Sumithira G. et al., / Vol. 05 / Issue 02 / 2017 / 45 – 58.

Research Article

Available Online at: www.ijphr.com
An African Edge Journal

International Journal of Pharmaceuticals and Health care Research

SJ Impact Factor – 5.546

ISSN: - 2306 – 6091

EVALUATION OF NEUROPROTECTIVE EFFECT OF *PLECOSPERMUM SPINOSUM* TREC IN EXPERIMENTALLY INDUCED DIABETIC NEUROPATHIC PAIN IN RATS

*Sumithira G, Ashma A, Akhilan D, Muhammed Shabeer A, Kavya V
Department of Pharmacology, The Erode College of Pharmacy and Research Institute
Vaeppampalayam, Perundurai main road, Vallipurathanpallaiyam (Post)
Erode- 638112, TN, India

Abstract

The present study was designed to screen the Neuroprotective effect of *EEPS* on Streptozotocin induced diabetic neuropathic pain in rats. Diabetes was induced in overnight fasted experimental rats with a single Intraperitoneal injection of Streptozotocin (55 mg/kg b.w). Diabetic rats exhibited significant hyperglycemia and rats were left untreated for first four weeks. Thereafter, treatment was initiated and continued up to week- 8. Treatment with *EEPS* (200&400 mg/kg) and pregabalin for 4 weeks significantly attenuated the nociception in behavioral models. Various biochemical parameters were assessed by collecting blood from retro orbital sinus puncture. Treatment with freshly prepared *EEPS* significantly reduced blood glucose in diabetic rats. *EEPS* also inhibited the AST, ALT and ALP and STZ induced loss of body weight, Total protein and Albumin was attenuated by the *EEPS*. The Effect of *EEPS* (400 mg/kg) is comparable to the standard drug Pregabalin (10 mg/kg). At the end of the study animals were sacrificed and explored the sciatic nerve & liver tissues which were isolated for further histopathological studies and oxidative stress parameters. *EEPS* increased the SOD, CAT, GSH and decreased the LPO levels in dose dependent manner. In comparison to STZ alone treated rats, *EEPS* exhibited significant increase in the pain threshold response. The result of nociceptive, oxidative stress parameter, histological and biochemical markers indicate the protective anti-nociceptive, anti-oxidant and neuroprotective properties of *Plecospermum spinosum* in preventing the progression of diabetic neuropathy.

Keywords: Diabetes, Streptozotocin, *Plecospermum Spinosum*, Pregablin.

Received on- 04.05.2017; Revised and accepted on- 17.05.2017; Available online- 22.05.2017

Introduction

Diabetes is a heterogeneous group of diseases characterized by chronic elevation of glucose in blood (or) it is a syndrome of disordered metabolism usually due to combination of hereditary & environmental causes resulting in abnormally high blood sugar levels (hyperglycemia).According to the International Diabetes Federation, India's population of people living with diabetes today is 65.1 million as compared to 50.8 million in 2010.That number is expected to cross 100 million by 2030[1].

Long standing diabetes mellitus leads to multiple organ damage and it is associated with an increased prevalence of microvascular disease (Nephropathy, Neuropathy& Retinopathy) and macro vascular diseases (Peripheral vascular disease, Ischemic heart disease& Stroke). Poor glycemic control, a

Author for Correspondence:
Sumithira G
Email: ashmamahab93@gmail.com

factor that has been observed in the Indian population with diabetes put them at risk of complication including neuropathy-24.6%, cardiovascular disease-23.6%, kidney problem-21.1%, retinopathy- 16.6% and foot ulcer-5.5%.Diabetic neuropathies are nerve damaging disorder associated with diabetes mellitus. It occurs when the high blood sugar interferes with the ability of the nerves to transmit signals; It also weakens the walls of the small blood vessels (capillaries) that supply the nerves with oxygen & nutrients[2]. In addition, inherited factors probably unrelated to diabetes may make some people more susceptible to nerve disease than others. Neuropathy may be diffuse, affecting many parts of the body, or focal, affecting a single, specific nerve and part of the body. When the nerves are damaged it often results in loss of reflexes and muscle weakness. The foot often becomes wider and shorter, the gait changes, and foot ulcers appear as pressure is put on parts of the foot that are less protected. Because of the loss of sensation, injuries may go unnoticed and often become infected[3]. Diabetic neuropathy can cause a number of serious complications include loss of a limb, Charcot joint, Urinary tract infection, hypoglycemia unawareness, low blood pressure, digestive problems, sexual dysfunction and increased or decreased sweating[4].Diabetic autonomic neuropathy account for silent myocardial infarction and shortens the lifespan resulting in death in 25%-50% patients within 5-10 years of autonomic diabetic neuropathy.

At present, the available synthetic drugs for the treatment of diabetic complications includes antioxidants selective serotonin reuptake inhibitors, antidepressants, anti-arrhythmics, polyphenols, anticonvulsants and opioids, which has met limited success in clinical trials. A large number of plants used in the traditional medicine have now become a part of the modern world health care system as they show promising therapeutic effect, minimal side Effects, cheap and easily available. Various medicinal plants/plant extracts containing flavonoids, alkaloids, phenolic compounds, terpenoids and glycosides type chemical constituents were found to be effective in the management of diabetic complications. This effect might be attributed to amelioration of persistent hyperglycemia, oxidative stress and modulation of various metabolic pathways involved in the pathogenesis of diabetic complications.[5]

Plecospermum Spinosum Trec., is commonly called as “Paper cup flower". It is one of the traditional plant grown in India as it have several phytoconstituents and pharmacological activities it is used as medicinal plant for treating disease like myocardial infarction, diabetes, cancer, cholera, cold, jaundice and tooth ache[6]. Major Phytochemicals present in this genus contain different natural compounds mainly phenols, Terpenoids, Flavonoids, Tannins, alkaloids and glycosides. One of the major chemical constituent of *Plecospermum Spinosum* is Flavonoid. The present study was designed to evaluate the neuroprotective effect of *Plecospermum Spinosum* in induced diabetic neuropathy.

Experimental section

Plant materials

The Aerial parts of *Plecospermum Spinosum* Trec., was purchased from a ABS garden in Salem and it was authenticated by Dr. A.B. Subramanium, ABS botanical garden, Kaaripatti, Salem.

Extract preparation

The aerial parts of *Plecospermum Spinosum* were collected and dried under shade and grinded into powder. Ethanolic extract of *Plecospermum Spinosum* Trec., was done by using soxhlet apparatus.

Acute toxicity study

Acute toxicity study of Ethanolic extract of the aerial parts of *Plecospermum Spinosum* Trec., was determined in Wistar albino rats (150-200 gm) according to OECD guidelines[7] No: 423. Based on performed toxicity tests the LD_{50}. Dose were selected as 200 and 400 mg/kg.,po.

Drugs and chemicals

Streptozotocin was obtained from Sigma and Pregabalin was purchased from Ranboxy Laboratories Pvt. Ltd. Bombay , India.

