinal uses are similar to those of *Euphorbia convolvuloides* crushed leaves, mixed with palm oil, are applied to dry up the rashes associated with measles, chickenpox and formerly smallpox. The crushed leaves are taken to treat diarrhoea and an infusion of the dried leaves is taken against dysentery. In contrast, an infusion of the whole plant is taken orally or as an enema for its laxative effects. An extract of the plant is taken to treat coughs, a sore throat, asthma and bronchitis. In Tanzania a decoction of the whole plant, together with the whole plant of *Euphorbia convolvuloides*, is taken to treat dysentery. The latex is rubbed on the breasts to stimulate milk flow.

Literature survey proved that ETL is well established drug for diabetic mellitus (Type-II). Flavonoid present in this plant is responsible for the antidiabetic activity. But this drug is never used for the treatment of diabetic neuropathy which is a secondary complication arising due to high level of glucose in the blood therefore; we propose to test this plant in the diabetic neuropathic condition. Similarly we plan to check this plant for its antihyperlipidemic properties.

Materials and methods

Plant collection

The plant ETL were collected from Sagar Institute of Pharmaceutical Sciences Sagar (M.P.) and authenticated by Department of Botany, Dr. H. S. Gour Central University, Sagar (M.P.). A voucher specimen of the plant is preserved in the herbarium of the Department of Pharmaceutical Sciences, Dr. H. S. Gour Central University, Sagar (MP) (Plant Authentication No. BOT/HER/B/1667).

Chemicals

Streptozotocin (STZ) was purchased from Sigma chemicals, Germany. Glucose, triglycerides, total cholesterol and HDL-cholesterol kits were purchased from Span Diagnostics, Gujarat. All other chemicals used in the study were of analytical grade.

Extract preparation

Shade dried, coarsely powder plant material was packed well in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with ethanol for 18 h or till clear extraction obtained. The extract filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desiccators till experimentation. Obtained extract was weighed and percentage yield was calculated in terms of air-dried powdered crude material.

Animals

Wistar rat of either sex, weighing about 150-250 g were used in the study. Animals were maintained under standard environmental conditions, i.e. ambient temperature of (22±2)°C, at 45-55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rats diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied ad libitum. All the studies were conducted in accordance with the Animal Ethical Committee of the college (CPCSEA, Registration No. SIPS/EC/2013/35).

Induction of diabetes

Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitonial injection of 65 mg/kg body weight STZ (streptozocine), 15 min after the intraperitonial administration of 110 mg/kg body weight of nicotinamide. STZ and nicotinamide was dissolved in citrate buffer (0.1M, pH 4.5) and normal saline respectively. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. The animals with blood glucose concentration more than 200 mg/dl were used for the study of diabetic neuropathy [13,14].

Experimental design

All the diabetic animals were randomly divided into eleven groups with six animals each and treated once a day for 21st days as follows:

Group 1- Positive control (Vehicle treated)

Group 2- Negative control (Disease induced)

Group 3- Standard [Met. (500 mg/kg body weight) + pyoglitazone (25 mg/kg body weight) + glimipride (1 mg/kg body weight)]

Group 4- Test group I [Ethanolic extract of *Euphorbia thymifolia* Linn. {EEET} (red) 100 mg/kg]

Group 5- Test group II [EEET (red) 200 mg/kg]

Group 6- Test group III [EEET (red) 100 mg/kg + **a**mitriptyline 25 mg/kg]

Group 7- Test group IV [EEET (red) 200 mg/kg + amitriptyline 25 mg/kg]

Group 8- Test group V [EEET (green) 100 mg/kg]

Group 9- Test group VI [EEET (green) 200 mg/kg]

Group10- Test group VII [EEET (green) 100 mg/kg + **a**mitriptyline 25 mg/kg]

Group 11- Test group VIII [EEET (green) 200 mg/kg + amitriptyline 25 mg/kg]

Dose

The dose were selected on the basis of OECD guidelines 423, the dose were administered on the difference of 50 under 200, 500 and 1000 & 2000 mg/kg. The significant dose

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was obtained at 1000 and 2000 mg/kg. So, the 1/10 dose of these particular dose was 100, 200 mg/kg according to OECD guidelines.

Physical examination (Body weight)

Body weight of animals was measured by analytical balance [15].

Thermal pain sensitivity by Eddy's hot plate method

Weight the animals and number them. Basal reaction time was taken by observing hind paw licking or jump response in animals when placed on the hot plate maintained at constant temperature (55 °C). Normally animals show such response in 6-8 sec. A cut off period of 15 sec was observed to avoid damage to the paws. Drug was injected to animals and notes the reaction time of animals on hot plate after the drug administration. As the reaction time increase with drug, 15 sec is taken as maximum effect. Percent increase in reaction-time was calculated at each time interval [16].

Muscle grip strength by Rota rod

Weight the animals and number them. Turn on the rota- rod. Select an appropriate speed (20-25 rpm is ideal). Place the animals one by one on the rotating rod. Note down the fall of time when the rats fall from the rotating rod. A normal rat generally falls off within 3-5 min. Drug was injected to the rats. Fall off time was noted. Compare the fall off time of animals before and after drug treatment [17].

