Supplementation of Corosolic acid prevents the development of Neuropathic pain in Streptozotocin induced diabetic rats

AMAN BABANRAO UPAGANLAWAR[1]

[1]Department of Pharmacology, SNJBs SSDJ College of Pharmacy, Neminagar, Chandwad. India, Egypt

Received 11 August 2016
Accepted 7 October 2016

ABSTRACT

Objective: The present study was designed to screen the neuroprotective and antioxidant activity of corosolic acid in painful diabetic neuropathy (DN).
Methods: Diabetes was induced in rats by a single dose of STZ (60mg/kg, i.p). Diabetic rats were tested every week for the development of pain, at the 5th week, rats showed sensations of pain. At the 6th week the rats developed significant neuropathic pain. They were divided into different groups and treated with Corosolic acid (2 and 4 mg/kg, p.o) for a further two weeks. The pain was assessed in the diabetic rats by mechano-tactil allodynia, mechanical hyperalgesia and cold allodynia. At the end of the treatment period the rats were scarified and biochemical changes such as their plasma glucose level, endogenous antioxidants (Lipid peroxidation, reduced glutathione, superoxide dismutase and catalase) in sciatic nerve were evaluated. Further Na^+/K^+ ATPase and nitric oxide content was also evaluated.
Results: Treatment with the corosolic acid for two weeks restored their altered body weight and elevated blood sugar levels. Further corosolic acid showed a dose dependent reduction in pain in neuropathic animals. The level of endogenous antioxidants enzymes, Na^+/K^+ ATPase and nitric oxide were significantly prevented.
Conclusions: The result of the present study suggests the antidiabetic, antioxidant and neuroprotectieve property of corosolic acid in diabetic rats with neuropathic pain.

KEY WORDS: Streptozotocin, Corosolic acid, Diabetes, Neuropathy

Introduction

Diabetes mellitus is the most common endocrine disorder occurring worldwide. Diabetic neuropathy (DN) is a microvascular complication of diabetes and is associated with considerable morbidity and diminished quality of life [1]. Neuropathic pain in diabetes can be developed by hyperglycemia and associated metabolic imbalances. Numerous biochemical mechanisms of neurovascular and nerve damage have been reported in diabetic neuropathy, excessive production of free radicals and oxidative stress is thought to be a common etiological factor [2].

Corosolic acid, a triterpenoid named 2α-hydric ursolic acid, has been discovered in many Chinese medicinal herbs. It is mainly extracted from Banaba leaves (*Lagerstroemia speciosa*) as an active constituent [3]. It has been reported to have strong antidiabetic activity [4], antioxidant, anti-inflammatory and anti-hypertensive activities [5].

Treatment of DN is always a challenging and expensive task. It begins with optimizing glycemic control first and then associated pain. As oxidative stress is an ancillary player in DN, compounds with antioxidant property can be used as a supplement with the conventional treatment. Based on the above assumption the present study was designed.

Materials and Methods

Drugs and Chemicals

Streptozotocin, Superoxide Dismutase, Catalase, 1, 1, 3, 3-tetraethoxypropane was purchased from Sigma Aldrich Co. St. Louis. MO. USA. Corosolic acid was purchased from Biogenic Ltd, Bangalore.

All the reagents and chemicals used in the entire study were of analytical grade. Diagnostic kit for the measurement of glucose was purchased from Reckon Diagnostic Ltd. India.

Experimental Animals

All experiments were carried out on Male SD rats weighing 200-250gm, obtained from Lachmi Biotech, Pvt. Ltd, Pune

Correspondence to: Dr. Aman Babanrao Upaganlawar
Email: amanrxy@gmail.com

and were placed separately in polypropylene cages (approx. 2-4 per cage) with paddy husk as bedding. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi. (No.SSDJ/CPCSEA/IAEC/2013-14/01)

Experimental Design

Diabetes was induced by a single dose of Streptozotocin (60 mg/kg, i.p) in chilled citrate buffer, pH 5.5. Streptozotocin (STZ) was freshly prepared at the time of injection. Rats were fasted six hours before STZ injection and they had free access to water. They were provided with 10% sucrose water as the sole water source for 48 hours after STZ injection (to prevent sudden hypoglycemia). After 72 hours of STZ injection blood was withdrawn from each rat by retro orbital flexor under light ether anesthesia. Rats which showed blood glucose level more than > 250 mg/dl were considered diabetic and used for the further study. Blood glucose level was checked every week till the development of neuropathic pain and then after up to the completion of study. It was found that rats will develop significant neuropathic pain at the 6^{th} week. At this stage rats were divided into various groups, each containing six rats.

Group I: served as the control and received vehicle throughout the study.

Group II: STZ induced diabetic neuropathic pain (DNP).

Group III: neuropathic pain animals treated with Corosolic acid (2 mg/kg, p.o) for 2 weeks.

Group IV: neuropathic pain animals treated with Corosolic acid (4 mg/kg, p.o) for 2 weeks.