Animals used

Wistar albino rats of male sex weighing between 150-200 gm were gathered from the Nandha College of pharmacy, Erode. The rats were housed in cages under standard laboratory conditions 23±2° c with 2 h light and dark cycle and had free access to water with standard chow diet. Animals care should be taken as per guidelines of the committee for the purpose of control and supervision of

experiments on animals (CPCSEA). Approval was taken from the Institutional Animal ethics committee for the study. (IAEC No: NCP/IAEC 2016-17-04).

Experimental design

Experimental induction of diabetes

Streptozotocin (STZ) was dissolved in 0.1 M sodium citrate buffer, pH 4.4 and administered at the dose of 55 mg/kg through i.p. route.[8] Blood samples were taken from the tail vein 48 h after STZ injection. Animals with fasting blood glucose levels over ≥ 250 mg/dl were considered diabetic and used for the further study.

Diabetic rats were randomly divided into five groups each group consists of 6 animals.

Group I - Served as control, received 0.5% N.S (1ml/kg; p.o).

Group II – Diabetic control receives Streptozotocin (55 mg/kg, b.wt; ip) + Insulin (5 IU/kg) freshly prepared in citrate buffer

Group III – STZ + Insulin + Standard pregabalin (10mg/kg) dissolved in 0.5% CMC.

Group IV – STZ+ Insulin+ EEPS (200 mg/kg, b.wt; p.o) suspended in 0.5% CMC

Group V – STZ+ Insulin + EEPS (400 mg/kg, b.wt; p.o) suspended in 0.5% CMC

In the first four weeks of the study all the groups were left untreated. From week 4 onwards the drug treatment started and continued up to the week 8 after STZ injection. Blood glucose and Body weight were measured and Behavioral assessments like Tail flick, Tail clip, Hot plate and formalin test were performed on 8th week of the study. At the end of the study the biochemical assessment (AST, ALP, ALT, Total protein and Albumin) were tested by collecting blood from retro orbital sinus puncture with mild anaesthesia and then the organ weights were noted on standard electronic weighing machine and measurement taken with wooden organ measuring boards. After collection of blood, all the treated animals were sacrificed. At the end of the experiment study, the major organs were harvested, weighed and histological evaluation of sciatic nerve was performed, further liver were isolated for antioxidant levels.

Behavioral assessment

Tail flick test

The nociceptive response was evaluated regarding the latency to withdrawal of the tail in response to noxious radiant heating. Animals were placed into individual restraining cages leaving the tail hanging out freely. The animals are allowed to adapt to the cages for 30 min before testing. The apparatus used is tail flick analgesiometer, the tip of tail of rat is placed on hot metal wire and latency of withdrawal is calculated manually by stop watch[9, 10].

Tail clip test

Tail clip test was performed by the method of Haffner[11]. A metal artery clip was applied to the root of each mouse's tail to induce pain. A sensitivity test was carried out, and animals that did not attempt to dislodge the clip within 10s were discarded. The responsive rats were placed in groups of 6 each containing 10 animals. The tail clip was applied 0, 30, 60 and 90 mins. The reaction time at various intervals was noted.

Hot plate test

In this hot plate method animals from the each group were placed on the hot plate (Eddy's hot plate) which is commercially available consists of an electrically heated surface. Temperature of this hot plate is maintained at 55°C- 56°C. This can be a copper plate or a heated glass surface. The observation is done up to the time until paw licking or jumping was noted the cut- off time was 10 sec[12, 13].

Formalin test

Animals were administered 0.05 ml of 10% formalin into the dorsal portion of the front paw. The test drug is administered orally. Each individual rat is placed into a clear plastic cage for observation. Pain responses are indicated by elevation or favoring of the paw or excessive licking and biting of the paw. Analgesic response or protection is indicated if both paws are resting on the floor with no obvious favoring of the injected paw. Nociception was evaluated by measuring the number of seconds that the animals spent on licking or bitting the injected area with the front paw[14, 15].

Evaluation of biochemical parameters

Total protein level

The protein concentration of the serum was estimated by the method of Lowry[16]. In alkaline solutions, protein forms a complex with copper ions and this copper – protein complex reacts with Folin - ciocalteau reagent to give a blue colour due

to the reduction of phosphomolybidate by thyrosine and tryptophan present in the protein. The intensity of the colour is proportional to the concentration of protein. By using spectrophotometer the changes in the absorbance was recorded at 720 nm. The values were expressed as gm/dl in serum.

Albumin level

The Albumin concentration of the serum was estimated by Bromocresol green method.[17] Under acidic conditions, serum albumin binds specifically with bromocresol green to form a green coloured complex. The absorbance is read at 640 nm. The values were expressed as gm/dl in serum.

Evaluation of liver marker enzymes

Aspartate aminotransferase (AST) level

The enzyme activity was assayed by Reitman and Frankel method[18]. The change in the absorbance is due to conversion of L-aspartate and oxoglutarate to oxaloacetate and L-gluatamate. By using spectrophotometer the changes in the absorbance was recorded at 520 nm. The values were expressed as U/L in serum.

Alanine aminotransferase (ALT) level

The enzyme activity was assayed by Reitman and Frankel method[18]. The change in the absorbance is due to conversion of L-alanine and oxoglutarate to oxaloacetate and L-gluatamate. By using spectrophotometer the changes in the absorbance was recorded at 520 nm. The values were expressed as U/L in serum.

Alkaline phospatase (ALP) level

The method used was that of King and Armstrong[19] in which disodium phenyl phosphate is hydrolysed with liberation of phenol and inorganic phosphate. The liberated phenol was measured at 700 nm with Folin-Ciocalteau reagent. The activity was expressed as units/L in serum.

Evaluation of antioxidant enzymes and lipid peroxide levels

Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 min, supernatant was collected and used for antioxidant enzymes and Lipid peroxide levels.

Superoxide dismutase (SOD)

SOD activity was assayed in the liver homogenate according to the method of Misra and Fridovich.[20] The enzyme catalyzes the dismutation of superoxide anion ($O2^{-\cdot}$) to hydrogen peroxide and molecular oxygen. The absorbance was recorded at 480 nm. The activity was expressed as units/gm protein.

Catalase (CAT)

Catalase activity was assayed in the liver homogenate according to the method of Sinha[21]. Hydrogen peroxide (H_2O_2) decomposition by CAT was monitored spectrophotometrically by following the decrease in absorbance at 610 nm. The activity of enzymes was expressed as µmoles of H_2O_2 decomposed/min/gm protein.

Reduced glutathione (GSH)

GSH content was estimated by following the method of Ellman[22]. In this assay, GSH reduced 5, 5'- dithiobis (2-nitrobenzoic acid) to 5-thio-2-nitrobenzoic acid. The absorbance was measured spectrophotometrically at 412 nm. The amount of GSH was expressed in µM/ gm protein.

Lipid peroxidation level (LPO)

The quantitative measurement of lipid peroxidation was performed in the liver homogenate according to the method specified by Ohkawa[23]. The amount of malondialhyde (MDA) and other thiobarbituric acid reactive substance (TBARS) were quantified by their reactivity with TBA in acidic conditions. The reaction generated a pink colour chromophore which was measured by a UV spectrophotometer at 532 nm. The results were expressed as nmoles MDA/gm protein.

