Neuroprotective potential of *Phyllanthus amarus* and Esculetin in STZ-induced neuropathy in rats

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**ABSTRACT**

Hyperglycemia-induced overproduction of free radicals causes the development of diabetic neuropathy (DNP), leading to peripheral nerve degeneration. The current study reports the neuroprotective effects of *Phyllanthus amarus* extract (PAE) and Esculetin (ESC) on Diabetic peripheral neuropathy (DPN) in streptozotocin (STZ)-induced diabetic male rats. Injection of STZ (60mg/kg/b.w, i.p) significantly induced diabetes mellitus (DM), verified by having a blood glucose level of more than 250 mg/dl. Randal-Selitto assessed neuropathic analgesia was assessed by randal-selitto and hot-plate methods and the sciatic nerves were collected and assessed for biochemical parameters at different interval of 7,14 and 21 days. Histopathological changes were also evaluated at the end of the experiment. Treatments with *Phyllanthus amarus* extract (400mg/kg/b.w/d) and esculetin (45mg/kg/b.w/d), Significantly ameliorated the STZ induced diabetic peripheral neuropathy by attenuating, such as decrease in blood glucose level, water intake level, aldose reductase activity (AR), thiobarbituric acid reactive substances (TBARS) and increase in catalase, superoxide dismutase (SOD), GSH, GPx along with body weight. Treatment with *Phyllanthus amarus* and esculetin significantly reversed the pain sensitivity and myelination, degenerative changes of sciatic nerve fibre in both the groups compared to the diabetic control group. Thus, *Phyllanthus amarus* and esculetin possess potential neuroprotective effect in a rat model of diabetic peripheral neuropathy in respect of neuropathic behaviour, improved morphological changes and significant antioxidant activity.

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INTRODUCTION

Diabetic neuropathy is associated with peripheral nerve damage in a length-dependent manner, followed by their structural and functional loss (Said, 1996). Chronic hyperglycemia leads to the progressive development of long-term microvascular complication such as diabetic peripheral neuropathy (DPN) which causes disease and finally leads to death among those affected persons (Said, 1996; Maritim et al., 2003). The prevalence of DPN also increases with time due to poor glycemic control and increased free radical production creating oxidative stress has been involved in related pathogenesis (Maritim et al., 2003). Hyperglycemia mediated oxidative stress is the main cause along with altered polyol pathway, AGE’s formation, mitogen-activated protein kinase over activation and PKC etc., (Oyenihi et al., 2015). Although many antioxidant compounds are used and tested on animal models; still hyperglycemia-mediated oxidative stress is an effective target towards diabetic peripheral neuropathy. In the hyperglycemic neuron,
mitochondria are especially most vulnerable, because the production of ROS species can damage their DNA and membranes.

Natural products have been an ultimate source of discovery and development of therapeutic drugs. *Phyllanthus amarus* is commonly known as "Bhui amla", of Euphorbiaceae family, appear as a weed in cultivated and wastelands. It has a powerful anti-inflammatory and anti-inflammatory properties (Sangeeta et al., 2017) thus, playing a great role for several experimental investigations. Presence of pure triterpenoids named ursolic acid, oleanolic acid and lupeol present in aerial parts of the *P.amarus* were shown to inhibit α-amylase activity (Hasenah, 2013). Besides, the polyphenolic content of *P.amarus* extract shows kinetic inhibition of carbohydrate-hydrolysing enzymes (Mohamad et al., 2014) and reduce hyperglycemia levels in streptozotocin-induced diabetic rats (Owolabi et al., 2011). Molecular docking and pharmacophore modelling studies have shown that phyllanthin (an active lignan present in various *Phyllanthus* species) had an affinity for aldose reductase, which can be used against T2DM68 (Masur et al., 2015). *Phyllanthus amarus* shows antioxidant and anti-nociceptive effects in sedentary men by improving exercise recovery. It exhibits anti-allodynic and anti-oedematous properties in persistent inflammatory and neuropathic pain models. Bhumyamalaki was able to significantly revert the decreased sensory perception and decrease the changes in numbness, tingling and burning pain in lower limbs in human diabetic neuropathy (Patel et al., 2011). On the other hand, *Phyllanthus amarus* found to induce the regeneration of injured sciatic nerve (Panakkaporn, 2012).

