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Research Article

Neuroprotective Activity of *Premna latifolia* on Streptozotocin Induced Diabetic Neuropathy in Rats

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ABSTRACT

The leaves of plant *Premna latifolia* Lam are traditionally used for treating diabetes and related complications. Presently ethanolic extract of leaves of plant *P. latifolia* explored for the neuroprotective potential in rat model of streptozotocin induced diabetic neuropathy. The intra-peritoneal route administered streptozotocin 60 mg/kg body weight to induce experimental diabetes, then treatment were not given for two weeks to develop diabetic complications. After two weeks of induction, animals were treated with extract 100 and 200 mg/kg per day for the duration of five weeks. The neuroprotective effect was assessed using Eddy's hot plate and tail immersion method. Also, measuring blood sugar and cytokines cause inflammations like interleukins, tumor necrosis factor and a neurotrophin in the sciatic nerve. Thiobarbituric acid reactive substances reduced glutathione and activity levels of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase measured in the sciatic tissue. Extract normalizes blood glucose in diabetic neuropathy rats brought on by streptozotocin. The diabetic neuropathy rats show significant reductions in the period when the tail retracts and the paws are licked or jumped. In these rats pro-inflammatory cytokines level in sciatic nerve was significantly high. In addition, the oxidative stress bio-markers level altered significantly in sciatic nerve tissue. Diabetic neuropathy-developing rats treated with extract for five weeks reduce the levels of nerve growth factor, oxidative stress indicators, and pro-inflammatory cytokines rise in the sciatic nerve tissue. Ethanolic extract of *P. latifolia* possesses neuroprotective action in streptozotocin-induced diabetic neuropathy.

Introduction

Diabetes Mellitus (DM), a metabolic disease it significantly contributes to the burden on global health, now affects 463 million adult populations worldwide and is anticipated to grow to 578 million people by 2030 and 700 million people by 2045.[1] Around 1.5 million fatalities globally are attributed to diabetes each year. According to estimates from the World Health Organization (WHO), 77 million Indians over the age of 18 are anticipated to have diabetes as of 2023; by 2030, that figure could rise to 100 million. Presently, 4 to 11.6% of urban and 3% of the rural people with more than 15 year age in India is identified to be diabetic. According to 2023 data of the WHO, globally 537 million adults are suffering from diabetes mellitus, mainly in developing countries.[2] Diabetes is characterized by hyperglycemia, related to altered metabolism of fats, proteins, and carbs following defective insulin discharge, its action or resistance to insulin, and pancreatic β cell dysfunction.[3] If hyperglycemia persist for long period can leads various organ dysfunction or failure. DM is also associated with high levels of blood lipids, a well-known risk factor for coronary and peripheral artery disease.[4] If DM not treated within time leads to pathological consequences called diabetic complications.[5] Approximately 10 to 15% of diabetic patients would have neuropathy. It's a major & long term impediment of both type 1 and 2 diabetes, which is categorized by sensory and motor nerve deficits.[6,7] Symptoms may include; numbness and tingling of extremities, loss of urinary bladder control, eyelid drooping, muscle weakness and speech impairment.

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First approach to treat the DN is to control over blood glucose level. Many anti-depressants drugs like duloxetine, amitriptyline, desipramine, and anti-epileptic drugs like pregabalin, gabapentin and opioids are used for treatment of DN but these drugs can produce variety of side effects including hematological effects and disturbances of the liver and kidney functions.[8] Anti-epileptic drugs cause severe allergic reactions, anti-depressant drugs causes variety of side effects like drowsiness, constipation and dry mouth. It is not possible to reverse and there is no ultimate cure for DN. But its progression can be slow down by maintaining normal blood glucose level and changing life style. There is an urgent need to permanently cure, effective DN drug without side effects. Herbal preparations are frequently considered to be less toxic and low cost. Numbers of plant source materials reported for useful in diabetes and DN.[9] The plant *Premna latifolia* belongs to family Verbinacae. It's a small tree, found in the region of South India. As per traditional claims leaves are used for diabetes, as diuretic and spasmolytic. Stem bark are used to lower blood glucose levels.[10,11] Other parts of plant have been claimed to be used in fever, fomentation in piles, glandular swelling, musculoskeletal disorder and rheumatism.[12, 13] This plant was reported for anti-inflammatory[14] and antioxidant activity.[15] However lack of scientific study about beneficial effects in diabetes related complications like DN to support the traditional claim. Therefore, the present research planned to evaluate neuroprotective effects of *P. latifolia* ethanol extract (PLEE) leaves in streptozotocin (STZ)-induced diabetic neuropathy.

