ORIGINAL ARTICLE

Neuroprotective Effects of Ellagic Acid, Rutin and p-Coumaric Acid on Diabetic Neuropathy Rats.

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Abstract

Background: The peripheral nervous system has regenerative capability which mainly dependent on Schwann cells plasticity. The administration of phenolic compounds may reduce damage to the peripheral nerve due to diabetic peripheral neuropathy. Hence, this study was conducted to determine the neuroprotective effect of phenolic compounds on *in vivo* and *in vitro* models of diabetic neuropathy.
Methods: Streptozotocin-induced diabetic rats were treated orally with ellagic acid (100 mg/kg), rutin (100 mg/kg), p-coumaric acid (100 mg/kg) and metformin (500 mg/kg), respectively. The control and diabetic groups were given 5% DMSO in 0.9% sodium chloride. After 28 days, fasting blood glucose was determined and the pancreas and sciatic nerves processed for H&E staining. ANOVA analysis was performed. $p<0.01$ was considered statistically significant.
Results: No reduction in blood glucose level of the severely hyperglycaemic diabetic rats was observed following treatment with the phenolic compounds. However, they do show protective effect on the pancreatic islets of the diabetic rats. Slow progression of sciatic nerve damage was observed with a slight increase in Schwann cells proliferation.
Conclusion: In conclusion, while reduction in blood glucose level was not observed, the phenolic compounds may have a neuroprotective effect by promoting pancreatic repair. Proliferation of Schwann cells may play a protective role in hyperglycaemia by protecting the peripheral nerve and promoting regeneration.

Keywords: *Ellagic acid, rutin, p-coumaric acid, diabetic neuropathy*.

Introduction

Peripheral neuropathy, which resulted from persistent hyperglycaemia, is the most common complication of diabetes mellitus. The persistently high glucose condition causes diversion of glucose metabolism to an alternative glucose metabolism pathway due to hexokinase saturation, thereby increasing risk of nerve dysfunction through several mechanisms: reduction in Na^+/K^+-ATPase activity due to the accumulation of glucose-derived toxic products, insulin resistance, and increased osmotic and oxidative stress. Furthermore, activation of the glucose overutilisation pathway may disrupt blood flow to the nerves, resulting in nerve injury. In animal study, neuropathy can be seen as early as three weeks after diabetic induction.[1]

The peripheral nerve has a great regenerative capability. It is said that the capacity of the peripheral nerve to regenerate is mainly dependent on the flexibility of the supporting Schwann cells. Schwann cells are not only important during the development of peripheral nerve and maintenance, but these supporting cells are crucial during nerve injury. However, it is uncertain whether the Schwann cells capability following diabetic injury is sufficient for recovery in early peripheral nerve regeneration or if the peripheral nerve requires exogenous sources to support early regeneration. Some studies have linked peripheral nerve injury, i.e., diabetic peripheral neuropathy with oxidative stress and its recovery with the administration of phenolic compounds.

Phenolic compounds are the major secondary metabolites found abundant in plants and natural products such as honey. The application of natural products is gaining popularity due to the claim that they can treat various illnesses and health issues, possibly through the secondary metabolites. Since the stingless bee honey is in great demand in the community recently, it is worthwhile to investigate if the secondary metabolites found in honey are potentially responsible for neuroprotective effects in diabetic peripheral neuropathy. Ellagic acid, rutin and p-coumaric acid are the phenolic compounds that have been detected in stingless bee honey.[2-4] Stingless bees collect nectar secreted by plants to produce honey; this is how the secondary metabolites are transferred from the nectar to the honey.[4] The elucidation of the beneficial effects of these phenolic compounds, may provide the scientific evidence that can add value to the stingless bee honey. Furthermore, this may indirectly support the stingless beekeeping industry, particularly the small and medium-scale entrepreneurs which are the major players in this industry.

To date, most studies mainly focus on the central nervous system which looked at the effects of the phenolic compounds on oxidative stress-induced neuronal degeneration in animal models and *in vitro*. The ability of ellagic acid, rutin and p-coumaric acid to protect nervous tissue is to be expected based on their antioxidant [5-13] and anti-inflammatory [14] properties. Furthermore, these compounds have also been shown to have a protective on neuroglial cells of central nervous system.[10, 15-23]
Nevertheless, the effects of ellagic acid, rutin and p-coumaric acid on peripheral nervous system are not well understood although their protective activity so far has been linked to the antioxidative property. Studies on the antioxidative effect of these compounds are not limited to the central nervous system but have also been reported in the peripheral nervous system, though not as extensive as in the central nervous system. Ellagic acid (50 mg/kg) and rutin (50, 100 and 200 mg/kg) have been shown to attenuate malonaldehyde, total oxidant capacity, oxidative stress index, nitric oxide level and to enhance total antioxidant capacity in serum, sciatic nerve and dorsal root ganglion of diabetic rats.[20, 24-27] Similarly, these improvements can also be seen in ischaemic-reperfusion induced and chemically-induced peripheral nerve injury in rats treated with p-

coumaric acid (100 mg/kg, single intraperitoneal injection))[28] and rutin (50 and 100 mg/kg).[29] In addition, protective role of these phenolic compounds on peripheral neuroglial is also not well understood. Therefore, this study was to determine the effect of ellagic acid, rutin and p-coumaric acid on the peripheral nerve of streptozotocin-induced diabetic rats.