Biochemical estimations

Blood glucose level estimation

Blood samples was collected by retro-orbital sinus/plexus bleeding method, in which, in anaesthetized animal tip of capillary tube was gently inserted below the eye at approximately a 45° angle into the space between the globe and the lower eyelid. At a point; capillary tube was feel rest on the orbit, at that time tube was twisted between thumb and forefinger. After that, sinus/plexus was ruptured and blood was flow through the tube, blood sample was collected and multiple tubes required collecting total volume. Blood glucose level was measured by glucometer. The blood glucose estimation was done weekly before and after administration of test compound.

Total serum protein estimation

5 ml of Biuret reagent was pipette out into each of 7 test tubes. 5 ml of the Biuret blank reagent was pipette out into each of 7 test tubes. Reagent-series was prepared by adding 100 µL of each of the protein standards to five separate test tubes filled with the Biuret reagent. Reagent blank was prepared by adding 100 µL of water to a sixth different test tube filled with Biuret reagent. Serum unknown was prepared by adding 100 µL of serum to a seventh test tube filled with Biuret reagent. Each tube was mixed by placing a piece of a parafilm on the top and inverting several times. Blank-series was prepared by adding 100 µL of each of the protein standards to five separate test tubes filled with the Biuret blank reagent. Reagent blank was prepared by adding 100 µL of water to a sixth different test tube filled with Biuret blank reagent. Serum unknown was prepared by adding 100 µL of serum to a seventh test tube filled with Biuret blank reagent. Each tube was mixed by placing a piece of a parafilm on the top and inverting several times. Cuvettes were allowed standing at room temperature for 30 min. Reagent-series blank was used, zeroing the Spec 20 at 540 nm and absorbance of the reagent series was measured including the serum unknown. All the test tube was inverted before that measurement. Blank was used re-zero the Spec 20 and absorbance of the blank series including the serum unknown was measured. Blank subtraction was conducted by subtracting the absorbance of the blank-series from its reagent series counterpart. Concentration of the unknown was determined by the plotting of graph between absorbance vs. concentrations [18].

Protein estimation in urine

The rat's urine was collected through activity cage. The protein was precipitated with trichloroacetic acid (final concentration was 0.33 mol/liter). After mixtures had stands for 30 min at room temperature, the precipitates were centrifuged for 20 min at 110 kg. The precipitate was processed and after reaction with biuret reagent, absorbency was measured by colorimeter [19]. The total protein concentration was determined as by

$$\text{Total protein concentration } \left(\frac{\text{g}}{\text{dl}}\right) = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 6.5$$

The formula was used for both determination of protein in serum as well as in urine samples.

Creatinine estimation

Creatinine in a protein free solution reacts with alkaline picrate and produces a red colored complex, which is measured by colorimeter at 520 nm.

Deproteinized test sample

0.5 ml of serum sample was mixed well with 0.5 ml distilled water and 3 ml picric acid (Reagent 1). It was kept in boiling water bath exactly for one minute and cooled immediately under running tap water and centrifuged. 2.0 ml of supernatant from the above step were mixed with 1.0 ml sodium hydroxide solution (Reagent 2). 0.5 ml of distilled water and working creatinine standard mixed with 1.5 ml picric acid and 0.5 ml sodium hydroxide solution served as blank and standard respectively. All the tubes were allowed to stand at room temperature after thorough mixing for 20 min. The absorbance of blank, standard and samples were measured immediately against distilled water at 520 nm [20].

$$\text{Serum creatinine (mg/100 ml)} = \frac{\text{Test OD} - \text{Blank OD}}{\text{Standard OD} - \text{Blank OD}} \times 4$$

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Optical Density (OD) =log I_0/I

I_0=Intensity of light incident upon sample cell.

I= Intensity of light leaving sample.

Histopathology

Histopathological study of sciatic nerve was done before and after treatment. Experimental rats (150-200 g) were implemented as a mammalian model for *in vivo* sciatic nerve experiments (Histopathological study). In preparation for surgery, rats were anesthetized with ketamine. Once anesthetized, animals were placed in the prone position and the sciatic nerve exposed over the length of the femur. An incision was made posterior-laterally extending from the gluteus muscles to the popliteal region. This allowed access to the sciatic nerve from its pelvic cavity exit to the level of the knee and visualization of specific motor branches (N. fibularis and N. tibialis) to the biceps femoris, gastrocnemius, and distal muscles. This surgical procedure served to expose sufficient area for electrical and optical stimulation and electrical recording of CNAPs along the nerve and CMAPs from the biceps femoris and gastrocnemius muscles. The muscle fascia overlying the nerve was carefully removed to expose the nerve surface with its epineuria covering maintained intact. Removal of this fascia was greatly decreasing the energy required for stimulation. Nerves were continually moistened with normal saline to avoid desiccation during the acute study. The typical rat sciatic nerve stimulated in this study was approximately 2 mm in diameter. The typical fascicle thickness is constant across all mammalian species (although the number of fascicles per nerve varies greatly) and tends to be between 200 and 400 µm [21].