Materials and Methods

Materials

Plant material

P. latifolia leaf material gathered in July from Dakshina Kannada area of Karnataka and authenticated by the Head, department of botany, KUD and Certified at ICMR, Belagavi (voucher specimen RMRC-967). For further reference, the plant is stored at the Regional Medical Research Center, ICMR, Belagavi.

Preparation of plant extract

The plant leaves cleaned in tap water and shade dried. Leaves were coarse powdered and passed in sieve no. 60. A total of 70% ethanol was considered for the extraction because it gives maximum yield.[16] Dried and coarsely powdered leaves were extracted using 70% of ethanol in soxhlet extractor by the method hot continuous percolation. Then filtered, concentrated by using a rotary evaporator at 40°C and allowed it to dry completely on hot water bath. It was stored in an airtight container and used for the activity.

Preparation of drug

Streptozotocin was mixed in citrate buffer (cold, pH 4.5). Solutions of PLEE prepared in 1% Tween 80 solution for the pharmacological research activities.

Preliminary phytochemical test

Dried ethanolic extract was investigated for presence of various phytochemicals.

Chemicals

Diagnostic kits were purchased from Erba Diagnostic Pvt. Ltd. Streptozotocin was purchased from Sisco Research Laboratories, Pvt. Ltd. Mumbai. Additional chemicals and reagents were bought from Hubballi, Karnataka, India.

Animals

Albino wistar rats were procured from the animal house of KLE college of Pharmacy, Hubli having body weight of 150 and 200 g. Animals were kept in a climate-controlled environment with 12:12 h of natural darkness and light (22.2°C). Animals were given access to clean water and high-quality food. Animals were divided into four groups and acclimated for seven days. Investigations were conducted in compliance with Committee for Control and Supervision of Experiments on Animals (CCSEA). The protocol was authorized by the KLE College of Pharmacy's Institutional Animal Ethical Committee (IAEC) in Hubballi, Karnataka, India. (IAEC: KLEU's-09-IAEC.HBL-31/Aug 2013).

Methods

Acute toxicity study (AOT)

Female albino mice (18–25 g) were used for acute oral toxicity studies as per recommendation by Organization for Economic Co-operation and Development (OECD, 423). Distilled water and tween 80 (1%) were used for the preparation of the extract. The dose of PLEE was administered 2000 mg/kg body weight by *p.o.* and food was withheld for 4 hours. Animals were observed at 0.5, 1, 2, 3, 4, 24 hours and once daily intervals for14 days.[16] On the basis of AOT studies, the doses of the extract were $1/10^{th}$ and $1/20^{th}$ of safe dose i.e. 100 mg/kg lower dose and 200 mg/kg. higher dose. These doses were selected for the further studies.

Assessment of PLEE effect on STZ- induced neuropathy

Streptozotocin 60 mg/kg body weight by *i.p* administered to 16 hours fasted animals to induce diabetes and neuropathy. Diabetes was confirmed on 3^{rd} day by measuring blood glucose level in fasted animals using kits. Animals with blood glucose level above 250 mg/dl included in groups for activity. Treatment was not given to these animals for the first two weeks, leading to DN development. After two weeks, neuropathy developing animals were treated with PLEE for 35 days.[17]

Experimental design

Normal rats included in control group (Group I). STZ-induced diabetic neuropathy rats with blood glucose levels over 250 mg/dl were randomly divided into three groups, containing six animals in each group (Group II, III, & IV).

Group I: Normal + vehicle 1% Tween 80. Group II: Diabetic neuropathy + vehicle 1% Tween80. Group III: Diabetic neuropathy + PLEE (100 mg/kg. lower dose). Group IV: Diabetic neuropathy + PLEE (200 mg/kg. higher dose). The effect of vehicle, plant extract at a lower and higher dose was determined in animals. DN is measured using antinociceptive activity and biomarkers in animal blood and sciatic tissue.[18] The antinociceptive activity were done by measuring animals' reactions to non-harmful stimuli; it was assessed by hot-plate & tail flick tests, before induction of neuropathy (on zero day) and during initial stage of neuropathy development (on 14th day) and also over the course of treatment (on days 26, 38, and 49).[19, 20]

Hot plate method

Thermal hyperalgesia was assessed in all group animals on 0th day i.e. before induction and also after induction i.e. during the treatment period on 14th, 26th, 38th and 49th day by hot plate test. The end point is jumping or paw licking (paw withdraw latency). The animals were kept on hot plate maintained at 55 ± 1^{o}C temperature. Response time was noted in seconds and mean reaction time was calculated. The cut off time was 15 seconds.