Materials and methods

Chemicals

Streptozotocin and toluidine blue were purchased from Sigma-Aldrich (Germany). Ellagic acid (97%), rutin (97+%), p-coumaric acid (p-Hydroxycinnamic acid, 98%) were obtained from Acros Organics (Belgium). Sodium dihydrogen phosphate anhydrous, disodium hydrogen phosphate anhydrous, formaldehyde (37-40%), and dibutylphthalate polystyrene xylene (DPX) were purchased from R&M Chemicals (Malaysia). Mayer's hemalum solution, D-glucose, dimethyl sulfoxide (DMSO), and sodium chloride were purchased from Merck (Germany). Paraffin wax and 100% alcohol were purchased from Leica (USA). Sodium hydroxide and 95% ethanol were purchased from HmbG Chemicals (Germany). Trisodium citrate dihydrate, citric acid anhydrous and hydrochloric acid were purchased from QReC (New Zealand). Xylene was purchased from Ajax FineChem (Australia), and Eosin Y 1% was purchased from Labchem (Malaysia).

Animal model

Adult male Sprague Dawley rats were purchased from a local supplier. Animals were acclimatized for a week before the commencement of the experiment. The rats were housed in plastic cages at a standard controlled temperature (24-26℃) with a natural (12-hour) light/dark cycle. Food and water were provided *ad libitum*. Rat cages were clean when necessary. All experiments were carried out in accordance with the approval granted by the Universiti Kuala Lumpur Royal College of Medicine Perak (UniKL RCMP) Animal Ethics Committee.

Diabetic induction

Rats were fasted overnight before glucose determination. Glucose was measured at the tail vein using the Avometer Avant glucometer. Induction of diabetes in the rats was done using freshly dissolved streptozotocin in cold citrate buffer (pH 4.5). A single dose of streptozotocin (60 mg/kg; 2 m/kg) was injected intraperitoneally in the lower right abdominal quadrant away from the midline. The control group was only injected with cold citrate buffer. The rats were observed daily for any mortality due to streptozotocin toxicity. After 72 hours of induction, the fasting blood glucose was measured for each induced rat, including the control group. The streptozotocin-induced rats with blood glucose level of 11.1 mmol/L (200 mg/dl) or above were then selected for further study and are known as diabetic rats. The rats that had received a citrate buffer injection and had blood glucose levels less than 7.5 mmol/L were classified as the control group.

Treatment with test compound

A total number of 24 rats were divided into the control, diabetic and treatment groups. Each treatment group was treated orally (5 ml/kg) with the test compound: ellagic acid (n=5) (100 mg/kg), rutin (n=4) (100 mg/kg), p-coumaric acid (n=4) (100 mg/kg) and metformin (n=3) (500 mg/kg), respectively. Both the control (n=4) and diabetic (n=4) groups received the vehicle containing 5% DMSO in 0.9% sodium chloride. Administrations of the test compounds and vehicle were performed daily and completed for 28 days.

Necropsy of rat

At the end of the treatment with the selected test compound, the fasting blood glucose was determined for each rat. The rats were euthanised using carbon dioxide (CO_2) asphyxiation followed by cervical dislocation. The pancreas and sciatic nerves were dissected and fixed in 10% formalin. The tissue specimens were trimmed and put into labelled plastic cassettes.

Tissue preparation for light microscopy

The pancreatic and sciatic nerve tissue specimens were processed using the automated tissue processor (Leica TP1020, Germany) where firstly, the specimens were washed in phosphate buffer solution (pH 7.4). After washing, the specimens were dehydrated in a graded series of alcohols and immersed in xylene for clearing. Each of the specimens were then immersed and embedded in a paraffin wax. The specimen blocks were allowed to harden on the cold plate. The specimen blocks were then trimmed and cut (5 µm) using a microtome (Leica RM2135, Germany) to obtain thin sections of each block. The sciatic nerve tissues were cut into longitudinal and transverse sections while the pancreatic tissues were randomly cut. The sections were mounted on a labelled slide and allowed to dry.