Esculetin (6,7-dihydroxy coumarin) is a potent coumarin-derived antioxidant. It occurs in various plants (Anthony et al., 2015), a variety of foods and is the main active ingredient of Cortex fraxini (Gennian et al., 2015). Esculetin has broad prospect of developing effective drugs because of its anti-inflammatory, antioxidant, neuroprotective, anticancer properties and so on. It has been reported that esculetin is having aldose reductase inhibitory activity in vitro and cataractogenesis (Chan-Sik et al., 2016). Esculetin is showing the anti-hyperglycemic effect in STZ-induced diabetic rats by modulating carbohydrate metabolic enzymes (Prabakaran et al., 2012). It has anti-proliferating activity by initiating mitochondrial-dependent apoptosis pathway against hepatocellular carcinoma in vivo and in vitro (Wang et al., 2015). Recent studies have shown the protective effect of esculetin on nociceptive pain of rats in non-inflammatory and inflammatory models (Przemyslaw et al., 2015). Esculetin increases antioxidant activity by inhibiting adipogenesis in 3T3-L1 Cells (Younghwa et al., 2017). Esculetin also protects against neuronal apoptosis caused by cerebral I/R injury by attacking Bcl-2, Bax and caspase-3 proteins (Wang et al., 2012). Besides, it has shown the ability to protect cells against neurodegenerative diseases occur by the death of brain cells and degeneration of axons or dendrites (Kadakol, 2016). Another side, In vitro studies, has shown the protective effect of esculetin on the AD (Alzheimer's disease) by inhibiting BACE1, BChE and AChE enzymes (Ali et al., 2016). Esculetin can restore GSH/GSSG ratio and COX activity in acute restraint stress mice model having the inability of contextual memory (Martin et al., 2016). So far many clinical and exploratory studies have proposed the role of esculetin on vascular proliferative disorders, various skin diseases, hepatotoxicity, diabetest (Atsushi et al., 2008), human bacterial infections, cancer and adipogenesis (Gennian et al., 2015; Kadakol et al., 2015). There is no data about its neuropotential effects against diabetic peripheral neuropathy.

To accomplish this, we investigated the beneficial effects of *Phyllanthus amarus* ethanolic extract and Esculetin in diabetic peripheral neuropathy in relation to traditional and scientific way with respect to hyperglycemic control and anti-oxidative effects using behavioural, histological and biochemical parameters in type II diabetic rats.

**MATERIALS & METHODS**

**Animals**

36 male Wistar rats (Rattus norvegicus) weighs 200±50g were procured from NCLAS, National Institute of Nutrition (NIN), Hyderabad, India. Rats were maintained in standard laboratory conditions, in single rat polypropylene cages, at an ambient temperature of 22±2°C and humidity (45-50%), with a 12 hr light/dark period for minimum 7 days before and during the experimental period. To minimize the animal's suffering, measures were taken as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experimental Animals) given by animal ethical committee (CPCSEA No: 383/01/a/CPCSEA), Department of Zoology, Osmania University, Hyderabad, India. Animals were provided with drinking water and rat dry food of known weight ad libitum.

**Plant Extract Preparation**

The aerial parts of *Phyllanthus amarus* plant were picked-up from Dendukur Village, Khammam district, Telangana, India during July-august, 2016. They were washed with double distilled water, dried under shade for two weeks then, finely powdered using the blender. The powdered plant material (500g) was then subjected to Soxhlet...
extraction using 20% ethanol as the solvent for 40 hrs. Filtration of the extract was done using a 3-4 gauze cloth followed by Whatman No.1 paper (32% w/w). Then, the evaporation of filtrate was carried out with the rotary evaporator (DLAB, RE100-Pro) at 50°C (22% w/w). The obtained extract was shade dried and utilized further by keeping in -20°C refrigerator.