Statistical analysis

All the data were expressed as mean±SEM. Statistical analysis was carried using Student's t-test to analyze the significance between the groups.

Result and discussion

In the study EEET (red and green) were tested for their diabetic neuropathy activity in Swiss albino rats induced by STZ-nicotinamide. The body weight of positive control, negative control, standard and different dose of plant extract treated animals were estimated on 0, 7th, 14th, 21st, 28th, 35th days respectively. The body weight of normal control group were normal so, there was not a significant difference found in normal control group (257±6.67 vs 260±6.71). While negative control group STZ-nicotinamide induced diabetic rats showed a significant decrease in body weight on 21st, 28th and 35th days. On day 15 animals treated with standard and test groups (I-VIII) significantly decrease in body weight as compare to normal control groups. On 14th day the treatment started on all groups except PC and NC. EEET (red) showed no weight gain on 35th day (220±6.18), (P>0.001) at dose of 200mg/kg and EEET (green) also showed no weight gain on 35th days of treatment (236±6.40), (P>0.001) as compare to NC group (Table 1). The main side effect of anti-diabetics is weight gain. EEET (red and green) was significant.

The paw jumping response was measured by Eddy's hot plate. In normal control group there was not a significant difference. But in negative control group there were increase in paw jumping response (6.12±1.0 *vs* 14.12±1.5). In standard group there were significant increase in paw jumping response on 21st, 28th, 35th days (8.13±1.1 *vs* 6.85±1.0), (P>0.001). On 14th day the treatment started on all groups except PC and NC. The paw jumping response of test groups (I-VIII) decrease significantly on 21th, 28th and 35th days as compare to NC groups. The EEET (red) showed decrease on 35th day (6.12±1.0 vs 7.95±1.1), (P>0.001) at dose of 200 mg/kg and EEET (green) also showed decrease on 35th days of treatment (5.86±1.0 vs 8.16±1.1), (P>0.001) compare to NC group. 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 ETL (red & green) treatment. The response with dose of 200 mg/kg was found to be better than 100 mg/kg doses (Table 2). The analgesic action was found to be near normal. Significant increase in pain threshold was observed in diabetic animals treated only with STZ. EEET (red and green) showed significant results.

Measurement of muscles grip strength by rota rod in PC was normal (60.35±3.2 *vs* 62.12±3.2) so there was not a significant difference. In NC group muscle grip strength were reduced (60.23±3.2 *vs* 12.41±1.4) significantly in Swiss albino rats with STZ-nicotinamide induction. On 14th day the treatment started on all groups except PC and NC. The muscle grip strength of standard group and test (I-VIII) groups were increases significantly on 21st, 28th and 35th days as compare to NC group. The EEET (red) showed increase on 35th day (42.15±2.6), (P>0.001) at dose of 200mg/kg and EEET (green) also showed decrease on 35th days of treatment (42.15±2.6), (P>0.001) compare to NC group (Table 3). 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 ETL (red & green) has been observed. Treatment of ETL showed significant increase in grip strength when compared with diabetic control group, where significant increase of grip strength was observed in EEET (red & green) at dose of 200mg/kg.

The blood glucose level of rats in experimental groups, except the normal control group, increased significantly after the STZ nicotinamide injection until 14th day. On 21st, 28th and 35th days post induction, significantly increase in blood glucose level in NC groups (95±4.0 *vs* 315±7.3). On 14th day the treatment started on all groups except PC and NC. On

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repeated administration of standard drug and EEET for 21st, 28th and 35th days, a sustained and significant decrease in blood glucose level of diabetic rats was observed in dose dependent manner as compared to diabetic control group (Table 4). The EEET (red) showed decrease blood glucose level on 35th day (178±5.5), (P>0.001) at dose of 200 mg/kg and EEET (green) also showed decrease blood glucose level on 35th days of treatment (215±6.0), (P>0.001) compare to NC group. In this study the different fractions of EEET reduced blood glucose level significantly in the hyperglycemic rats. The antihyperglycemic activity of EEET (red & green) at dose of 200 mg/kg as compared to 100 mg/kg was significant cause hypoglycemia and it may be useful for the treatment of diabetes and associated complications.