Subsequently, the slides were stained with Haematoxylin and Eosin (H&E). Firstly, the sections were immersed in xylene to remove the paraffin and rehydrated in a graded series of alcohol. The sections were then stained with Mayer's haematoxylin and eosin. After staining, the sections were again dehydrated in a graded series of alcohol and cleared by xylene. Lastly, a few drops of DPX mounting agent were placed on each section and a coverslip was placed to cover the section before viewing under the light microscope (Leica DM750, Switzerland). The images were captured and examined for any histological changes (Leica ICC50 HD, Germany).

Statistical analysis

The data in this study were presented as mean ± SEM. The normally distributed data were analysed by ANOVA followed by Dunnett's test for multiple comparisons. $p<0.01$ was considered statistically significant.

Results

Fasting blood glucose

Fasting blood glucose is used to determine the success of diabetic induction and treatment with test compound. Before diabetic induction, all the groups had a mean fasting blood glucose between 4.43± 0.13 and 5.18 ± 0.26 mmol/L. These value range is similar among groups. After 72 hours of being injected with streptozotocin, all injected rats had a significant elevation ($p<0.01$) of mean fasting blood glucose compared to the control group (5.33±0.38) that received citrate buffer (pH 4.5): diabetic group (23.33±1.12), metformin group (21.20±2.40), ellagic acid group (22.88±0.97), rutin group (26.78±2.17), and p-coumaric acid group (26.65±2.43). After four weeks of treatment with metformin (500 mg/kg), ellagic acid (100 mg/kg), rutin (100 mg/kg), and p-coumaric acid (100 mg/kg), respectively; no significant difference is obtained in the mean fasting blood glucose among all the treated groups when compared to the diabetic group (25.30±1.29): metformin group (21.95±0.35), ellagic acid group (23.90±0.00), rutin group (26.65±0.75), and p-coumaric acid group (19.70±0.00) (Figure 1).

Microscopic features of diabetic pancreas

The pancreas is a target organ for streptozotocin. The normal morphology of a pancreas is presented by the control group. The islet of Langerhans, were observed as pale embedment in the exocrine tissue. The pancreatic islet cell had a nucleus in the centre with a clear cell boundary. The cells were arranged in cords or clusters. Capillaries were seen in abundance between the islet cells (Figure 2a). In the diabetic group, the morphology of exocrine and endocrine were altered after streptozotocin administration. The pancreatic cells were observed to have an irregular arrangement without a clear cell boundary. Cytoplasmic vacuolation and cellular degeneration were observed (Figure 2b). The cytoplasmic vacuolation of the pancreatic islet cells observed in the diabetic rat group appears to be reduced in the diabetic rats treated with ellagic acid, rutin, and p-coumaric acid, respectively, for 28 days. A similar observation was also made in the metformin group. However, the regular arrangement of cells observed in the control

group was not observed in these treated group (Figure 2c, d, e, f).

Microscopic features of sciatic nerve

A typical morphology of the sciatic nerve fibres is shown by the control group in a longitudinal section. The perineurium is observed covering the nerve bundle. The longitudinal section of the nerve fibres shows a wavy appearance. Some transversely sectioned fibres are also observed due to its wavily feature. The axons of the myelinated fibres are seen in the centre surrounded by the remnant of myelin sheath known as myelin neurokeratin. Nuclei of Schwann cells are observed along the nerve fibres. A node of Ranvier is also observed. The sciatic nerves of diabetic and treated groups did not show much different from the control group. However, relatively more nuclei are observed in these groups compared to the control group (Figure 3).

Transverse section of the sciatic nerve of the control group shows various sizes of the nerve fibres. Axon is located in the centre of a nerve fibre surrounded by the myelin neurokeratin. Schwann cells are observed encircling some of the nerve fibres in both control and diabetic groups. Irregularity in the shape of myelin sheath boundary, with absence or clearance of myelin neurokeratin, are seen in some of the nerve fibres. More of these features are found in the diabetic sciatic nerve fibres. On the contrary, the irregularity of the myelin sheath is reduced in the sciatic nerve of diabetic rats treated with metformin. Similar observation of less myelin shape irregularity is made in the sciatic nerve of ellagic acid and p-coumaric acid treated where much reduction is seen in the ellagic acid group. However, in the rutin group, the changes were not much different from those in the diabetic group (Figure 4).

Discussion

***In vivo* study using diabetic induced rats**

In this study, a diabetogenic agent streptozotocin is used to develop a diabetic animal model using adult male Sprague Dawley rats. The success of the induction in the present study may be attributed to the preparation of streptozotocin solution. The streptozotocin was freshly prepared to ensure its efficacy by reconstituting it in a cold sodium citrate buffer (pH 4.5) immediately before injecting the drug into the rat's peritoneal cavity.