**Induction of Type 2 diabetes and Experimental design**

Non-insulin-dependent (type 2) DM was induced in 18 Wistar male overnight fasted rats by injecting single dose (60 mg/kg b.w, i.p) of streptozotocin mixed in 0.1 M sodium citrate buffer, pH 4.5. Remaining 18 rats were served as non-diabetic. A drop of blood was collected from the tail vein after ~72 h of streptozotocin injection and hyperglycemia was considered using a blood glucose test strip and glucometer (Dr.Morepen Gluco One, Model: BG-03). Only rats with glucose concentration more than 250 mg/dL in the blood were considered diabetic and were taken for the present study (Erbas O et al, 2016). Animals were divided into 6 experimental groups.

1. **Group I**: Control animals (CT) - provided normal drinking water.
2. **Group II**: Diabetic control (DC) - received 60mg/kg b.w, i.p STZ in 1ml of buffer.
3. **Group III**: Diabetic + PAE - received STZ (60 mg/kg b.w, i.p) and then received ethanolic extract of *Phyllanthus amarus* (PAE) plant (400 mg/kg b.w/ml/day) orally from Day1 to 21 after induction of diabetes.
4. **Group IV**: Diabetic+ESC - received STZ (60mg/kg b.w, i.p) and then received esculetin (45 mg/kg b.w/ml/day), day1 to 21 after induction of diabetes.
5. **Group V**: PAE - received only ethanolic extract of *Phyllanthus amarus* (400 mg/kg b.w/ml/day) day1 to 21, which are non-diabetic rats.
6. **Group VI**: ESC - received only esculetin (45 mg/kg b.w/ml/day), day1 to 21, which are non-diabetic rats.

After confirmation of diabetes, from 4th day onwards after STZ injection, PAE and esculetin were administered daily for 21 days. Rats of day 7, 14 and 21 were selected for the experimental studies. Rats were sacrificed and the sciatic nerves were dissected from day 7, 14 and 21 rats and then used for biochemical and histopathological studies. During the experimental period, the gain in body weight of rats was monitored daily. Water intake level was also monitored daily at 10 a.m. High glucose levels were confirmed using a digital glucometer in all rats by taking blood from a tail vein on day 10 and 21 after STZ injection.

**Chemicals**

Esculetin and Streptozotocin were obtained from Sigma-Aldrich Company, USA. The solvent for plant extraction and all other chemicals used for biochemical analysis and histopathological examination were of analytical grade from Himedia, India.

**Behavioural Studies**

**Thermal and Mechanical nociceptive responses:** Thermal pain sensitivity was evaluated by the hot plate test (Gunn et al, 2011). The pain reflexes were evaluated by footpad attachment on the hot plate for each rat. The time between placement of rat on the Remi hot plate (52.0±0.5°C) and the rat showing various behavioural sensations of heat pain (rearing, forepaw and hindpaw licking, face washing, body cleaning, hind leg withdrawal, and jumping off) was noted as a time of response in seconds. Mechanical nociceptive tolerance was assessed by using the Randall-Selitto paw withdrawal test (Randall et al, 1957) with an analgesic meter (TSE systems GmbH, Germany). It generates a linearly increasing limited applied force of up to 250g (calibrated force) to avoid skin damage. The results were expressed based on pressure (gm) maximally exerted by the rats in response to mechanical stimuli induced pain in the paw and the withdrawal of paw was noted. Rats were customized to the devices for 5 days before carrying out the tests. At each time point, rats were tested with five trials, and the values are averaged.

**Estimation of aldose reductase activity**

Aldose reductase assay was performed in sciatic nerve by Hayman and Kinoshita method (1965). Using 50mM potassium phosphate buffer, the sciatic nerve was homogenized at pH 7.2 followed by centrifugation at 25,000×g / 30 min at 0-4°C and supernatant was used for the assay. At room temperature, the reaction was pre-incubated for 5 min and the further reaction was started by adding NADPH. The decrease in absorbance was recorded for 5 min at 340nm spectrophotometrically. The activity of aldose reductase was indicated in µ moles/mg of protein.