Statistical analysis

The data of all the results were represented as Mean ± S.E.M. on statistically analyzed by one-way ANOVA followed by Duncun's Multiple range test[24] was used for statistical analysis $p<0.05$ was considered significant.

Results

Effect of *EEPS* on blood glucose, body weight and organ weight in diabetic rats

Administration of STZ (55 mg/kg) significantly elevated (p<0.001) Blood Glucose, Kidney weight and whereas the body weight, liver and heart weight were decreased when compared normal control group. Treatment with Pregabalin and *EEPS* (200 mg/kg& 400 mg/kg) significantly (p<0.001) decrease in Blood Glucose and kidney weight and increase in body weight, liver and heart weight when compared to Diabetic control group. (Table No: 1)

Table No. 01: Effect of *EEPS* on blood glucose, body weight and organ weight in Experimentally induced diabetic rat model

Group	Glucose (mg/dl)	Body weight (gm)	Organ weight (gm)		
			Liver	Kidney	Heart
Group I 0.9% NS	91.33±1.84	193.54±4.81	3.68±0.18	0.93±0.05	2.68±0.36
Group II STZ(55mg/kg)+ Insulin (5 IU/kg)	248.36±4.31***	158.18±3.16***	1.93±0.11**	1.84±0.06*	1.97±0.13*
Group III STZ(55mg/kg) + Insulin(5IU/kg) + Pregabalin(10 mg/kg)	118.46±1.31ns^{aa}	182.18±5.16ns^{aa}	3.14±0.54ns^{a}	1.09±0.05ns^{a}	2.21±0.73ns
Group IV STZ(55mg/kg)+ Insulin (5 IU/kg)+ *EEPS*(200 mg/kg)	141.31±1.94*^{a}	169.55±6.18*ns	2.96±0.24*ns	1.21±0.08ns	2.46±0.16^{a}
Group V STZ(55mg/kg)+ Insulin (5 IU/kg)+ *EEPS*(400 mg/kg)	104.56±1.56^{aa}	191.85±7.54^{aa}	3.48±0.13^{aa}	0.98±0.07^{aa}	2.51±0.27^{a}

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).
*,**,***indicates significance p<0.05, p<0.001 & p<0.0001 when compared to control group
a, aa indicates significance p<0.05 and p<0.0001 when compared to Diabetic control
NS indicates non-significant

Effect of *EEPS* on total protein and albumin in diabetic rats

The levels of Total protein and Albumin in plasma were reduced significantly (p>0.001) in STZ induced diabetic group compared to normal control group. The level of Total protein and albumin recovered to normal after treatment with Pregabalin and *EEPS* (200& 400 mg/kg). (Fig No. 01)

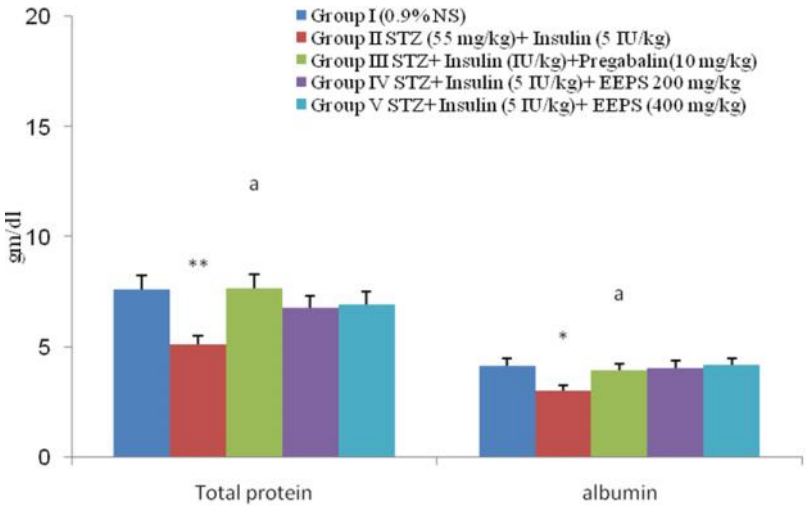

Fig. No. 01: Effects of *EEPS* on Total protein and Albumin in experimentally induced Diabetic rat model

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).
*,** indicates significance p<0.05, p<0.001 when compared to control group
a indicates significance p<0.05 and p<0.0001 when compared to Diabetic control
NS indicates non-significant

Effect of *EEPS* on serum aspartate aminotransferase (AST) level, serum alanine aminotransferase (ALT) level and serum alkaline phospatase (ALP) level

The activities of ALT, AST and ALP were significantly (p>0.0001) elevated in STZ diabetic control when compared with the normal control. Rats administered *EEPS* showed significant reduction in these marker enzymes activites to normal level which were not significantly different when compared to control group. (Fig No:2)

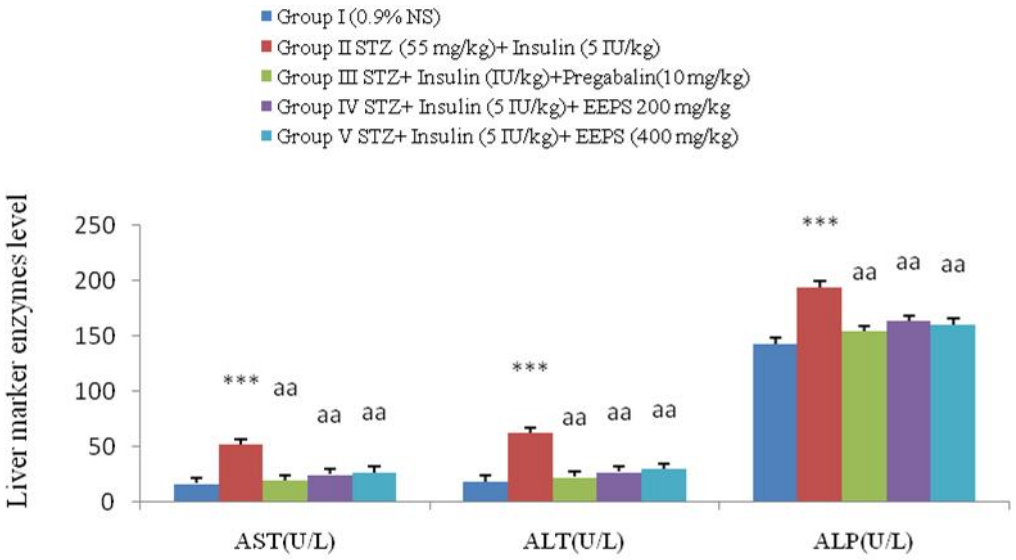

Fig. No. 02: Effects of *EEPS* on Liver marker enzymes (AST, ALT and ALP) in Experimentally induced Diabetic rat model

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).
*** indicates significance p<0.0001 when compared to control group
aa indicates significance p<0.05 and p<0.0001 when compared to Diabetic control
NS indicates non-significant