Citrate buffer is used as the medium for dissolving streptozotocin as its use in other studies have shown that it is more soluble in sodium citrate buffer than in water, while dissolving it in normal saline may produce bubbles, which may influence its diabetogenic effectiveness; also, keeping the streptozotocin solution in a cold environment can protect it from degradation.[30-33] The buffer medium was kept at 4.5 as the study on the effect of different pH on streptozotocin stability have shown that this drug is highly stable at about pH 4; and at this pH, streptozotocin produces a higher concentration of nitrate, which corresponds to its diabetogenic potency.[34-35]

In this study, a single dose of streptozotocin (60 mg/kg) was able to induce blood glucose elevation in rats. Our result showed that before the diabetic induction, the fasting blood glucose of all the rats in this study was within the normal range but after 72 hours of induction with a single dose of streptozotocin, the blood glucose level significantly increased to a level above the normal upper limit of 7.5 mmol/L. Also, the blood glucose level of the diabetic induced group remained high throughout the 28 days of the experiment, indicating stable hyperglycaemia, while the control group maintained a low glucose level throughout the experiment.

The maintenance of the high blood glucose level found in the streptozotocin-induced diabetic rats is corroborated by our histological findings which shows tissue damage leading to persistently high blood glucose level. There is evidence of pancreatic islet damage in the pancreatic tissue of the streptozotocin-induced diabetic rats which would have led to a consistently high blood glucose level. Furthermore, evidence of polydipsia, polyuria and body weight loss which are associated with persistently high blood glucose level were also observed in the streptozotocin-induced diabetic rats while none was observed in the control rats.

Thus, we can conclude that induction with streptozotocin at 60mg/kg on adult male Sprague Dawley rats are able to produce a stable diabetic animal model which is consistent with the earlier study.[36] Diabetic induction by streptozotocin is the preferred method in this study due to the unavailability of genetically modified diabetic animals and the slow development of diabetes by dietary induction.

Antihyperglycaemic effect of phenolic compounds

The phenolic compounds are well studied and are well known for their antioxidants and anti-inflammatory activities which plays an important role against cellular damage.[37-40] Phenolic compounds have been suggested to have the ability to reduce blood glucose levels in diabetic rats.[41-44] The phenolic compounds selected for this study are ellagic acid, rutin and p-coumaric acid. Their activity was compared against the antidiabetic drug, metformin.

Reduction of blood glucose level in streptozotocin-induced diabetic rats

In our study, the phenolic compounds that we employed did not reduce the glucose level in streptozotocin-induced diabetic rats after four weeks of treatment. Similar finding was also observed in the positive control group, i.e., the metformin treated group. In a study using ellagic acid, it was found that blood glucose of streptozotocin (60 mg/kg) induced rats did not reduced after four weeks of ellagic acid treatment (25 and 50 mg/kg).[26]

The high blood glucose in our study may be attributed to the severity of diabetic induction by streptozotocin. Induction with a high concentration of streptozotocin (60 mg/kg) may have caused massive damage to the rat pancreatic islets resulting in diabetes with severe hyperglycaemia; thus, the four weeks of treatment may not be enough for the phenolic compounds and metformin to show their anti-diabetic effect in these severely hyperglycaemic rats. This point of view might be justified as other earlier studies have shown a substantial decrease in blood glucose levels in diabetic rats with moderate hyperglycaemia when treated with ellagic acid and p-coumaric acid with doses similar to the dosage used in our present study. [45-46]

Extending the duration of the duration of treatment in diabetic rats with severe hyperglycaemia may be an option to allow for extensive pancreatic islet regeneration that would improve insulin secretion, resulting in significant blood glucose level reduction. This idea is supported by an earlier study which showed reduction of blood glucose level in severely hyperglycaemic rats when treated with ellagic acid (50 mg/kg) for eight weeks.[47] Our study was conducted for four weeks, and it shows that the purported anti-diabetic activity of ellagic acid, rutin and p-coumaric acid are not strong enough to lower the high blood glucose level in the severely hyperglycaemic rats within the four weeks of treatment.

Pancreatic islet recovery in streptozotocin-induced diabetic rats

Our study showed that despite its inability to lower blood glucose level, the phenolic compounds are able to promote pancreatic islets recovery in streptozotocin induced diabetic rats after four weeks of treatment. Although the

pancreatic islets morphology did not return to normal, a positive change was observed in these phenolic compounds-treated diabetic rats compared to the untreated diabetic rats. The anti-oxidant properties of these phenolic compounds may be responsible for the recovery of the pancreatic islets seen in this study. The phenolic compounds may attenuate the oxidative stress caused by hyperglycaemia or the cytotoxic effect of streptozotocin, preventing further pancreatic islets damage in these rats.