**Enzymatic oxidative stress markers**

**Estimation of Thio-Barbituric Acid Reactive Substances (TBARS)**

The lipids in the sciatic nerve will undergo peroxidation, was estimated according to Bhuyan et al. (1981). 1 ml of sciatic nerve homogenate and 1 ml of 20% Tri chloro acetic acid (TCA) were mixed, then heated using water bath at 70°C/10 min and
then cooled at room temperature. After centrifuging the samples at 30,000×g at 25°C for 10 min, 0.4ml of the supernatant was collected, which is devoid of proteins. It was mixed with 0.2ml of 0.5% aqueous solution of thiobarbituric acid (TBA), heated for 10 min then cool at room temperature. The absorbance of the pink coloured condensation Trimethine product determined spectrophotometrically at 533nm. Results were expressed in nanomoles of TBARS/gm tissue (MDA concentrations) and were calculated from the standard curve of tetra ethoxy propane (TEP).

The Sciatic nerve sample was homogenized in 0.05M sodium phosphate buffer, centrifuged at 750×g at 4°C for 20 min. The supernatant was spun at 12000×g from which 50µl was used for catalase estimation and remaining supernatant was spun at 35000×g at 4°C for 25min, from which 50µl was used for determining SOD activity.

**Estimation of Catalase Activity (CAT)**

Catalase activity was measured according to the method of Aebi et al. (1984). Catalase activity was measured by following UV-spectrophotometric method. In the ultraviolet range, H₂O₂ undergo de- decomposition followed by the reduction in extinction per unit time at 240 nm. The reaction mixture (3ml) was prepared by mixing 50µl of the tissue sample, 1.95 ml of sodium-phosphate buffer and 1ml of 30mM H₂O₂ which was added at the time of taking O.D readings. Catalase activity was measured by taking the difference in absorbance per unit time.

**Estimation of Superoxide Dismutase Activity (SOD)**

The sciatic nerve superoxide dismutase activity was measured by the method of Marklund and Marklund (1974). The method includes inhibition of O₂-dependent autoxidation of pyrogallol by SOD. The assay mixture includes 50 µmole of Tris HCl buffer at pH 8.2, 0.05 µmole di-ethylene tri-amine Penta-acetic acid (DETAPA), 0.04 µmole pyrogallol and 50 µl of the enzyme. After the addition of pyrogallol, the increased absorbance was noted over 30 seconds at 420 nm, spectrophotometrically and the activity of SOD was expressed as units/mg protein.

**Glutathione Peroxidase (GPx)**

GPx activity was estimated by the method of Rotruck et al. (1973). 1 ml of DTNB reagent (1% sodium citrate containing 0.04% DTNB) was added to 3 ml of the supernatant obtained after centrifugation of tissue homogenization with Tris-HCl buffer. The developed colour was measured spectrophotometrically at 412 nm. GSH activity was expressed as μ mol of GSH consumed/min/mg protein.

**Nonenzymatic Antioxidants**

**Reduced Glutathione (GSH)**

Reduced GSH content in the sciatic nerve was assessed by the Ellman method (1959). The reaction was established on the ability of GSH group to reduce DTNB, (5, 5′-dithiobis-(2-nitrobenzoic acid) (Ellman’s reagent)) to yield a yellow compound 5′-thio-2-nitrobenzoic acid (TNB), measured at 412 nm. The results were expressed as μg/mg protein.

**Histopathological Studies**

After the experimentation, the sciatic nerve was taken out according to the procedure described by Mizisin (2004). The animals were sacrificed and the sciatic nerve was exposed by removing the skin on the lateral surface and an incision was made on the left thigh. Sciatic nerves were carefully removed and preserved in 10% v/v formalin solution for histopathological analysis. The nerves were paraffin embedded and cut thin (3-5 μm) sections by using microtome and observed under a light microscope with haematoxylin and eosin (H&E) stain (Lillie et al., 1976). Sections were evaluated on their severity of the pathological changes including nerve degeneration and oedema.