Tail flick method

Cold allodynia was assessed in all group animals on 0th day i.e., before induction and after induction i.e. during the treatment period on 14th, 26th, 38th and 49th day by tail immersion method. The end point was tail withdrawal latency (time in seconds) of each animal, which was noted by keeping the animals' tail in the cup with cold water. The temperature of water was maintained constant at 10°C ± 1^{o}C. The cut off time was 15 seconds.

Biochemical estimation

After 49 days, animals were fasted and sacrificed under mild anesthesia. Blood sample and sciatic tissues were collected and the same was used for biochemical estimations. After centrifugation separated serum was stored at −20°C & used for investigation. Sciatic tissue washed with ice cold saline, blotted on filter paper then kept at 2°C for further biochemical analysis. After five weeks of treatment, i.e. on 49th day animal was sacrificed, blood along with sciatic nerve tissue was taken to measure cytokines, neurotrophic factors, oxidative stress and other bio-markers. R&D Systems, Inc.'s ELISA kits were used to measure the serum concentrations of glucose and cytokines such as tumor necrosis factor (TNF), interleukin (IL) 1 and IL 6, and neurotrophic factors such as NGF were measured in the sciatic nerve. The concentrations of thiobarbituric acid reactive compounds (TBARS) in the sciatic tissue was calculated by measuring LPO product i.e. malondialdehyde (MDA) by spectrophotometric method.[17] GSH levels were tested in accordance with Sedlak and Lindsay's instructions.[21] Superoxide dismutase (SOD) activity levels were estimated in accordance with (Kono)'s[22] description. The enzymes glutathione reductase (GR) and glutathione peroxidase (GPx) were measured using colorimetric kits, and catalase (CAT) level was computed in accordance with the method given by (Aebi *et al.*).[23]

Statistical Analysis

Results were presented as Mean values ± S.E.M. (standard error of mean). There were six animals (n = 6) in each group. One-way analysis of variance (ANOVA) done for statistical analysis, followed by Tukey's multiple comparison. Significance conveyed by the *p* values, if it is less than 0.05 thought to be significant.

Results

Phytochemicals in PLEE Leaves

Phytoconstituents include glycosides, flavonoids, carbohydrates, steroids, tannins, phenolic compounds and alkaloids were found in PLEE, according to preliminary examination results.

Effect of PLEE on Hot and Cold Stimuli in DN Rats

Normal animals responded normally to the non-noxious stimuli. These animals showed no variation in response time during study period. Diabetic neuropathy group rats were hyper sensitive for hot & cold stimuli, they demonstrated significant ($p < 0.001$) reduction in paw withdraw duration and tail flick time related to control rats, which can be attributed to thermal hyperalgesia and cold allodynia. Low and high dose of PLEE treated animals exhibited notable ($p < 0.001$) rise in paw withdrawal and tail flicking ability in a dose dependent manner on 38th and 49th day. These results indicated animals' normal ability to

Table 1: PLEE effect on paw withdraw latency in STZ induced neuropathy rats

Group/Duration	*Normal control*	*Diabetic neuropathy*	*DN + PLEE (100 mg/kg)*	*DN + PLEE (200 mg/kg)*
Before induction (0th Day)	7.43 ± 0.70	7.39 ± 0.56	6.30 ± 0.63	6.49 ± 0.41
After induction (14th Day)	7.62 ± 0.61	4.54 ± 0.3^{c}	3.67 ± 0.50	3.89 ± 0.34
Day 26th	7.46 ± 0.65	3.67 ± 0.5^{c}	4.13 ± 0.49	4.65 ± 0.34
Day 38th	7.69 ± 0.54	2.98 ± 0.4^{c}	4.53 ± 0.49	5.42 ± 0.3^{x}
Day 49th	8.01 ± 0.37	2.16 ± 0.3^{c}	4.95 ± 0.4^{z}	6.08 ± 0.3^{z}

Values are Mean ± S.E.M; (n = 6). Statistical study by one way Analysis of variance then by Tukey's multiple comparison test. $^{c}p < 0.001$, related to normal rats; $^{x}p < 0.05$, $^{y}P < 0.01$, $^{z}p < 0.001$, related to diabetic neuropathy (DN); PLEE– *Premna latifolia* ethanol extract.