Table 1: Body weight observation

S.No	Groups	Body weight (g)					
		0 day	7th day	14th day	21st day	28th day	35th day
1	Positive control [Vehicle treated]	257±6.67	257±6.67	257±6.67	260±6.71	260±6.71	260±6.71
2	Negative control [Disease induced]	265±6.75	240±6.45	231±6.33	223±6.22	205±5.96	198±5.86
3	Standard [Pyo+Gli+Met]	265±6.75	243±6.49	235±6.38	235±6.38***	237±6.41***	237±6.41***
4	Test I-[EEET (red)100mg/kg]	265±6.78	240±6.45	230±6.31	225±6.25**a**	220±6.18**a**	218±6.15**a**
5	Test II-[EEET(red) 200mg/kg]	270±6.84	243±6.49	232±6.34	223±6.22**b***	220±6.18**b**	220±6.18**a**c***
6	Test III-[EEET(red)100mg/kg+Ami]	255±6.65	245±6.52	230±6.31	227±6.27**a**	225±6.25**a**	225±6.25**a**
7	Test IV- [EEET(red)200mg/kg + Ami]	260±6.71	240±6.45	230±6.31	225±6.25**b**	223±6.22**a**	223±6.22**a**b**
8	Test V- [EEET(green) 100mg/kg]	265±6.78	242±6.48	228±6.29	223±6.22***	227±6.27**a**	232±6.34**a**
9	Test VI- [EEET(green) 200mg/kg]	270±6.84	245±6.52	235±6.38	228±6.29**a*	232±6.34**a*	236±6.40** a**
10	Test VII- [EEET(green)100mg/kg+ Ami]	263±6.75	240±6.45	230±6.31	221±6.19**b*	230±6.31**a**	234±6.37**a**
11	Test VIII- [EEET(green)200mg/kg+Ami]	265±6.78	245±6.52	232±6.34	224±6.23**a***	230±6.31**a***	238±6.42**a***

No of animals per group (n) = 6, value are in mean ±SEM, Statistical significant with control using one way ANOVA followed by Dunnet t test. Pyo:-Pyoglitazone, Gli:-Glimipride, Met:-Metformine, Ami:- Amitriptiline, EEET:- Ethanolic extract of *Euphorbia thymifolia* Linn. a=Compared with positive control, b=Compared with standard group, c=Compared with negative control.

Table 2: Thermal pain sensitivity (paw jumping response) by Eddy's hot plate

S.No.	Groups	Thermal Pain Sensitivity (sec)					
		0 day	7th day	14th day	21st day	28th day	35th day
1	Positive control [Vehicle treated]	6.24±1.0	5.14±0.9	5.12±0.9	5.10±0.9	5.42±0.9	6.12±1.0
2	Negative control [Disease induced]	6.12±1.0	7.52±1.1	9.13±1.2	11.12±1.3	12.19±1.4	14.12±1.5
3	Standard- [Pyo+Gli+Met]	6.41±1.0	8.85±1.2	9.21±1.2	8.13±1.1***	7.16±1.9***	6.85±1.0***
4	Test I- [EEET (red)100mg/kg]	5.45±0.9	8.45±1.2	9.20±1.2	8.72±1.2***	8.16±0.9***	7.37±1.1***
5	Test II- [EEET(red) 200mg/kg]	6.12±1.0	8.75±1.2	8.92±1.2	8.82±1.2***	8.23±1.1***	7.95±1.1**a***
6	Test III- [EEET(red)100mg/kg+Ami]	6.13±1.0	8.46±1.2	8.46±1.2	8.35±1.2***	8.28±1.1***	8.16±1.1***
7	Test IV- [EEET(red)200mg/kg + Ami]	5.70±0.9	8.16±1.1	8.40±1.2	8.26±1.1***	8.18±1.1***	7.98±1.1***
8	Test V- [EEET(green)100mg/kg]	6.13±1.0	8.45±1.2	9.15±1.2	8.82±1.2***	8.52±1.2***	8.23±1.1***
9	Test V- [EEET(green)200mg/kg]	5.86±1.0	8.13±1.1	9.12±1.2	8.85±1.2***	8.61±1.2***	8.16±1.1**a***
10	Test VII- [EEET(green)100mg/kg+ Ami]	5.62±0.9	7.90±1.1	8.95±1.2	8.45±1.2***	8.32±1.2***	8.12±1.1***
11	Test VIII -[EEET(green)200mg/kg+Ami]	5.51±0.9	7.83±1.1	8.87±1.2	8.70±1.2***	8.43±1.2***	8.12±1.1***

No of animals per group (n) = 6, value are in mean ±SEM, Statistical significant with control using one way ANOVA followed by Dunnet t test. Pyo:-Pyoglitazone, Gli:-Glimipride, Met:-Metformine, Ami:- Amitriptiline, EEET:- Ethanolic extract of *Euphorbia thymifolia* Linn. a=Compared with positive control, b=Compared with standard group, c=Compared with negative control.

The blood protein levels in all experimental groups except the normal control were significantly decreased after STZ-nicotinamide injection. On 21st, 28th, 35th days post induction the blood protein level decrease significantly in NC group (6.35±1.0 *vs* 3.28±0.7) as compare normal control group. On 14th day the treatment started on all groups except PC and NC. The blood protein levels were detected in animals treated with standard drug and different fraction of EEET on 21st, 28th, 35th days, increase in blood protein level as compare to negative control group. On 14th day the treatment started on all groups except PC and NC. The EEET (red) showed increase in blood protein on 35th day (4.62±0.8), (P>0.001) at dose of 200mg/kg and EEET (green) also showed increase in blood protein on 35th d of treatment (4.48±0.8), (P>0.001) as compare to NC group (Table 5). EEET (red and green) was significant.