**Statistical Analysis**

The obtained results were expressed as the Mean ± SEM (standard error of the mean) and one-way analysis of variance (ANOVA) followed by t-test to determine the statistical significance between the groups.

**RESULTS**

The behaviour and health status of all animals were checked throughout the study.

**Changes in Blood glucose, body weight and water intake levels**

The blood glucose levels, body weights and water intake levels of all groups were shown in tables 1, 2 and 3. STZ-injected rats depicted basic symptoms of diabetes like loss of body weight, polyuria, polydipsia and polyphagia as expected. Significant value differences between the groups revealed the results using one way ANOVA and t-test. Rats exhibited a significant increase (p < 0.001) in fasting blood sugar (FBS) level within 48 h after STZ injection and the hyperglycemia was continued till the end of the experiment compared to non-diabetic rats (Table 1). The animals in control, PAE and ESC groups showed normal blood glucose levels throughout the experiment. After inducing diabetes with STZ, predictable loss of body weight (P<0.001) with age-matched controls (Table 2).
was observed. The water intake levels and final body weight were normal in the control, PAE and ESC groups. Diabetic rats were showing high water intake levels (P<0.001) and there is no gain of body weight till the end of 3 weeks (Table 3).

These symptoms are common for type 2 diabetes in humans as well as in animal models. However, daily administration of the P.amarus extract and esculetin to diabetic rats after 21 days generated a significant gain of body weight and restoration of water capacity to some extent (P<0.05), which indicates proper utilization of glucose in the rats. Whereas, the results obtained by the esculetin were significantly higher than the plant extract treated group. The noticed results could be assigned to the improved glycemic control due to the ameliorative effect of the Phyllanthus amarus extract and esculetin in managing glucotoxicity, lipotoxicity, induced adipogenesis, oxidative stress and inflammation.
Nociceptive responses: Diabetic rats also showed significantly reduced heat sensitivity on a hot plate as measured to the control group (p<0.001). *P<0.001 against control group and **P<0.01 against diabetic group.
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(p<0.001) for three weeks daily, the pain threshold values on Randall Selitto were significantly got normalized. The percent of change in nociceptive responses (Hotplate and Randall Selitto tests) of Day 21 rats is more than Day 7 and day 14 rats of similar treatment. The results of PAE and ESC separate groups were found to be nearer to that of the control group.

Besides, the esculetin treated group is showing more improvement than the PAE treated group (Fig.1a and b).

**Aldose reductase assay**

Aldose reductase activity was significantly greater in diabetic rats when compared to the control rats (p<0.001) in sciatic nerve tissue confirmed the activation of the polyol pathway and progression of diabetic peripheral neuropathy. On treatment with PAE and ESC, there is a significant decrease in AR activity (p<0.05) may be due to their inhibitory effect (Fig.2).

**Oxidative stress biomarkers**


LPO, CAT, SOD, GPx, GSH levels for all groups (n=6) on Day 7, 14 and at the end of the experiment (Day 21) are shown in fig (3). The initiation of diabetes in the rats resulted in significantly (P<0.001) enhanced levels of TBARS in the sciatic nerve compared to the control group. The treatment of diabetic rats daily for three weeks with 400mg/kg b.w of PAE extract and 45mg/kg b.w of ESC exhibited significant impediment of diabetes-induced increase of TBARS (Fig.3a) in the sciatic nerve(P<0.05). Furthermore, the diabetic control rats exhibited lower enzymatic marker levels of CAT (P<0.01), SOD (P<0.001), GPx (P<0.05) and reduced levels of GSH (P<0.05) when compared with the control rats (Fig. 3b, 3c, 3d, 3e respectively). The decreased activity of antioxidants GSH, SOD, CAT, GPx levels in the sciatic nerve were gradually improved (P<0.05) in the preventive groups (D+PAE, D+ESC) than that in the STZ group, while those in PAE and ESC separate groups, no change is observed (p < 0.05). Phyllanthus amarus etha-
nolic extract and Esculetin are thus significantly effective in lowering the free radical or oxidative stress level at 400 mg/kg b.w and 45 mg/kg b.w doses respectively. While, enhanced levels of these assays (P<0.05), were observed in D+ESC group rats in relation to D+PAE rats.