Our study also provided evidence of cellular regeneration by the phenolic compounds in the pancreatic islets of streptozotocin-induced diabetic rats possibly by increasing cell proliferation. The pancreatic islet cells of the phenolic compounds treated diabetic rats are dense and irregularly arranged. In addition, the changes seen in these groups are better than the metformin treated group. This finding is in line with studies that showed the promotion of β cell proliferation *in vivo* and *in vitro* hyperglycaemia through the upregulation of Cyclin D1 via the Akt pathway by the same group of phenolic compounds. [46, 48-49] While metformin could also restore pancreatic islets damage caused by diabetes, our study shows that the restoration activity is not able to match the ones observed in the phenolic compound-treated groups.
The observations here suggest that the phenolic compounds have a protective effect on the pancreatic islets of diabetic rats and these may have been exerted through their anti-oxidative and proliferative properties.

Neuroprotective effect of phenolic compounds
Our findings in this study suggests that ellagic acid and p-coumaric acid may protect the sciatic nerves of diabetic rats from further damage caused by hyperglycaemia. These anti-oxidant-rich phenolic compounds may protect the peripheral nerve by controlling diabetes through pancreatic tissue repair or directly protecting the peripheral nerve. While like the phenolic compound, metformin did not reduce hyperglycaemia in the diabetic rats after the four weeks of treatment, our histological observation of the sciatic nerve also suggest that metformin exert similar protective effect on the sciatic nerve. This finding is similar to a recent study on diabetic rats that showed the same neuroprotective effect through the preservation of the sciatic nerve even when blood glucose was high.[50] Thus, the protective effect of metformin may not have been solely mediated by its glucose-lowering effect; it may also be targeting the sciatic nerve. This notion is in agreement with others. [50-51]
Our study showed a slow progression of sciatic nerve damage after four weeks of diabetes where a rather mild nerve damage is observed. Because the sciatic nerve is the largest peripheral nerve, injury to this nerve may be delayed or may take a while to manifest compared to the smaller nerve fibres.
The four weeks period accorded to our observation study may not have been enough to allow for better development of sciatic nerve damage in the sciatic nerve. Therefore, extension of the four weeks period may have allowed for the signs of neuropathy in the sciatic nerve to be observed better and would have confirmed diabetic peripheral neuropathy in the animal model. Also, utilisation of an electron microscopic evaluation may have been able to provide stronger additional evidence. This, however, is not employed in this study due to some unavoidable constraints.

Another possibility for the slow progression of sciatic nerve damage could also be due to the protective mechanism played by the Schwann cells in response to injury, due to diseases such as diabetes mellitus, which may slow the progression of diabetic peripheral neuropathy. In the early stage of diabetes mellitus, protection of sciatic nerve or delay to the sciatic nerve damage could have been exerted by an increase in Schwann cells proliferation. In this present study, a slight increase in the number of Schwann cells were seen in the sciatic nerve of diabetic rats. This

observation is similar to the observation made in other diabetic study where Schwann cells proliferation in diabetic rats was observed.[52] Therefore, cell proliferation may be one of the defence mechanisms provided by the Schwann cells in diabetic nerve injury. This could explain why the signs of nerve damage caused by early diabetes are mild in our present study. In this condition, because phenolic compounds may have dampened the diabetic damage, the response of Schwann cells to proliferate may have also reduced in these groups.

Histological analysis versus biochemical analysis

It is interesting to highlight the sensitivity of the histological analysis in the present study. In animal studies, biochemical and histological methods are frequently utilised as the two components complement each other in an effort to obtain a more comprehensive finding. In our present study, the histological findings were found to be more robust and sensitive than the biochemical analysis. The morphological recovery of the pancreatic islets manifested earlier and thus was able to be observed earlier than that of the biochemical analysis. In that instance, the pancreatic islets regeneration was observed after four weeks of treatment with the selected phenolic compounds while the blood glucose level remains high during the four weeks period.

The only negative point in the histological study is that morphological evidence can only be obtained after the animals were sacrificed. Nevertheless, the histological finding is highly dependable as it provides strong morphological evidence and relatively accurate data. On the other hand, fasting blood glucose level are easily influenced by internal and external factors; because of these factors, the blood glucose levels can sometimes differ between animals, necessitating more samples to reduce standard error in statistical analysis. However, where ethics of animal usage is concern, the use of a large number of animals in research is usually not recommended. Therefore, histological analysis is an acceptable method to support animal studies despite the need to sacrifice animal as it reduces animal usage and provides strong morphological evidence with a relatively accurate data.

Limitations of the study

There were some limitations to the study. One of the limitations is the small number of animals. as some died during the experiment, and the study could not be extended due to Covid-19 pandemic. A quantitative analysis of histological changes was not possible due to the small number of samples. Thus, the histological analysis was based solely on observation.