The urinary protein level in positive control group were normal but in negative control group the urinary protein level were increase(0.35±0.2 *vs* 3.48±0.7) significantly after STZ-nicotinamide injection. On 21st, 28th 35th days post induction

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decrease in urinary protein level were detected in animals treated with standard drug and different fraction of EEET as compare to negative control group. On 14th day the treatment started on all groups except PC and NC. The EEET (red) showed decrease in urinary protein on 35th day (1.47±0.5), (P>0.001) at dose of 200mg/kg and EEET (green) also showed decrease in urinary protein on 35th days of treatment (1.65±0.5), (P>0.001) as compare to NC group (Table 6). EEET (red and green) was significant.

Serum creatinine level in positive control group were normal but in negative control group the creatinine level were increase (0.75±0.36 *vs* 1.46±0.50) significantly after STZ-nicotinamide injection. On 21st, 28th and 35th days post induction decrease in creatinine level were detected in animals treated with standard drug and different fraction of EEET as compare to negative control group. On 14th day the treatment started on all groups except PC and NC. On repeated administration of standard drug and EEET for 21st, 28th and 35th d, a sustained and significant decrease in creatinine level of diabetic rats was observed in dose dependent manner as compared to diabetic control group. The EEET (red) showed decrease on 35th day (0.85±0.38), (P>0.001) at dose of 200 mg/kg and EEET (green) also showed decrease on 35th days of treatment (0.86±0.38), (P>0.001) compare to NC group (Table 7).

Creatinine is a metabolic product of creatinine which is derived from the muscle protein. This caused a rise in serum creatinine level in diabetic mellitus. In this study the different fractions of EEET reduced creatinine significantly in the hyperglycemic rats. The significant antihyperglycemic activities of EEET (red & green) at dose of 200 mg/kg as compare to 100 mg/kg lowered hyperglycemia and it may be useful for the treatment of diabetes and associated complications.

Table 3: Muscle grip strength by Rota rod apparatus

S.No.		Muscle Grip Strength (sec)					
	Groups	**0 day**	**7th day**	**14th days**	**21st day**	**28th day**	**35th day**
1	Positive control [Vehicle treated]	60.35±3.2	69.18±3.4	65.73±3.3	64.50±3.3	65.48±3.3***	62.12±3.2***
2	Negative control [Disease induced]	60.23±3.2	44.36±2.7	32.2±2.3	21.5±1.9	18.46±1.7	12.41±1.4***
3	Standard [Pyo+Gli+Met]	60.10±3.2	40.30±2.6	34.33±2.4	38.26±2.1***	45.12±2.7***	50.12±2.9***
4	Test I [EEET (red)100mg/kg]	65.21±3.3	41.34±2.6	33.48±2.3	35.35±2.4***	39.15±2.5***	42.13±2.6***
5	Test II [EEET (red) 200mg/kg]	60.25±3.2	39.12±2.5	32.15±2.3	34.12±2.4***	38.15±2.5***	42.15±2.6**a***
6	Test III [EEET (red)100mg/kg+Ami]	65.24±3.3	42.23±2.6	31.15±2.2	32.48±2.3***	38.45±2.5***	47.15±2.8***
7	Test IV [EEET (red)200mg/kg + Ami]	61.23±3.2	41.16±2.6	31.15±2.2	33.12±2.3***	37.12±2.5***	49.48±2.9***
8	Test V [EEET (green) 100mg/kg]	65.25±3.3	41.25±2.6	31.12±2.2	30.47±2.2***	36.64±2.5***	42.23±2.6***
9	Test VI [EEET (green) 200mg/kg]	62.24±3.2	42.25±2.7	32.14±2.3	34.12±2.4***	32.19±2.5***	42.15±2.6**a***
10	Test VII [EEET (green)100mg/kg+ Ami]	61.12±3.2	40.19±2.6	30.12±2.2	35.45±2.4***	38.13±2.5***	44.15±2.7***
11	Test VIII [EEET (green)200mg/kg+Ami]	65.23±3.3	42.12±2.6	30.18±2.2	34.12±2.4***	38.00±2.5***	47.02±2.8***

No of animals per group (n) = 6, value are in mean ±SEM, Statistical significant with control using one way ANOVA followed by Dunnet t test. Pyo:-Pyoglitazone, Gli:-Glimipride, Met:-Metformine, Ami:- Amitriptiline, EEET:- Ethanolic extract of *Euphorbia thymifolia* Linn. a=Compared with positive control, b=Compared with standard group, c=Compared with negative control.