Table 2: PLEE effect on tail flick latency in STZ induced neuropathy rats

Group/Duration	*Normal control*	*Diabetic Neuropathy*	*DN + PLEE (100 mg/kg)*	*DN + PLEE (200 mg/kg)*
0th Day (Before induction)	10.41 ± 0.28	10.46 ± 0.32	10.16 ± 0.49	10.35 ± 0.29
14th Day (After induction)	10.18 ± 0.27	6.28 ± 0.29^{c}	5.81 ± 0.42	5.45 ± 0.38
26th Day	10.23 ± 0.18	5.25 ± 0.25^{c}	6.45 ± 0.39	6.47 ± 0.40
38th Day	10.36 ± 0.15	4.06 ± 0.31^{c}	7.10 ± 0.39^{z}	7.54 ± 0.42^{z}
49th Day	10.51 ± 0.22	2.85 ± 0.23^{c}	7.88 ± 0.36^{z}	8.55 ± 0.42^{z}

Values are Mean ± S.E.M; (n = 6). Statistical study done by one way Analysis of variance then by Tukey's multiple comparison test. $^{c}p < 0.001$, related to normal rats; $^{x}p < 0.05$, $^{y}p < 0.01$, $^{z}p < 0.001$, related to diabetic neuropathy (DN); PLEE– *P. latifolia* ethanol extract.

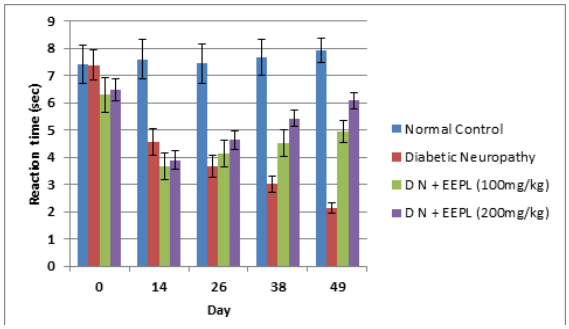

Fig. 1: PLEE effect on paw withdraw latency in STZ induced neuropathy rats. Diabetic neuropathy (DN); EEPL–ethanol extract of *P. latifolia*.

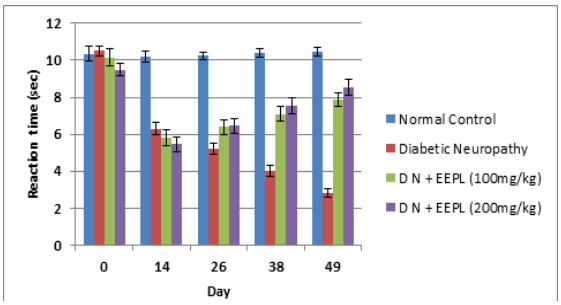

Fig. 2: PLEE effect on tail flick latency in STZ induced neuropathy rats. Diabetic neuropathy (DN); EEPL–ethanol extract of *P. latifolia*.

respond to non-noxious stimuli in various treated groups. (Table 1, 2 & Fig. 1, 2).

Influence of PLEE on Inflammatory Markers

Normal animals have not shown variations in blood glucose or pro-inflammatory and sciatic nerve growth factors throughout the study. Diabetic neuropathy animals exhibited notable ($p < 0.001$) rise in blood sugar, sciatic nerve tissue IL 1β, TNF α and IL 6 levels, however significant ($p < 0.001$) decline in NGF levels, related to normal rats. Low and high dose of PLEE treatment showed significant reduction of blood glucose, sciatic nerve tissue IL 1β, TNF α and IL 6 levels and also showed notable ($p < 0.001$) rise in NGF levels as related to diabetic neuropathy. PLEE showed maximum blood glucose, IL 6, TNF α and IL 1β and reducing, but NGF improvement effect at dose 200 mg/kg in neuropathy animals as related to its low dose. (Table 3 & Fig. 3).