Conclusion

Treatment of ellagic acid (100 mg/kg), rutin (100 mg/kg) and p-coumaric acid (100 mg/kg) for 28 days do not cause a reduction in blood glucose level of diabetic rats induced with 60 mg/kg streptozotocin. Nevertheless, these phenolic compounds have a protective effect on the pancreatic islets of diabetic rats and these may have been exerted through their anti-oxidative and proliferative properties. Four weeks period may not be enough for the development of sciatic nerve damage in the Sprague Dawley rats, despite the severe hyperglycaemia produced. Another possibility for the slow progression of sciatic nerve damage could be due to the protective mechanism played by the Schwann cells in response to diabetic injury where slight increase in its proliferation may have provided protection to the sciatic nerve or delay damage to the nerve. Ellagic acid and p-coumaric acid may protect the sciatic nerves of diabetic rats by controlling diabetes through pancreatic tissue repair or directly protecting the peripheral nerve.

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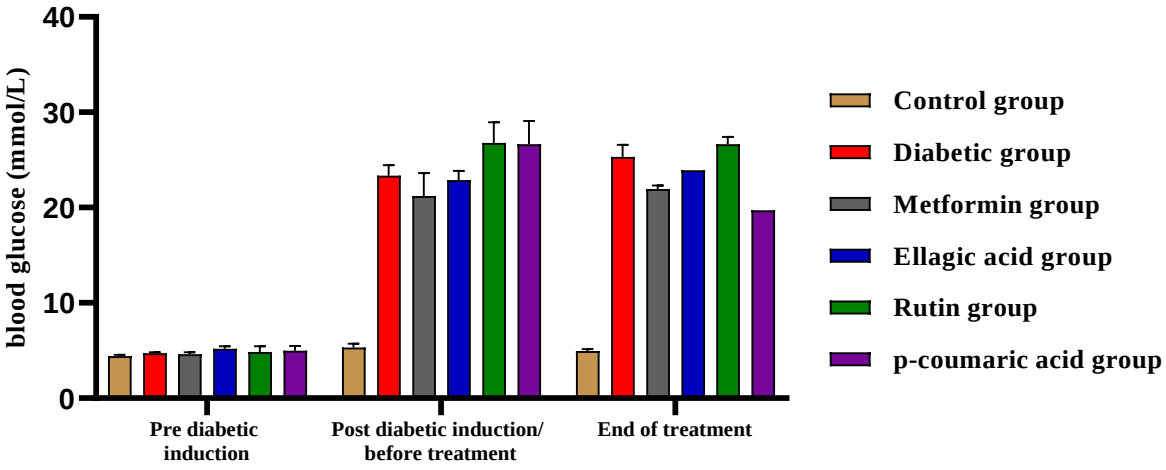

Figure 1. Mean fasting blood glucose for pre- and post-diabetic induction and at the end of treatment with ellagic acid (100 mg/kg), rutin (100 mg/kg), p-coumaric acid (100 mg/kg) and metformin (500 mg/kg) for 28 days. * indicates a significant difference between treated and control groups ($p<0.01$).