Table 4: Glucose estimation

S.		Blood Glucose Level (mg/dl)					
No.	**Groups**	**0 day**	**7th day**	**14th day**	**21st day**	**28th day**	**35th day**
1	Positive control [Vehicle treated]	95±4.0	95±4.0	93±4.0	95±4.0	95±4.0	98±4.0
2	Negative control [Disease induced]	95±4.0	178±5.5	271±6.8	281±6.9	295±7.1***	315±7.3***
3	Standard [Pyo+Gli+Met]	93±4.0	182±5.5	288±7.0	232±6.3***	192±5.7***	141±4.9***
4	Test I [EEET (red)100mg/kg]	95±4.0	180±5.5	275±6.8	262±6.7***	243±6.4***	228±6.2***
5	Test II [EEET (red) 200mg/kg]	95±4.0	183±5.6	283±7.0	250±6.5***	225±6.2***	178±5.5**a***
6	Test III [EEET(red)100mg/kg+Ami]	96±4.0	180±5.5	281±6.9	271±6.8***	265±6.7***	252±6.5***
7	Test IV [EEET(red)200mg/kg+Ami]	93±4.0	185±5.6	279±6.9	248±6.5***	230±6.2***	182±5.5***
8	Test V [EEET (green) 100mg/kg]	95±4.0	182±5.5	278±6.9	268±6.5***	251±6.5***	241±6.4***
9	Test VI [EEET (green) 200mg/kg]	96±4.0	180±5.5	283±7.0	261±6.7***	238±6.4***	215±6.0**a***
10	Test VII [EEET(green)100mg/kg+Ami]	95±4.0	195±5.7	285±7.0	265±6.7***	253±6.6***	243±6.4***
11	Test VIII [EEET(green)200mg/kg+Ami]	95±4.0	188±5.7	283±7.0	258±6.6***	240±6.4***	205±5.9***

No of animals per group (n) = 6, value are in mean ±SEM, Statistical significant with control using one way ANOVA followed by Dunnet t test. Pyo:-Pyoglitazone, Gli:-Glimipride, Met:-Metformine, Ami:- Amitriptiline, EEET:- Ethanolic extract of *Euphorbia thymifolia* Linn. a=Compared with positive control, b=Compared with standard group, c=Compared with negative control.

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Table 5: Protein estimation in blood

S. No.	Groups	Serum Protein Level (mg/dl)					
		0 day	7th day	14th day	21st day	28th day	35th day
1	Positive control [Vehicle treated]	6.38±1.0	6.38±1.0	6.32±1.0	6.30±1.0	6.31±1.0	6.32±1.0
2	Negative control [Disease induced]	6.35±1.0	4.75±0.8	4.12±0.8	3.85±0.7	3.55±0.7	3.28±0.7
3	Standard [Pyo+Gli+Met]	6.36±1.0	4.74±0.8	4.14±0.8	4.32±0.8***	5.53±0.9***	5.76±1.0***
4	Test I [EEET (red)100mg/kg]	6.38±1.0	4.73±0.8	4.17±0.8	4.25±0.8***	4.34±0.8***	4.46±0.8***
5	Test II [EEET (red) 200mg/kg]	6.32±1.0	4.74±0.8	4.15±0.8	4.28±0.8***	4.42±0.8***	4.62±0.8**a***
6	Test III [EEET (red)100mg/kg+Ami]	6.37±1.0	4.72±0.8	4.17±0.8	4.27±0.8***	4.31±0.8***	4.38±0.8***
7	Test IV [EEET (red)200mg/kg+Ami]	6.35±1.0	4.70±0.8	4.13±0.8	4.30±0.8***	4.43±0.8***	4.54±0.8***
8	Test V [EEET (green) 100mg/kg]	6.36±1.0	4.70±0.8	4.12±0.8	4.31±0.8***	4.36±0.8***	4.41±0.8***
9	Test VI [EEET (green) 200mg/kg]	6.38±1.0	4.73±0.8	4.12±0.8	4.28±0.8***	4.35±0.8***	4.48±0.8**a***
10	Test VII [EEET (green)100mg/kg+ Ami]	6.36±1.0	4.72±0.8	4.14±0.8	4.29±0.8***	4.34±0.8***	4.46±0.8***
11	TestVIII [EEET(green)200mg/kg+Ami]	3.37±1.0	4.74±0.8	4.13±0.8	4.32±0.8***	4.38±0.8***	4.47±0.8***

No of animals per group (n) = 6, value are in mean ±SEM, Statistical significant with control using one way ANOVA followed by Dunnet t test. Pyo:-Pyoglitazone, Gli:-Glimipride, Met:-Metformine, Ami:- Amitriptiline, EEET:- Ethanolic extract of *Euphorbia thymifolia* Linn. a=Compared with positive control, b=Compared with standard group, c=Compared with negative control.