Effect of *EEPS* on antioxidant enzymes and lipid peroxide levels

Pregabalin and *EEPS* (200 & 400 mg/kg) treated diabetic group exhibited significant (p>0.001) increase in the SOD activity in the Liver homogenate in comparison to Diabetic group. (Fig No. 03)

Significant (p>0.001) increase in CAT activity in the liver homogenate was observed in *EEPS* (200 & 400 mg/kg) and Pregabalin when compared to diabetic group. (Fig No. 03)

Treatment of Pregabalin and *EEPS* (200 & 400 mg/kg) exhibited a significant (p>0.001) increase in the GSH levels in comparison to diabetic group.(Fig No. 03)

Lipid peroxidation level

Diabetic group showed significant (p>0.0001) increase in LPO levels when compared to normal control group. Pregabalin and *EEPS* (200 & 400 mg/kg) significantly (p>0.001) decrease the LPO levels in comparison to diabetic group. (Fig No. 04)

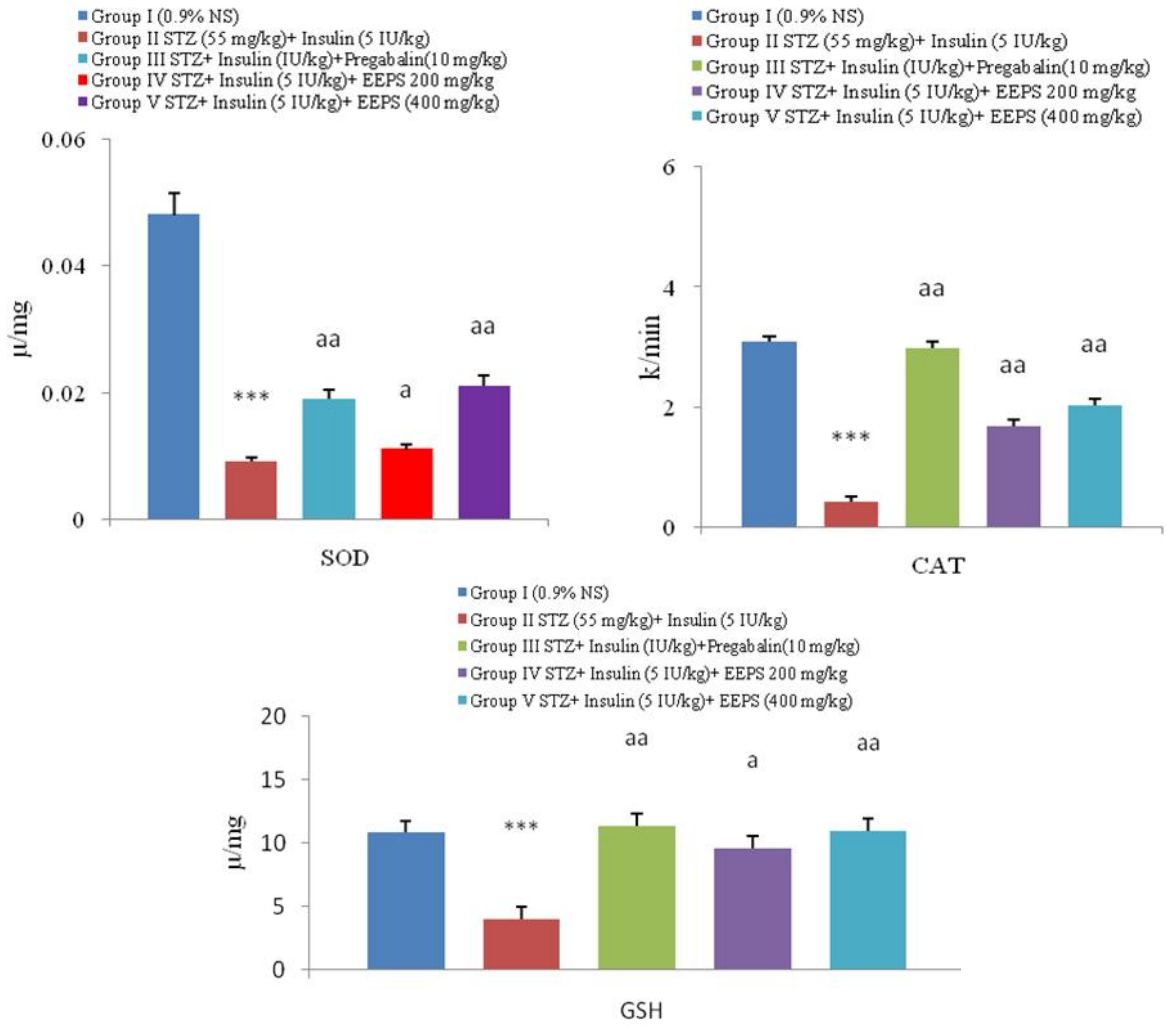

Fig. No. 03: Effects of *EEPS* on antioxidant levels (SOD, CAT and GSH) in Experimentally induced diabetic rat model

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*** indicates significance p<0.0001 when compared to control group

aa indicates significance p<0.0001 when compared to Diabetic control

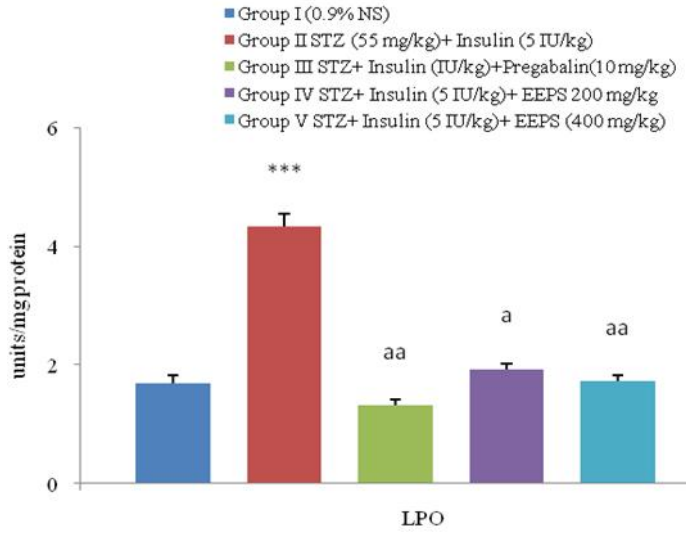

Fig. No. 04: Effects of *EEMP* on Lipid peroxidation level in Experimentally Diabetic induced rat model.