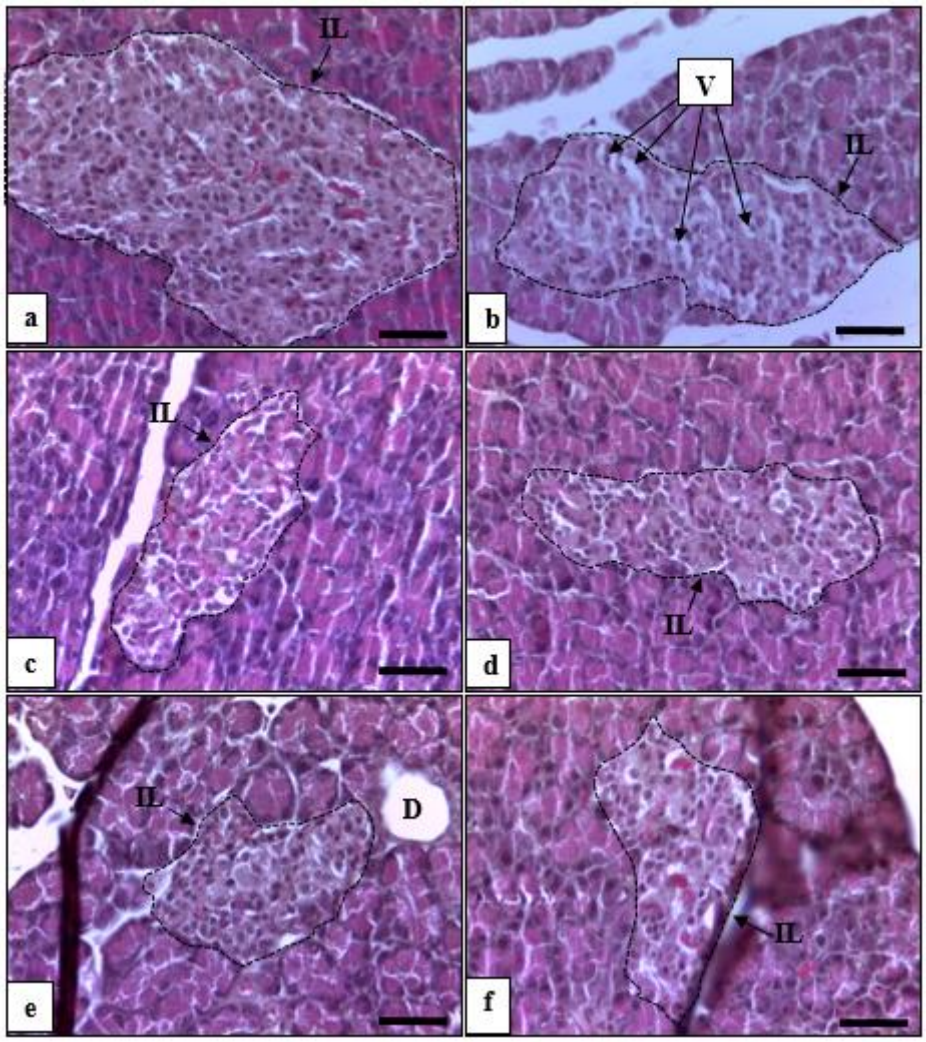

Figure 2. Photomicrographs of the pancreas stained with H&E. (a) control, (b) diabetic (c) metformin treated (500 mg/kg), (d) ellagic acid treated (100 mg/kg), (e) rutin treated (100 mg/kg), and (f) p-coumaric acid treated (100 mg/kg). Note: Cytoplasmic vacuolation (V) of islet of Langerhans (IL) was reduced in all the treated groups (28-day treatment) compared to the diabetic group. The islet of the treated groups had an irregular cellular arrangement. D=duct. 400X magnification. Scale bar = 50 µm.