Table 6: Protein estimation in urine

S. No.	Groups	Urine Protein (mg/dl)					
		0 day	7th day	14th day	21st day	28th day	35th day
1	Positive control [Vehicle treated]	0.38±0.2	0.37±0.2	0.32±0.2	0.34±0.2	0.32±0.2	0.30±0.2
2	Negative control [Disease induced]	0.35±0.2	1.75±0.5	2.15±0.5	2.28±0.6	3.07±0.7	3.48±0.7
3	Standard [Pyo+Gli+Met]	0.37±0.2	1.75±0.5	2.13±0.5	1.58±0.5***	1.21±0.4***	1.08±0.4***
4	Test I [EEET (red)100mg/kg]	0.38±0.2	1.74±0.5	2.17±0.5	1.90±0.5***	1.83±0.5***	1.62±0.5***
5	Test II [EEET(red) 200mg/kg]	0.34±0.2	1.73±0.5	2.18±0.5	1.92±0.5***	1.74±0.5***	1.47±0.5**a***
6	Test III [EEET (red)100mg/kg+Ami]	0.36±02	1.74±0.5	2.13±0.5	1.94±0.5***	1.85±0.5***	1.72±0.5***
7	Test IV [EEET (red)200mg/kg + Ami]	0.35±0.2	1.75±0.5	2.15±0.5	1.97±0.5***	1.86±0.5***	1.53±0.4***
8	Test V [EEET (green) 100mg/kg]	0.37±0.2	1.78±0.5	2.16±0.5	1.07±0.5***	1.95±0.5***	1.83±0.5***
9	Test VI [EEET(green) 200mg/kg]	0.35±0.2	1.77±0.5	2.12±0.5	1.98±0.5***	1.87±0.5***	1.65±0.5**a***
10	Test VII [EEET (green)100mg/kg+Ami]	0.38±0.2	1.76±0.5	2.15±0.5	2.09±0.5***	1.96±0.5***	1.85±0.5***
11	Test VIII [EEET (green)200mg/kg+Ami]	0.35±0.2	1.78±0.5	2.19±0.5	2.10±1.4	1.86±0.5	1.63±0.5

No of animals per group (n) = 6, value are in mean ±SEM, Statistical significant with control using one way ANOVA followed by Dunnet t test. Pyo:-Pyoglitazone, Gli:-Glimipride, Met:-Metformine, Ami:- Amitriptiline, EEET:- Ethanolic extract of *Euphorbia thymifolia* Linn. a=Compared with positive control, b=Compared with standard group, c=Compared with negative control.

Table 7: Serum creatinine

S. No.	Groups	Serum Creatinine level (mg/dl)					
		0 day	7th day	14th day	21st day	28th day	35th day
1	Positive control [Vehicle treated]	0.72±0.35	0.72±0.35	0.74±0.35	0.72±0.3	0.70±0.34	0.74±0.35
2	Negative control [Disease induced]	0.75±0.36	0.98±0.41	1.09±0.43	1.12±0.44	1.31±0.47	1.46±0.50
3	Standard [Pyo+Gli+Met]	0.73±0.35	0.98±0.41	1.08±0.43	0.99±0.41***	0.95±0.40***	0.82±0.37***
4	Test I [EEET (red)100mg/kg]	0.72±0.35	0.95±0.40	1.10±0.43	1.05±0.42***	0.95±0.40***	0.91±0.39***
5	Test II [EEET (red) 200mg/kg]	0.70±0.35	0.97±0.41	1.09±0.43	0.98±0.39***	0.92±0.39***	0.85±0.38**a***
6	Test III [EEET (red)100mg/kg+Ami]	0.73±0.35	0.99±0.41	1.07±0.43	0.92±0.39***	0.85±0.38***	0.81±037***
7	Test IV [EEET (red)200mg/kg + Ami]	0.71±0.35	0.92±0.39	1.05±0.43	0.98±0.41***	0.82±0.37***	0.76±0.36***
8	Test V [EEET (green) 100mg/kg]	0.73±0.35	0.98±0.41	1.05±0.43	0.96±0.40***	0.91±0.39***	0.83±0.37***
9	Test VI [EEET (green) 200mg/kg]	0.72±0.35	0.97±0.41	1.07±0.43	0.99±0.41***	0.92±0.39***	0.86±0.38**a***
10	Test VII [EEET (green)100mg/kg+ Ami]	0.71±0.35	0.99±0.41	1.90±0.43	0.85±0.38***	0.81±0.37***	0.76±0.36***
11	Test VIII [EEET (green)200mg/kg+Ami]	0.74±0.35	0.92±0.39	1.09±0.43	0.98±0.41***	0.93±0.40***	0.86±0.38***

No of animals per group (n) = 6, value are in mean ±SEM, Statistical significant with control using one way ANOVA followed by Dunnet t test. Pyo:-Pyoglitazone, Gli:-Glimipride, Met:-Metformine, Ami:- Amitriptiline, EEET:- Ethanolic extract of *Euphorbia thymifolia* Linn. a=Compared with positive control, b=Compared with standard group, c=Compared with negative control.