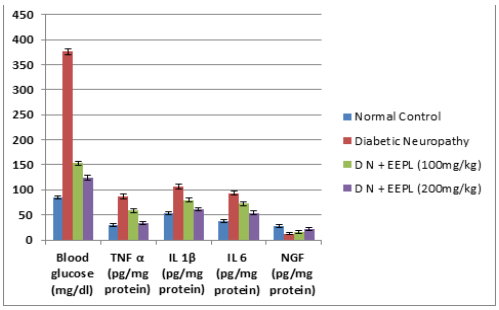

Fig. 3: Effect of PLEE on blood glucose, IL6, TNFα, IL1β and NGF levels in sciatic nerve of STZ-induced neuropathy rats. Diabetic neuropathy (DN); EEPL–ethanol extract of *P. latifolia*.

Influence of PLEE on Oxidative Markers

In sciatic nerve of normal rats have no significant variations of TBARS, GSH and SOD levels. Diabetic neuropathy group animal showed significant ($p < 0.001$) rise in TBARS but a significant ($p < 0.001$) fall in GSH and SOD was seen, related to normal rats. PLEE treatment made notable ($p < 0.001$)

Table 3: PLEE effect on blood glucose, interleukins, TNFα and NGF levels in sciatic nerve of STZ-induced DN rats

Group/Test	Blood glucose (mg/dl)	IL6 (pg/mg protein)	IL1β (pg/mg protein)	TNFα (pg/mg protein)	NGF (pg/mg protein)
Normal control	85.15 ± 3.53	38.14 ± 2.78	54.16 ± 3.07	29.08 ± 2.69	27.05 ± 2.84
Diabetic neuropathy	375.8 ± 5.86^{c}	92.56 ± 4.63^{c}	105.32 ± 5.31^{c}	86.91 ± 4.58^{c}	12.53 ± 1.73^{c}
Diabetic+ PLEE (100 mg/kg)	153.2 ± 4.283^{z}	72.53 ± 3.86^{z}	79.51 ± 3.89^{z}	58.47 ± 3.72^{z}	16.08 ± 2.56^{z}
Diabetic+ PLEE (200 mg/kg)	124.7 ± 5.438^{z}	54.17 ± 3.48^{z}	61.49 ± 2.78^{z}	33.85 ± 2.96^{z}	21.75 ± 2.49^{z}

Values are Mean ± S.E.M; (n = 6). Statistical study by one way Analysis of variance then by Tukey's multiple comparison test. $^{c}p < 0.001$, related to normal rats; $^{x}p < 0.05$, $^{y}p < 0.01$, $^{z}p < 0.001$, related to diabetic neuropathy (DN); PLEE– *P. latifolia* ethanol extract. TNFα - Tumor necrosis factorα; IL1β – Interleukin1β; IL6- Interleukin6; NGF- Nerve growth factor.

Table 4: Extracts effect on SOD, GSH and TBARS in sciatic nerve of STZ-induced neuropathy rats

Group/Test	*SOD (protein U/mg)*	*GSH (protein nM/mg)*	*TBARS (protein nM/mg)*
Normal control	02.72 ± 0.36	12.36 ± 1.53	14.07 ± 1.59
Diabetic neuropathy	01.53 ± 0.31^{c}	07.38 ± 1.23^{c}	38.75 ± 1.86^{c}
Diabetic + PLEE (100 mg/kg)	01.87 ± 0.41^{z}	08.63 ± 1.72^{z}	31.08 ± 2.46^{z}
Diabetic + PLEE (200 mg/kg)	02.34 ± 0.36^{z}	11.47 ± 2.08^{z}	24.53 ± 2.08^{z}

Values are Mean ± S.E.M; (n = 6). Statistical study by one way Analysis of variance then by Tukey's multiple comparison test. $^{c}p < 0.001$, related to normal rats; $^{x}p < 0.05$, $^{y}p < 0.01$, $^{z}p < 0.001$, related to diabetic neuropathy (DN); PLEE– *P. latifolia* ethanol extract. TBARS - Thiobarbituric acid reactive substances; GSH- Reduced glutathione; SOD- Superoxide dismutase.