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).
*** indicates significance p<0.0001 when compared to control group
a, aa indicates significance p<0.05 and p<0.0001 when compared to Diabetic control

Behavioral assessment

Effect of *EEPS* on tail flick test:
(Thermal Hyperalgesia)

EEPS (200 & 400 mg/kg) and Pregabalin has significantly (p > 0.0001) increased the tail withdrawal latency of STZ induced diabetic group as compared to normal control and diabetic control. (Fig No. 05)

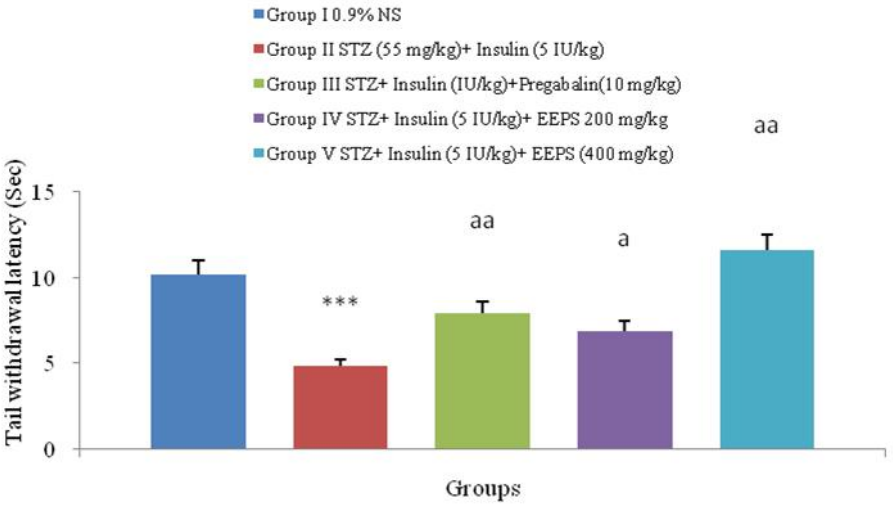

Fig. No. 05: Effects of *EEPS* on Tail flick test (Thermal hyperalgesia) in Experimentally induced Diabetic rat model

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).
*** indicates significance p<0.0001 when compared to control group
a, aa indicates significance p<0.05 and p<0.0001 when compared to Diabetic control

Effect of *EEPS* on tail clip test

The Diabetic control Group showed significant (p>0.0001) decrease in reaction in time when compared to the normal control Group. Pregabalin and *EEPS* (200& 400 mg/kg) showed statistically significant (p>0.0001) increase in reaction in time when compared to Diabetic control Group. (Fig No. 06)

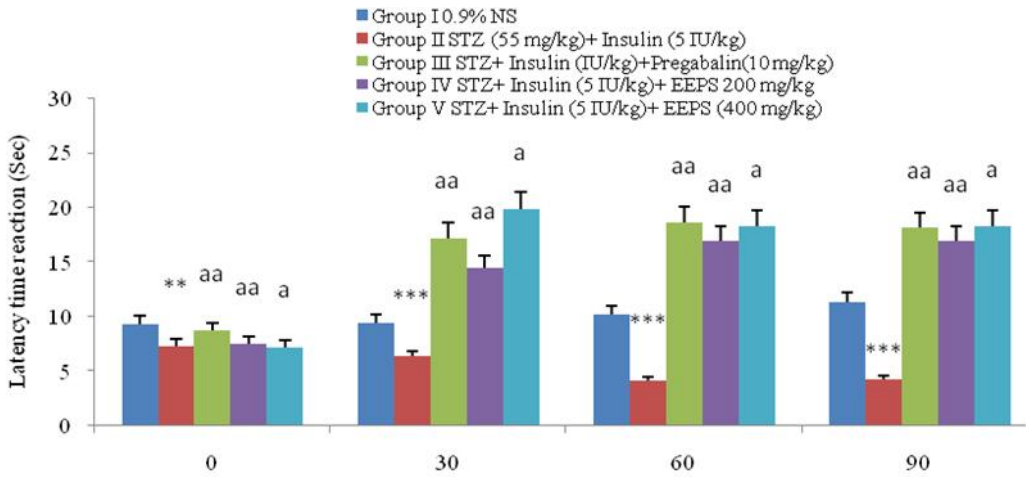

Fig. No. 06: Effects of *EEPS* on Tail Clip test in Experimentally induced Diabetic rat model

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).
,*indicates significance p<0.001 & p<0.0001 when compared to control group
a, aa indicates significance p<0.05 and p<0.0001 when compared to Diabetic control

Effect of *EEPS* on hot plate test: (Thermal hyperalgesia)

The Diabetic control Group showed significant ($p<0.0001$) decrease in Paw withdrawal latency when compared to the normal control Group. Pregabalin and *EEPS* (200& 400 mg/kg) showed statistically significant increase in Paw withdrawal latency when compared to Diabetic control Group. (Fig No. 07)

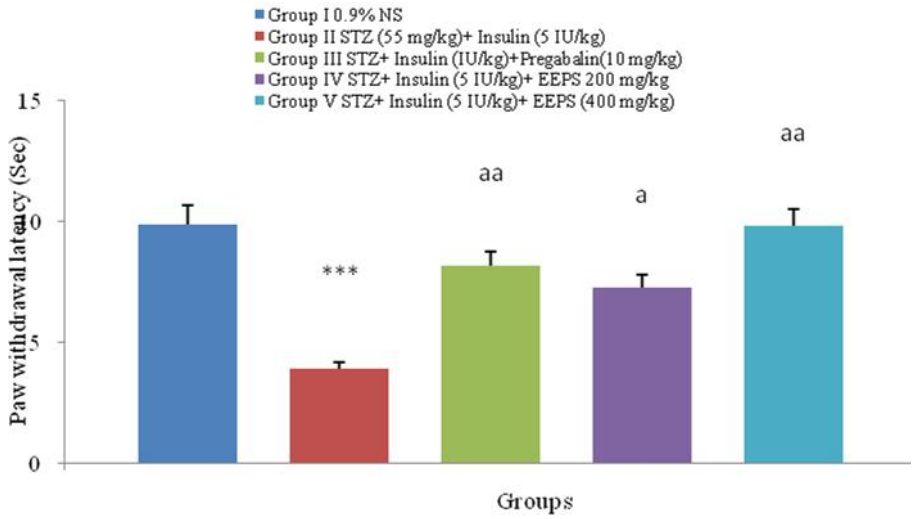

Fig. No. 07: Effects of *EEPS* on Hot Plate Test (thermal hyperalgesia) in Experimentally induced Diabetic rat model

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*** indicates significance $p<0.0001$ when compared to control group

a, aa indicates significance $p<0.05$ and $p<0.0001$ when compared to Diabetic control

Effect of *EEPS* on formalin test: (Nociception)

The Diabetic control Group showed significant ($p>0.0001$) decrease in intensified nociceptive response and analgesic response when compared to the normal control Group. Pregabalin and *EEPS* (200& 400 mg/kg) showed statistically significant ($p>0.0001$) increase in intensified nociceptive response and analgesic when compared to Diabetic control Group. (Fig No. 08)