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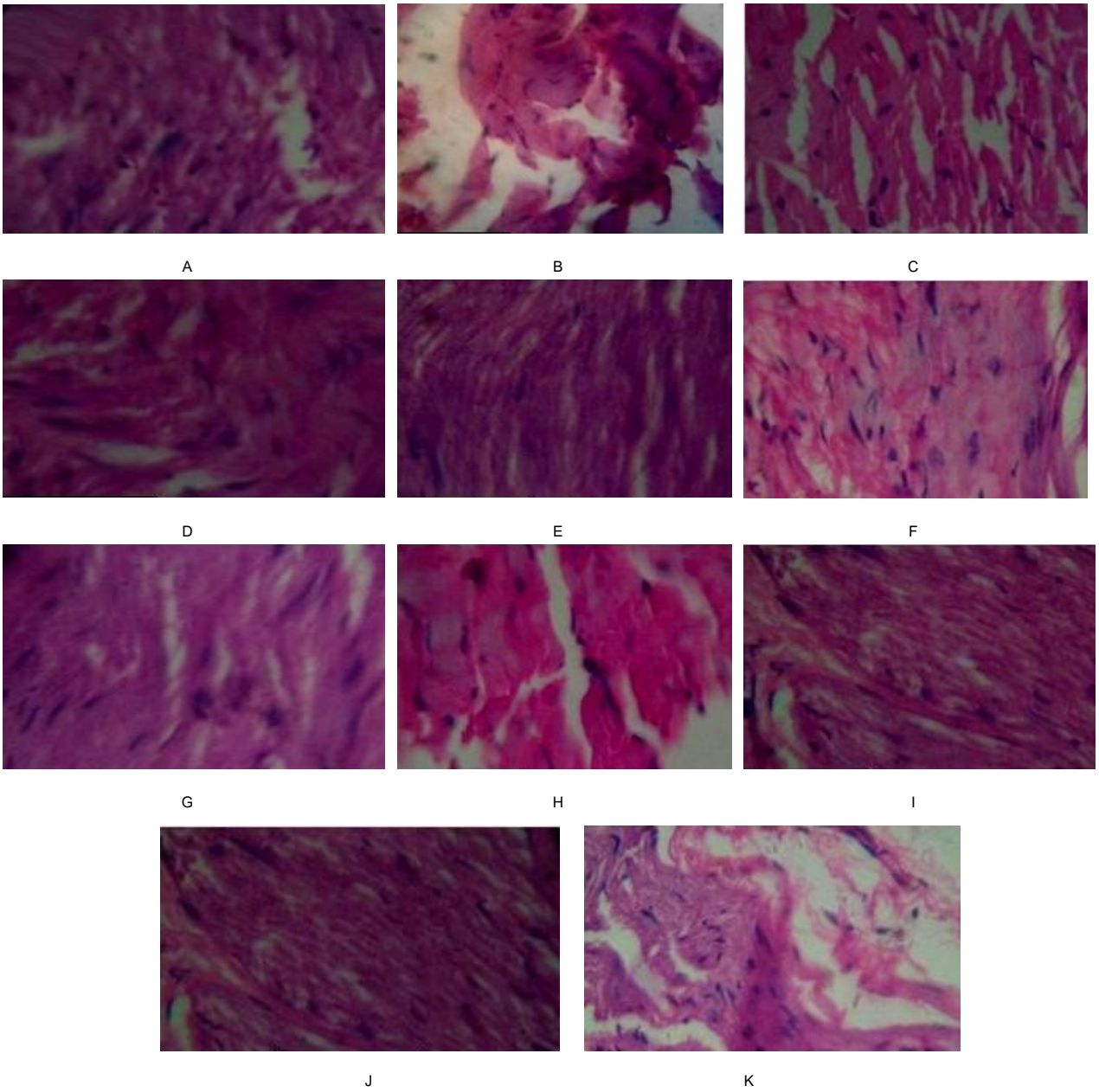

Figure 1(A-K): Shows histopathological study of sciatic nerve

Where Figure 1(A):- Positive control [Vehicle treated], Figure 1(B):- Negative control [Disease induced], Figure 1(C):-Standard (Pyo+Gli+Met), Figure 1(D):-Test I [EEET (red) 100mg/kg], Figure 1(E):-Test II [EEET (red) 200mg/kg], Figure 1(F):-Test III [EEET (red) 100mg/kg+Ami], Figure 1(G):-Test IV [EEET (red) 200mg/kg + Ami], Figure 1(H):-Test V [EEET (green) 100mg/kg], Figure 1(I):-Test VI [EEET (green) 200mg/kg], Figure 1(J):-Test VII [EEET (green) 100mg/kg+ Ami], Figure 1(K):-TestVIII [EEET (green) 200mg/kg+Ami]

Conclusion

The present study reveals that the EEL had antihyperglycemic activity. Our preliminary phytochemical analysis has indicated that flavonoids have been reported to exert potent hypoglycemic effect. Hence treatment with EEET (red & green) significantly increase in body weight, increase in grip strength and pain sensitivity and decreases the glucose, urinary protein, Creatinine and a significantly increase in blood protein. These results were further substantiated with the histopathological result. This indicates its protective role against damage to the neurons. Therefore, it can be concluded that EEL (red & green) has significant anti-diabetic and neuroprotective effects in experimental animals.

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