Table 5: PLEE effect on antioxidants in sciatic nerve of STZ-induced neuropathy rats

Group/Test	*CAT (protein U/mg)*	*GPx (nM/mg protein)*	*GR (nM/mg protein)*
Normal control	03.91 ± 0.54	21.49 ± 1.87	13.93 ± 2.79
Diabetic neuropathy	02.23 ± 0.38^{c}	11.42 ± 1.53^{c}	06.96 ± 1.41^{c}
Diabetic + PLEE (100mg/kg)	02.85 ± 0.52^{z}	14.67 ± 1.81^{z}	08.52 ± 1.67^{z}
Diabetic + PLEE (200mg/kg)	03.17 ± 0.86^{z}	19.08 ± 2.14^{z}	12.39 ± 2.04^{z}

Values are Mean ± S.E.M; (n = 6). Statistical study by one way Analysis of variance then by Tukey's multiple comparison test. $^{c}p < 0.001$, related to normal rats; $^{x}p < 0.05$, $^{y}p < 0.01$, $^{z}p < 0.001$, related to diabetic neuropathy (DN); PLEE– *P. latifolia* ethanol extract. CAT- Catalase; GPx- Glutathione peroxidase; GR- Glutathione reductase.

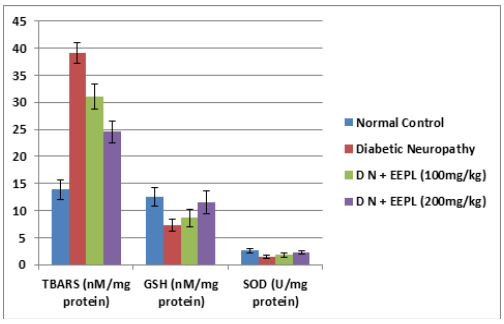

Fig. 4: Extracts effect on SOD, GSH and TBARS in sciatic nerve of STZ- induced neuropathy rats. Diabetic neuropathy (DN); EEPL–ethanol extract of *P. latifolia*.

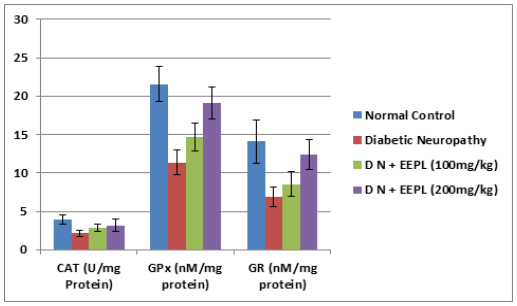

Fig. 5: Effect of PLEE on antioxidants levels in sciatic nerve of STZ-induced neuropathy rats. Each bar represents Mean ± S.E.M; (n = 6). Diabetic neuropathy (DN); EEPL–ethanol extract of *P. latifolia*.

fall of TBARS and notable ($p < 0.001$) rise in GSH and SOD related to diabetic neuropathy. PLEE showed maximum TBARS reduction, but GSH and SOD improved the effect at 200 mg/kg in neuropathy compared to its low dose. (Table 4, Fig. 4).

Normal animal not show significant variation in GPx, GR and CAT levels (in sciatic nerve). Neuropathy rats exhibited notable ($p < 0.001$) decrease in these levels during study, related to normal control. Low and high dose of PLEE treatment showed notable ($p < 0.001$) increase in CAT, GPx, and GR levels as compared to neuropathy group. In the PLEE 200 mg/kg treated animals maximum increase in all the antioxidant levels was observed as related to its low dose. (Table 5, Fig. 5).

DISCUSSION

Plants are the important natural sources, since humankind, they used to treat various ailments. To develop a new plant based drug, it has to be subjected for various studies in order to prove its efficacy, safety and validate scientifically. This study was undertaken to evaluate the neuroprotective property of *P. latifolia* on rats with diabetic neuropathy caused on by streptozotocin. Streptozotocin-induced diabetic neuropathy in animals is a highly useful model to examine to evaluate plant extract's ability to cause diabetic neuropathy. Significant hyperglycemia, hypoinsulinemia, weight loss, and altered lipid profiles are symptoms of streptozotocin-induced diabetes. By blocking the enzymes necessary for DNA synthesis, streptozotocin hinders DNA synthesis.[24] Hyperglycemic effect could be due to the destructive effect of streptozotocin on pancreatic islets.[25] Streptozotocin also enhances free radical formation and causes defects in antioxidant defense of pancreatic b –cells.[26]

Before the experiment, blood glucose levels were measured in all the group animals and confirmed that all animals were normal. STZ given 60 mg/kg body weight, after three days of administration glucose level measured and it is confirmed that animals were diabetic. Mechanism underlie raised in glucose level was; damaging DNA structure of beta cells; it gets alkylated by the nitrosourea group of STZ. Also NO is released by STZ binds to mitochondrial aconitase

enzyme and inhibits the formation of ATP but activates xathine oxidase. These mechanisms lead to formation of highly reactive free radicals like O_2^-, Peroxynitrate (ONOO), OH^- and H_2O_2 which produces oxidative stress and damaging of cells. Due to all these beta cells sensitivity for glucose was reduced and unable to synthesize normal quantity of the insulin and release. This leads to increase glucose level in blood, low insulin levels also cells slowly but surely develop resistance to the insulin.[27]