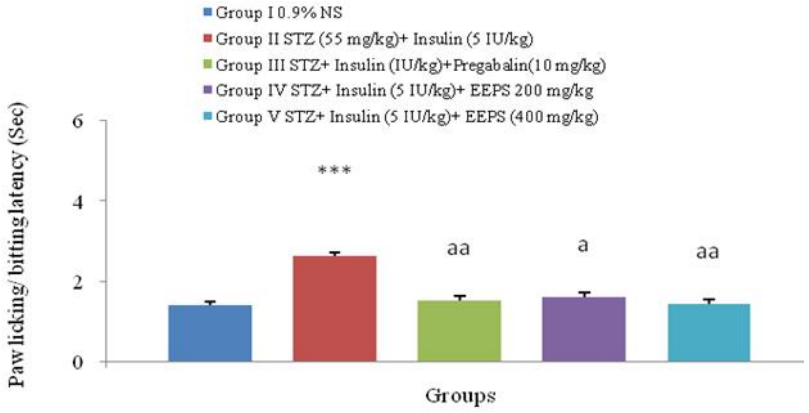

Fig. No. 08: Effects of *EEPS* on Formalin test (Nociception) in Experimentally induced Diabetic rat model

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*** indicates significance $p<0.0001$ when compared to control group

a, aa indicates significance $p<0.05$ and $p<0.0001$ when compared to Diabetic control

Histopathology of sciatic nerve

The Ehrlich's Haemaoxylin stained lateral section of diabetic control group (B) reduced nerve fiber density in the endoneurium when compared to normal control group (A). After treatment with Pregabalin and *EEPS* (400 mg/kg body weight) in diabetic rats showed effective regeneration of recovery (almost 80%) of sciatic nerve fibres is comparable to normal control group. (Fig No. 09)

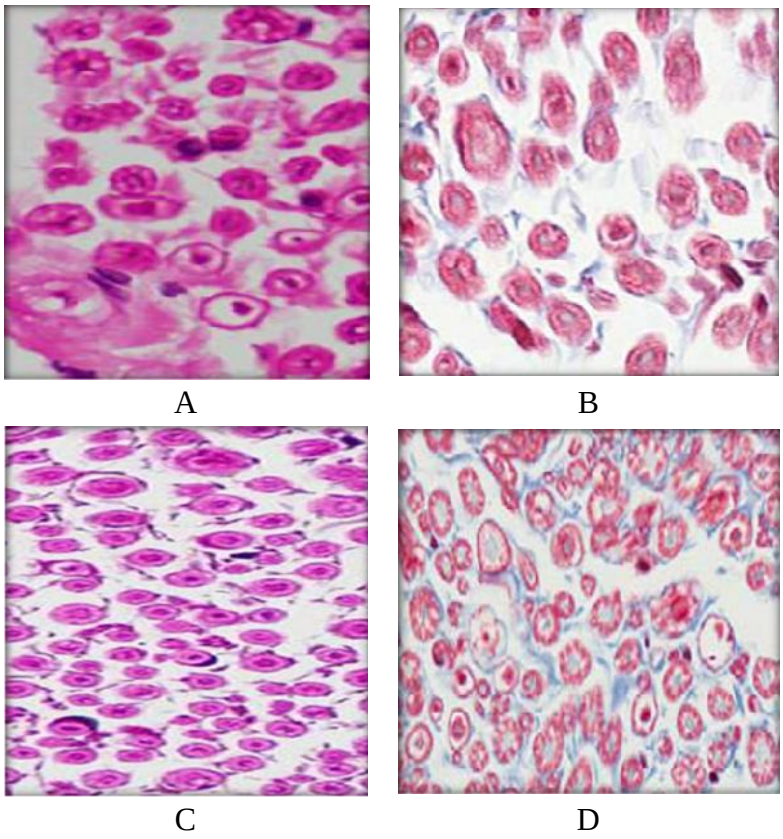

Fig. No. 09: Photomicrograph of H&E stained lateral sections of sciatic nerves

A. Normal control (Sciatic nerve fibre density is well preserved and the fibres are compactly arranged**), B. Diabetic control (**reduced nerve fiber density in the endoneurium**), C. STZ+Insulin (5 IU/kg)+ Pregabalin 10mg/kg (**Restored the normal architecture of sciatic nerve fibre which is similar to that of control group**), D. STZ+ Insulin (5 IU/kg)+*EEPS* 400mg/kg (**Effective regeneration of recovery (almost 80%) of sciatic nerve fibres.

Discussion

Diabetes mellitus is chronic metabolic disorder with late complications as diabetic neuropathy and is frequently painful, with the pain involving predominantly the distal extremities. Pain associated with diabetic neuropathy can occur either spontaneously or as a result of exposure to only mildly painful stimuli (hyperalgesia) or to stimuli not normally persued as painful (allodynia)[25]. Development of diabetic neuropathy involves depressed Na^+, K^+- ATPase activity, activation of cyclooxygenase, modulation of adenosinergic and opioidergic system and increased oxidative stress[26, 27, 28]. Hyperglycemia could enhance the generation of reactive oxygen species which in turn gave rise to the increased neuronal damage by oxidizing proteins, lipids and augmented levels of lipid peroxidation products in cellular membranes[29, 30].

Neuropathic pain associated with peripheral nerve injury is characterized by the sensory abnormalities such as unpleasant abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia) and pain in response to a stimulus that does not normally provoke pain (allodynia)[31].

In this study, *Plecospermum Spinosum* Trec., extract was given for the treatment of neuropathic pain in STZ induced diabetic rats.

The preliminary phytochemical analysis of *EEPS* revealed the presence of carbohydrates, flavanoids, terpenoids, glycosides, Alkaloids, tannins and phenols. It has been found that presence of flavanoids in the plant *plecospermum Spinosum* shows anti-diabetic action[32].

Acute toxicity studies revealed the non-toxic nature of the *EEPS* there was no lethality or any toxic reactions found with high dose (2000 mg/kg body weight) till the end of the study. According to the OECD 423 guidelines (Acute Oral Toxicity: Acute Toxic Classic Method), an LD_{50} dose of 2000 mg/kg and above was considered as unclassified so the *EEPS* was found to be safe.

Experimentally induced diabetes by STZ in rodents is a well-known animal model to investigate metabolic and pharmacological changes associated with diabetes[33]. Rats injected with 55mg/kg showed significant increase in blood glucose levels and decreased nociceptive thresholds. Similar thermal hyperalgesia, mechanical and formalin evoked pain in STZ induced rats have been demonstrated earlier[34, 35].

Pregabalin a selective Ca_2 (α2-δ subunit) channel anatagonist and anticonvulsant which is successfully being used to treat neuropathic pain syndrome has reversed the STZ- induced hyperalgesicresponse[36]. Treatment with *EEPS* extract in diabetic rats significantly increased the nociceptive threshold and decreased oxidative stress in a dose-dependent manner.

Diabetic rats showed a significant rise in blood glucose levels and decreased body weight &had lighter heart than the normal rats. Hyperglycemia in turn influence proteolysis in skeletal muscle and lipolysis in adipose tissues results in severe weight loss in the animal models& the lighter hearts were responsible for depressed ATPase values. ATP activity would be expected higher in lighter hearts.[37,38, 39] Our in vivo results showed that *EEPS* extract (200 and 400 mg/kg) treatment had an attenuating effect on the serum glucose of the diabetic animals with significant improvement in body weight it may be due to *EEPS* persisting insulin secretogoge action like sulfonylureas.