Histopathology
At the end of the experiment, morphological changes were examined in the sciatic nerve section for all the groups. Examination of sciatic nerve sections under the light microscope, in the control group exhibited normal structure, myelination and morphology (Fig. 5A) while diabetic control group showed drastic changes including reduction in the number of myelinated fibres and appeared to be more small fibres, axonal degeneration, necrosis, swelling, thin, loosely formed and disorganized myelin sheath and many Schwann cells (Fig. 5B) compared to non-diabetic rats. Treatment with *P. amarus* extract and esculetin (Fig. 5C, 5D) reduced the changes so that axonal regeneration and a few dispersed Schwann cells were observed in these preventive groups. No detectable injury was shown in PAE and ESC separate groups (Fig. 5E, 5F). D+ESC at 45 mg/kg b.w was found to be more effective than D+PAE at 400 mg/kg b.w.

Discussion
In the present study, the neuroprotective effects of ethanolic extract of *Phyllanthus amarus* (PAE) aerial parts and Esculetin (ESC, a coumarin derivative) on the development of diabetic peripheral neuropathy in streptozotocin (STZ) induced diabetic rats were evaluated. The experimental data has shown, sustained increase in blood glucose levels will cause diabetes mellitus (DM) and its complications, mainly causing peripheral nervous system malfunctions. In the present study, STZ-injected rats depicted common symptoms of diabetes like elevation of FBS levels, decrease in body weight, polydipsia, polyphagia and excessive passage of
urine after STZ injection and these symptoms were continued till the end of the experiment compared to non diabetic rats and these results were in agreement with the previous reports (Wu and Yan, 2015; Javidanpour et al., 2012; Pellegrino et al., 1998). Besides, STZ induced diabetic neuropathy is well studied in the rat model (Bina et al., 2017). Our results confirmed that *P.amarus* extract and esculetin with their respective doses caused a significant decrease in the blood glucose concentration, able to gain the body weight and lessen the water intake levels in relation to diabetic control (DC) rats. In this regard, the hypoglycemic effect of *Phyllanthus amarus* is may due to the presence of phyllanthin, chief component present in the plant aerial parts. Esculetin may lower the glucose levels from the existing β-cells of the pancreas by stimulating the insulin production. As per the earlier studies, diabetic rats have shown significantly increased aldose reductase (AR) activity when compared to the normal rats, which is the predominant polyol-pathway linked pathogenic factor in the diabetic peripheral nerve (Bhaskar and Pratap Reddy, 2014; Jung et al., 2011). *Phyllanthus amarus* and esculetin have significantly inhibited the AR activity, such that sorbitol accumulation may be decreased in the sciatic nerve, leads to improvement in the motor and sensory nerve conduction.

Although the proper mechanism of the nociceptive reaction is unsure, in existence, it is found that sensitized peripheral nerve nociceptive response is a common symptom related with diabetic peripheral neuropathy (Ristagno et al., 2012). Diabetic rats showed significantly reduced heat sensitivity and paw withdrawal threshold response on a hot plate and Randall Selitto respectively when compared to the normal group confirmed the pathophysiology of nerve damage, where the behaviour of rats was changed with diabetes-induced neurodegeneration (Bhaskar and Pratap Reddy, 2014). There is an extending aid for the neuroprotective effects of traditional, as well as recently found, present antioxidant agents in animal models of diabetic peripheral neuropathy (Ali et al., 2016; Erbas et al., 2016; Jameel, et al., 2016; Kadakol et al., 2016; Wang et al., 2012). In the current study, PAE and ESC treated diabetic rats could successfully improve the nociceptive responses on a hot plate and Randall Selitto by increasing the pain threshold values. The diabetic rats treated with *P.amarus* and esculetin significantly ameliorated the nociceptive responses, attributes to their anti-nociceptive, anti-inflammatory and neuroprotective activity in STZ-induced DPN.