Hyperglycemia if persists for long period leads to complications called diabetic complications. One of major complication is DN, due to defects in structure and functioning of neurons involved in this process. Diabetic untreated consequences are described below. This promotes polyol pathway, aldose reductase converts glucose to sorbitol. Sorbitol unable to cross cell, gets accumulated in nerves.[28] Because of enhanced turnover of NADPH and NAD+, decrease in the formation of glutathione, enhanced production of advanced glycation end products and diacylglycerol. Reduced glutathione level leads to oxidative stress and other toxic effects.[29] The other mechanism of development of oxidative stress is autoxidation of glucose, altered mitochondrial function, protein kinase C and activation of hexosamine pathway.[30,31] Reactive nitrogen species mainly peroxynitrite is also contribute for development of neuropathy. Microvascular complications like increase in thickness of blood vessels, that carry oxygenated blood to nerves, and reduced lumen size causes nerve ischemia. Because of ploy & hexosamine pathway activation, high blood glucose and altered lipid levels causes increased reactive oxygen species, DNA damage, mitochondrial dysfunction and low blood supply to neurons (hypoxia). All these mechanism leads to altered structure, electric activity and function of nerves.[32] These changes or developments occurs in animal models, normally after two weeks of diabetes induction, called it as diabetes induced neuropathy

Characteristic features or symptoms of DN are pain, hot sensation, tingling or loss of sensation in feet, hyper responsiveness to normal stimuli. It can be assessed by variety of tests: response of animals for non-noxious stimuli like hot stimulation tested by conducting eddy's hot plate test, another test response of animals for non-noxious stimuli like cold- it is tested by immersion of tail in cold water. Study report the diabetic untreated animals become hyperalgesia, they showed hypersensitivity for the hot and cold stimuli. This indicates there was defect in structure, functioning (or both) of nerves involved in this process. This was confirmed measuring biochemical's in sciatic nerve of animals. There was rise in TNF-α, IL-1β and IL-6 levels, but nerve growth factor level was significantly reduces. There was significant increase in the levels of TBARS, decreased glutathione reductase, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase. The extract normalizes the response of animals to the non-noxious stimuli, by restoring structure and function of sciatic nerve. This was because extracts improve the level of protective factors like nerve growth factor and suppress pro-inflammatory cytokines and reactive oxygen species in sciatic nerve. PLEE high dose showed superior effect as compared to its low dose.

Phytoconstituents present in PLEE are steroids, glycosides, carbohydrates, flavonoids, phenolic compounds, tannins and alkaloids. The phytochemicals present in the plant supports findings in this study. From free radicals cells are safeguarded by flavonoids.[33] Phenolic compounds increase protective enzyme levels, indirectly acting as antioxidants.[34] As per the earlier reports, phytochemicals like steroids of *Elephantopus scaber*,[35] glycosides in *picralima nitida*,[36] tannic acid, condensed tannins and polyphenols in *Passiflora ligularis*[37-39] and alkaloids in *Aerva lanata*,[40] had shown useful effects in the treatment of diabetes and also reduce the diabetic complications.[41,42] The neuroprotective effect of ethanolic extracts of plant *P. latifolia* is due to phytoconstituents' individual action or might be due to synergetic effect of all the constituents. Based on the neuroprotective study results, PLEE 200 mg/kg body weight was showed promising result.

Conclusion

Ethanolic extract of *P. latifolia* leaves extract (200 mg/kg) had significant neuroprotective activity in streptozotocin-induced diabetic neuropathy rats. Activity was due to the normalization of blood glucose level, pro-inflammatory cytokines, nerve growth factor, oxidative stress markers, and thiobarbituric acid reactive substances in sciatic tissue. Extraction normalizes the structure and functions of the pancreas and sciatic nerve tissue by regenerating cells.

Acknowledgement

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Ethics Statement

Research activities carried out in accord with the Committee for Control and Supervision of Experiments on Animals (CCSEA). The KLE College of Pharmacy's institutional animal ethical committee Hubballi, Karnataka, India, authorized the protocol. (IAEC: KLEU's-09-IAEC.HBL-31/Aug 2013).

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