The increase in kidney weights in diabetic rats may have been caused by hyper filtration, aggregation of lymphocyte and fat in filtrations and glomerular hypertrophy which indicates increase in size and area of the glomeruli. The pathological effects of hyperglycemia on the hepatic tissue have been demonstrated by elevated serum hepatic enzymes and liver hypertrophy. The AST, ALT and ALP are hepatic enzymes that are assessed to monitor hepatic integrity and cardiac injuries. Elevation of serum liver enzymes which is strongly point to hepatic injury.[40] In this current study, there was a marked increase in serum activities of hepatic enzymes in diabetic group when compared to control group. Increased serum activity of ALP in hyperglycemic environment was linked to the peroxidation of lipids in the cell membranes of hepatic cells whereas leakage of AST and ALT from the hepatocytes cytosol into the blood was reported to be caused by the disruption of hepatocytes' cell membranes due to accumulation of toxic free fatty acids (FFAs).[40] On treatment with the EEPS shows significant reverse in their enzyme levels.

Decreased levels of both albumin and total protein in diabetic rat may occur consequent to decreased protein synthesis rate. Protein synthesis in diabetics is derailed because the mechanisms involved in protein synthesis require ATP from glucose metabolism, in which glucose metabolism is attenuated in diabetes. Gluconeogenesis or protein catabolism occurs in diabetes in an attempt to balance ATP production[42]. In the present study, there were significant decreases in the serum levels of total protein and albumin in diabetic groups when compared to the normal control group. Reduced levels of the proteins may be due to hyperglycemia which targets proteins that would then be utilised in advanced glycation end products (AGEs) formation. Other reasons to explain the decrease involves increase in protein catabolism, decreased synthesis of proteins, liver damage, and renal loss (polyuria) due to renal impairment. On treatment with *EEPS* there is increased in the level of total protein and albumin.

In the present study, we have demonstrated that *EEPS* extract could attenuated hyperalgesia and the elevation of oxidative stress induced by sciatic nerve constriction injury in diabetic rats.

Oxidative stress is another major contributor in the development of neuropathy in diabetes[43]. Chronic hyperglycemia induces oxidative stress by the autoxidation of monosaccharides[44] leads to production of superoxide and hydroxyl radicals. Oxidative stress also causes vascular Impairment leading to endoneurial hypoxia which leads to impaired neural function and reduced nerve conduction velocity[45]. In the present study, significant increases in LPOs and reductions in

endogenous antioxidant enzyme (SOD and CAT) levels in diabetic rats have been observed. The role of oxidative stress in the pathogenesis of diabetic neuropathy is further supported by experimental and clinical studies where various antioxidants, including GSH, lipid soluble antioxidant, metal chelators, α lipoic acid and acetyl-L-carnitine have been shown to amilorate biochemical and functional nerve disorders[46]. In the present study EEPS exhibited significant decrease in LPOs and increase in endogenous antioxidant enzymes. This antioxidant activity of the EEPS could also have influenced its activity in diabetic neuropathic pain.

The most useful model of diabetic neuropathy should exhibit the key feature present in human pathology. Diabetic rodents show behavioral, structural, functional and molecular biomarkers and they are widely used as models to investigate etiology as well as to screen the efficacy of the potential therapeutic interventions[47].

Behavioral studies in diabetic rats often focus on the response to a painful or non-painful sensory stimulus, there by measuring hyperalgesia and allodynia respectively. The simplest of such tests measures the time to withdrawal of a limb such as the tail or a paw from a noxious heat source, with a faster withdrawal time being interpreted as hyperalgesia and a slower one as hypoalgesia[48]. The techniques used in this study to evaluate antinociceptive activity of *EEPS* were Haffner's Tail Clip Method, Hot plate method, Radiant Heat Method (Tail Flick Analgesiometer) and Formalin method[49].

These models are supposed to be behavioral biomarkers of diabetic neuropathy. The hot-plate and tail-clip tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level. The hot plate test involves two type of responses paw licking and jumping. Both responses integrate at supraspinal structure with the C and Aδ type I and II sensitive fibers participating in this model. The tail withdrawal response after thermal stimuli is elicited by the spinal motor reflex most probably via endogenous release of substance P in the spinal cord[50]. In Formalin test the results demonstrated that there is an intensified nociceptive response and exaggerated hyper-algesic behavoiur in response to noxious stimuli in diabetic control group rats.

The H&E stained lateral section of diabetic control sciatic nerve (B) showed reduced nerve fibre density in neuronal fibres and vacuolar changes compared to normal nerve (A). Treatment of Pregabalin and *Plecospermum Spinosum* (400 mg/kg body weight) in diabetic rats was shown to improve the structural integrity in neuronal fibres and vacuoles in dose dependent manner.

Conclusion

Plecospermum Spinosum extract was proposed that in addition to its antidiabetic, the antioxidant properties is the prominent features in attenuation of diabetes induced neuropathy and its generating pain. These findings suggest that *Plecospermum Spinosum* treatment must be beneficial to treat pain in diabetic animals.

References

1. http://www.diabetic.org/introductiontodiabetesmellitus/ 1104085113.
2. Ramachandran A, Snehalatha C and Vijay Viswanathan. Burden of type 2 diabetes and its complications – The Indian scenario.*Current Science*.2002 ; 83(12):1471-1476.
3. http://www.mayoclinic.org/diseasesconditions/diabeticneuropathy/ basics/ causes / con20033336. (Accessed 21st April 2015)
4. http://www.planetayurveda.com/diabeticneuropathy.htm
5. http://www.medicinenet.com/script/main/art.asp?articlekey=11289(Accessed 14th July 2013)
6. G.Sumithira, A.Ashma, H.Rajamathanky, V.Kavya, K.Muhammed Riyas. A review on Ethanobotanical uses and pharmacology of *Plecospermum Spinosum*. *Int. J. of Res in Pharmacology &Pharmacotherapeutics*. 2017; 6(1):45-49.
7. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423(Accessed17th december 2001)
8. Faisal Mohd. The Pharmacological Evaluation of Epigallocatechin-3-Gallate (EGCG) Against Diabetic Neuropathy in Wistar Rats.*Int J sci Res Rev.* 2012; 1(3): 75-87.
9. Kelli.A.Sullivan. Mouse Models of Diabetic Neuropathy. *Neurobiology Of Disease*.2007;28(1):276–285.
10. Vogel H. *Drug Discovery and Evaluation of Pharmacological Assays*. 2nd ed. Germany: Springer Publication. 2002