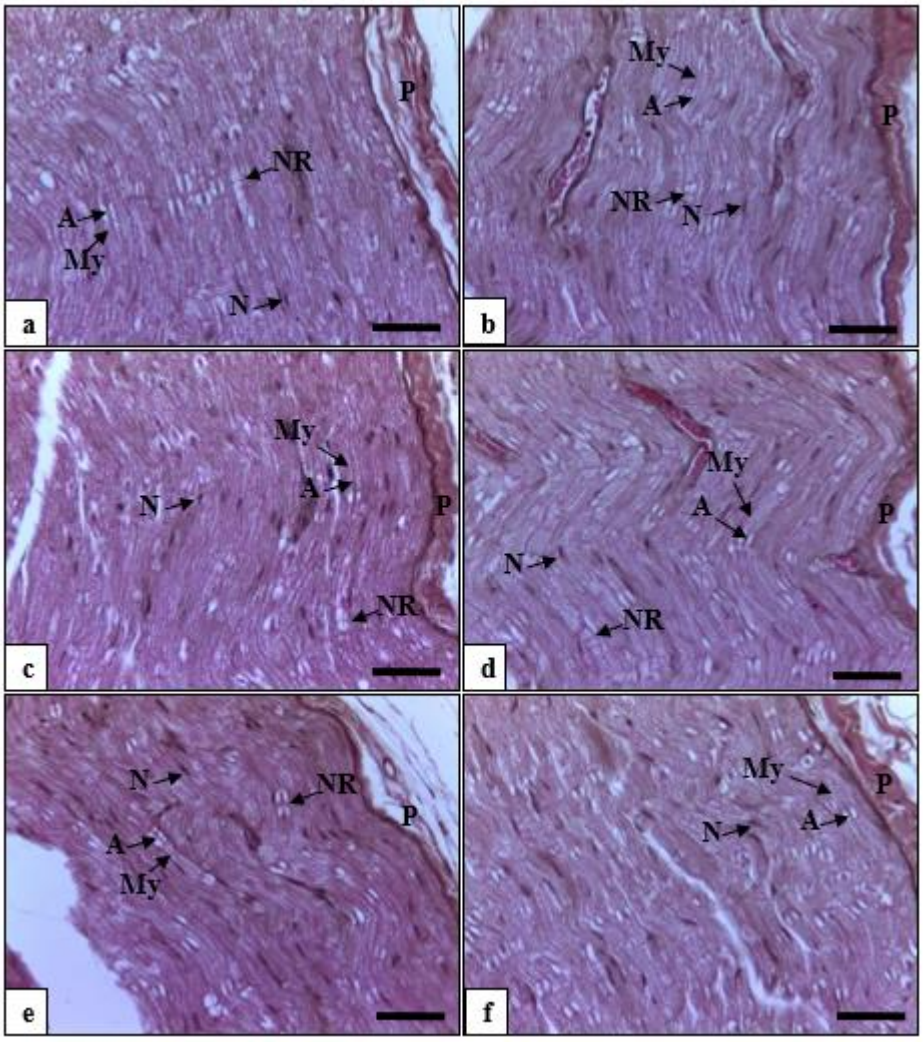

Figure 3. Photomicrographs of the longitudinal section of sciatic nerves stained with H&E. (a) control, (b) diabetic (c) metformin treated (500 mg/kg), (d) ellagic acid treated (100 mg/kg), (e) rutin treated (100 mg/kg), and (f) p-coumaric acid treated (100 mg/kg) groups after treated for 28 days. Note: More Schwann cell nuclei (N) were relatively observed in diabetic and all treated groups compared to control. A= axon, My= myelin neurokeratin, NR= node of Ranvier, P= perineurium. 400X magnification. Scale bar = 50 µm.

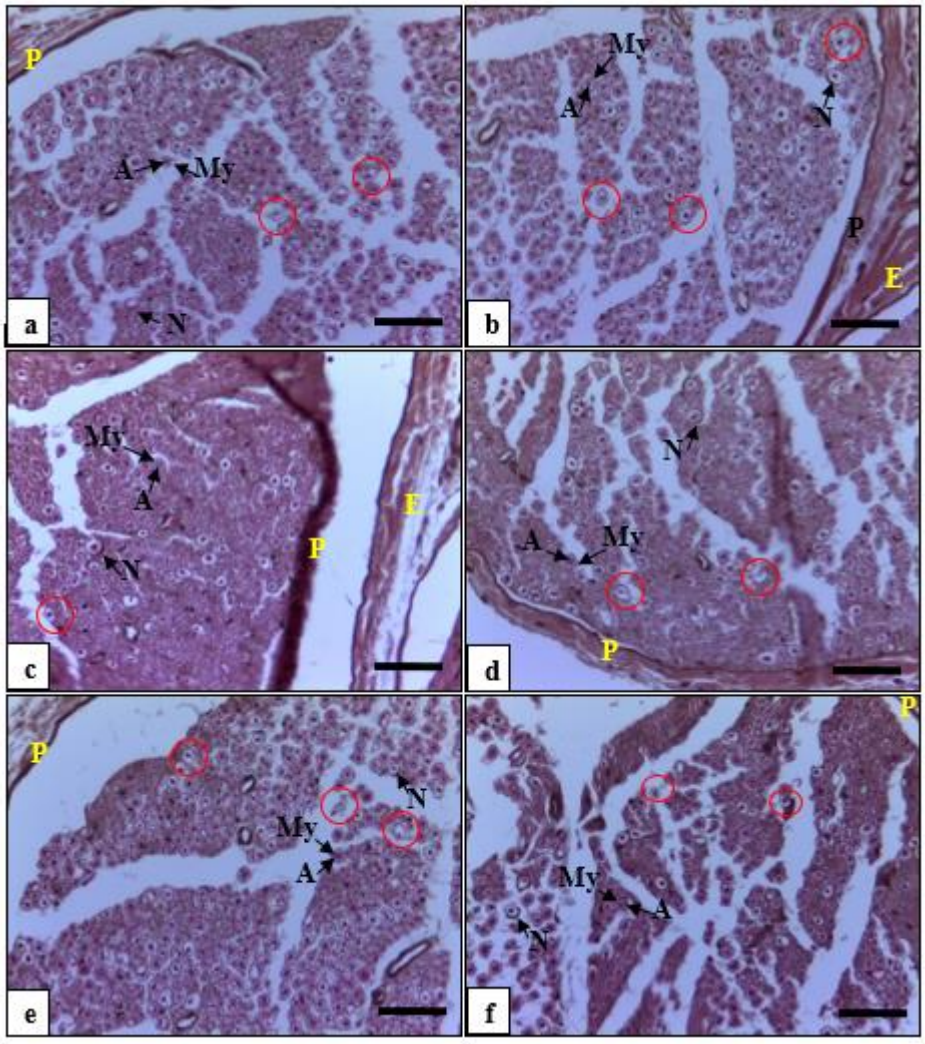

Figure 4. Photomicrographs of the transverse section of sciatic nerves stained with H&E. (a) control, (b) diabetic (c) metformin treated (500 mg/kg), (d) ellagic acid treated (100 mg/kg), (e) rutin treated (100 mg/kg), and (f) p-coumaric acid treated (100 mg/kg). Note: The treated group were given 28-day treatment. Irregularity in the shape of myelin sheath boundary with absence or clearance of myelin neurokeratin (red circle) was observed more in diabetic and rutin groups. A= axon, My = myelin neurokeratin, N= Schwann cell nucleus, E= epineurium, P= perineurium. 400X magnification. Scale bar = 50 µm.

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