In line with the previous reports, in diabetic control rats, we noticed a significant increase in lipid peroxidation (LPO), a significant decrease of catalase (CAT) and superoxide dismutase activity (SOD), and a significant decrease of reduced glutathione (GSH), glutathione peroxidase (GPx) content in the sciatic nerve. Hyperglycemia-induced oxidative damage and a further increase in free radicals lead to cellular dysfunction with damaged mitochondria, increased production of ROS, slow down of NCV, followed by axonal loss of nerve fibres via activation of programmed cell death or necrosis (Wu et al., 2015). The estimation of the oxidative and anti-oxidative position of study groups disclosed that *P.amarus* extract and esculetin were able to reduce the lipid peroxides and will enhance the SOD, CAT, GSH, GPx levels in the sciatic nerve tissue of diabetic rats. This study has revealed that, in addition to the decrease of glucose level in the blood, the results have shown the use of *P.amarus* extract and esculetin had antioxidative nature even after the start of neuropathy and continued after that, considering it is a progressive process. Studies by Mohamad et al., 2014 reported that *P.amarus* extract was able to scavenge the free radicals in diabetes mellitus, but there was no study on the role of *P.amarus* and its possible application in the alleviation of hyperglycaemia-bringing oxidative stress in sciatic nerve. Prabakaran et al., (2012) proved that treatment of STZ induced diabetic rat with esculetin exerts a protective effect against hepatic and renal tissues, but there is no study on its role against DPN by attenuating hyperglycemia-mediated oxidative stress, which plays a critical role in diabetic complications. Reactive oxygen and nitrogen species (ROS&RNS) are the potential sources to the pathogenesis of diabetic neuropathy (Oyenihi et al., 2015). In this regard, the evaluation of antioxidant activity of *P.amarus* extract and esculetin by enzymatic and non-enzymatic anti-oxidant markers on free radicals revealed their good anti-oxidant nature and our results were indicating that esculetin have high anti-oxidant properties. So, the herbal drugs with anti-hyperglycemic, anti-oxidant activities are yet to be commercially obtained as modern medicines, even though they are traditionally in use as therapeutics.

The results include fibre degeneration, endoneural swelling, partially separated myelinated nerve fibres, axonal atrophy of sciatic nerve tissue were reported in STZ induced DPN rats were in parallel with the findings of Omran (2012). If treatment is not established, these effects will cause degenerative changes in the peripheral nervous system. Morphological observation of sciatic nerve showed a significant decrease of oedema and caused regenerative changes and normal fibre arrangement were observed in *P.amarus* and esculetin treated diabetic rats. Behavioural, histological and biochemical results established that *P.amarus* extract and esculetin could decrease diabetes-induced
neurodegeneration in peripheral nerves. The results obtained in PAE and ESC alone treated groups, were in similar with the control group. Overall, these findings suggest clear evidence of the neuroprotective potential of *Phyllanthus amarus* extract and esculetin against hyperglycemia, oxidative stress and apoptotic cell death in diabetic peripheral neuropathy in rats.

**CONCLUSION**

The results of the present study along with the hypoglycemic effect showed neuroprotective effects of *Phyllanthus amarus* ethanolic extract and esculetin treatments in attenuating STZ-induced diabetic peripheral neuropathy possibly through anti-hyperglycemic, anti-nociceptive, antioxidant and neuro-regeneration developing mechanisms. Our results proved that *Phyllanthus amarus* extract and esculetin administration lowered the glucose levels, water-intake level and improved the metabolic pathways including antioxidant defence system, body weight and morphology of sciatic nerve. Esculetin seemed to be more potent than the *Phyllanthus amarus* extract in affecting most of these aspects. However, more advanced clinical studies are required to assess the efficiency and long-term protection of *Phyllanthus amarus* extract and esculetin which can be used as a treatment modality for diabetic neuropathy.

**Conflict of Interest**

The authors declare no conflict of interest.

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