11. Schleyerbach R. Analgesic, anti-inflammatory, and anti-pyretic activity. In Vogel HG editor. Drug discovery and evaluation of pharmacological assays. 2nd ed. Germany: Springer. 2002.
12. Talbot S.. Emerging Role of Microglial Kinin B1 Receptor in Diabetic Pain Neuropathy. *Experimental Neurology.*2011; 9.
13. Khalili M. The Effect of Oral Administration Of *Withania Somnifera* Root On Formalin-Induced Pain In Diabetic Rats. *Autumn*. 2009;1(1):233-235.
14. ShaikhAS. Animal Models and Biomarkers of Neuropathy In Diabetic Rodents. *Indian Journal of Pharmacol*. 2010;42(3):129-134.
15. Dubuisson D, Dennis SG. The Formalin test: A quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. *Pain*.1977;4:161–174.
16. Shibata M, Ohkubo T, Takahashi H. Modified formalin test; characteristic biphasic pain response. *Pain*.1989;38:347–352.
17. Lowry O.H, Rose brough N.J, Farr A.L, Randall, R.J. The original method. *J.Biol. Chem.* 1951;193: 265.
18. Ochei J, Kolhatkar A. Medical laboratory science : Theory and practice. New Delhi. Tata McGraw-Hill publishing company limited, 2000.
19. Reitman, S., Frankel, S.A. Colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol*.1957;28:56-63.
20. King Ej, Armstrong Ar. A Convenient Method for Determining Serum and Bile Phosphatase Activity. *Can Med Assoc J.*1934 ;31(4):376–381.
21. Misra HP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and simple assay of superoxide dismutase.*J.Biol. Chem*. 1972; 247:3170-3184.
22. Sinha AK, Colorimetric assay of catalase, Analytical Biochemistry. 1972;47(2): 389-394.
23. Bhesh Raj Sharma. Nelumbo nucifera leaf extract attenuated pancreatic β-cell toxicity induced by interleukin-1β and interferon-ý, and increased insulin secretion of pancreatic β-cell in streptozotocin-induced diabetic rats.
24. Okhawa H, Ohigni N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95:351-359.
25. Bridges D, Thompson SW, Rice AS. Mechanisms of neuropathic pain. *Br. J. Anaesth*. 2001;87:12–26.
26. Freshwater JD, Svensson CI Malmberg AB Calcutt NA. Elevated spinal cyclooxygenase and prostaglandin release during hyperalgesia in diabetic rats. *Diabetes*. 2002;51:2249–2255.
27. Kamboj SS, Vasishta RK, Sandhir R. N-acetylcysteine inhibits hyperglycemia-induced oxidative stress and apoptosis markers in diabetic neuropathy. *Journal of Neurochemistry.*2010;112:77–91.
28. Kumar S, Arun KH, Kaul CL, Sharma SS. Effects of adenosine and adenosine A2A receptor agonist on motor nerve conduction velocity and nerve blood flow in experimental diabetic neuropathy. *Neurolological Research.* 2005;27:60–66.
29. Hawkins CL , Davies MJ. Generation and propagation of radical reactions of proteins. *BiochemBiophysActa*.2001;1504:196-219.
30. Baydas G E, Sonkaya M, Tuzcu , Yasar , E. Donder. Novel role for gabapentin in neuroprotection of central nervous system in Streptozotocin-induced diabetic rats. *Acta Pharmacol Sin*. 2005; 26:417-422.
31. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms and management.*Lancet*.1999;353:1959-1964.
32. Mukesh S, Patil MB. Antidiabetic activity of Crateva nurvala stem bark extracts in alloxan-induced diabetic. *Journal of pharmacy and bioallied science*.2010;2:18-21.
33. LenzenS. The mechanisms of alloxan and Streptozotocin induced. *Diabetologia.* 2008;51:216-226.
34. Courteix C, Bardin M, Chantelauze J, LavarenneJ,Eschalier A. Study of the sensitivity of the diabetes- induced pain model in rats to a range of analgesics. *Pain*.1994;51:153- 160.
35. Calcutt NA, Jorge MC, Yaksh TL, Chaplan TL. Tactile allodynia and formalin hyperalgesia in Streptozotocin- diabetic rats: effects of insulin, aldose reductase inhibition and lidocaine. *Pain.*1996;68:293-299.

36. Stump P. Drugs Today (Barc). 2009;45:19-27.
37. Malhotra A, Huang S, Bhan AK. Subunit function in cardiac myosin: Effect of removal of LCi (18,000) on enzymatic properties. *Biochemistry*.1979;18:461-467.
38. Malhotra A. Penpargkul S. Scheuer J. The nature of contractile protein changes in hearts of diabetic rats. *Circulation Res*.1979;60(12).
39. Ashwani Malhotra, Somsong Penpargkul, Frederick S, Fein, Edmund H. Sonnenblick James Scheuer. The Effect of Streptozotocin-Induced Diabetes in Rats on Cardiac Contractile Proteins. *Circulation Research* .1981 ;49 (6) : 1243-1250.
40. Oluwafemi O, Oguntibeju, Samantha Meyer, YapoG.Aboua, Mediline Goboza. et al. Significantly Reduced Hyperglycaemia and Hyperglycaemic-Induced Oxidative Stress in the Liver and Kidney Tissues of Streptozotocin-Induced Diabetic Male Wistar Rats. *Evidence-Based Complementary and Alternative Medicine*.2016;1-10.
41. Gometi SA, Ogugua VN, Odo CE, Joshua PE. Effects of some antidiabetic plants on the hepatic marker enzymes of diabetic rats. *African Journal of Biotechnology*. 2014;13(7):905–909.
42. Murugan P, Pari L. Influence of tetrahydrocurcumin on erythrocyte membrane bound enzymes and antioxidant status in experimental type2 diabetic rats. *Journal of Ethno pharmacology*. 2007;113 (3):479–486.
43. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414:813–820.
44. Bonnefont-Rousselo D. Glucose and reactive oxygen species. *Curropin Clin Nutr Metab Care*. 2002;5:561-568.
45. Pop-Busui R, Marinescu V, Van HC. Dissection of metabolic Vascular and nerve conduction interrelationships in experimental diabetic neuropathy by cyclooxygenase inhibition and acetyl-L-carnitine administration. *Diabetes*. 2002;51:2619-2628.
46. Vincent AM, Russell JW, Low P. Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev*. 2004; 25:612-628.
47. Talbot S, Chahmi E, Dias Jp, Couture R. Key role for spinal dorsal horn microglial kinin B1 receptor in early diabetic pain neuropathy. *Journal of Neuro inflammation.* 2010;7(36):1-16.
48. Michael B. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414:813-820.
49. Chandra N, SeKha, JayaSree T, Shaikh Ubedulla, rohit dixit, Manohar V S, et al. Evaluation of Antinociceptive Activity of Aqueous Extract of Bark of *Psidium Guajava* in Albino Rats and Mice. *Journal of Clinical and Diagnostic Research*. 2014; 8(9):01-04.
50. Yashpal K, Radhakrishnan V, Coderre TJ. Henry JL. CP-96, 345 but not its stereoisomer, CP-96, 344, blocks the nociceptive responses to intrathecally administered substance P and to noxious thermal and chemical stimuli in the rat. *Neuroscience*. 1993;52:1039-1047.

Indexed by - Scientific index, Research bible, Jour–Informatics, Google Scholar, Inno-space.org, Cosmos: Germany

Registered & Approved by:
ISSN International Centre, Bibliographic Data Section, 45 rue de Turbigo, 75003 Paris, France.