Group III: neuropathic pain animals treated with standard drug Pregabaline (10 mg/kg, p.o) for 2 weeks.

Assessment of Neuropathic pain

Mechanical Allodynia

Mechanical allodynia was assessed using Standard Von Frey hairs (Aesthesio, Made in USA. Supplied by Samitek Instruments, New Delhi). Rats were placed in acrylic cages with mesh bottoms which allowed full access to the paws. They were acclimatized for 15 min to stabilize the cage exploration and major grooming activities. Activity was assessed as suggested by Chaplan *et al*, 1994 [6].

Mechanical hyperalgesia

Mechanical hyperalgesia was assessed using the pin prick test. Rats were individually placed in suspended acrylic chambers on a mesh floor. After the acclimatization period for 30 min, plastic filaments were applied perpendicularly to the planter surface of both hind paw with sufficient force to bend the plastic filaments; paw withdrawal (lifting) latency was recorded in second. The cut off time of the paw withdrawal was 15s. The paw withdrawal time was measured weekly after confirmation of neuropathic pain [7].

Cold allodynia

The method was suggested by various authors. In that they dip the hind paw gently in ice cooled water and observed the paw withdrawal latency. In the present study we modified the method and used acetone to produce cold allodynia. The cotton swab was deepened into fixed volume of acetone, it was then applied perpendicularly to the planter surface of both hind paws for measurement of the paw withdrawal (lifting) latency, and the cut-off time was 15 secs [8].

Assessment of Biochemical parameters

Removal and Processing of Serum and Tissues for Various Estimations

At the end of the treatment period, blood was collected from the retro-orbital plexus without any anti-coagulant and allowed to clot for 10 minutes at room temperature. It was then centrifuged at 2500 rpm for 20 minutes. The serum obtained was used for the assessment of glucose level (GOD-POD using diagnostic kit).

Tissues homogenization

The animals were euthanasiously sacrificed and isolate sciatic nerve quickly transferred to ice-cold Tris hydrochloric buffered saline (pH 7.4). It was blotted free of blood and tissue fluids, weighed on an Electronic Balance WENSAR, (Model - PGB200). The Sciatic nerves were cross-chopped with surgical scalpel into fine slices, suspended in chilled 0.25M sucrose solution and quickly blotted on a filter paper. The tissues were then minced and homogenised in

chilled tris hydrochloride buffer (10mM, pH 7.4) to a concentration of 10% w/v. The homogenate was centrifuged at 10,000 rpm at 0°C for 15 minutes using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the determination of lipid peroxidation, Reduced Glutathione, Nitric oxide for antioxidant activities.

Assessment of lipid peroxidation and endogenous antioxidants

Assay of Lipid Peroxidation (MDA content)

It was estimated using the method described by Slater and Sawyer (1971) [9]. 2.0 ml of the tissue homogenate (supernatant) was added to 2.0 ml of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation and 2.0 ml of clear supernatant solution was mixed with 2.0 ml of freshly prepared thiobarbituric acid (TBA). The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532nm against reagent blank. Different concentrations (0-23nM) of standard malondialdehyde were taken and processed as above for standard graph. The values were expressed as nM of MDA/mg protein.

Assay of Reduced Glutathione (GSH)

Reduced glutathione was determined as follows, equal volumes of tissue homogenate (supernatant) and 20% TCA were mixed. The precipitated fraction was centrifuged and to 0.25ml of supernatant, 2ml of DTNB reagent was added. The final volume was made up to 3ml with phosphate buffer. The colour developed was read at 412nm against reagent blank. Different concentrations (10-50µg) of standard glutathione were taken and processed as above for standard graph. The amount of reduced glutathione was expressed as µg of GSH/mg protein [10].

Assay of Superoxide dismutase (SOD)

Superoxide dismutase was estimated using the method developed by Misra and Fridovich (1972) [11]. 0.5ml of tissue homogenate was diluted with 0.5ml of distilled water, to which 0.25ml of ice-cold ethanol and 0.15ml of ice-cold chloroform was added. The mixture was mixed well using cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0.5ml of supernatant, 1.5ml of carbonate buffer and 0.5ml of EDTA solution were added. The reaction was initiated by the addition of 0.4ml of epinephrine and the change in optical density/minute was measured at 480nm against reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme unit. Calibration curve was prepared by using 10-125 units of SOD.

Assay of Catalase (CAT)

It was estimated by the method of Hugo Aebi as given by Colowick *et al*. [12]. To 2ml of diluted sample 1ml of hydrogen peroxide (30 mmol/l) was added to initiate the reaction. The blank was prepared by mixing 2ml of diluted sample with 1ml of phosphate buffer (50mmol/l; pH 7.0). The dilution should be such that the initial absorbance should be approximately 0.500. The decrease in absorbance was measured at 240nm. Catalase activity was expressed as µmoles of H_2O_2 consumed/min/mg protein.

Assessment of Sodium-Potassium dependent adenosine triphosphatase (Na^+/K^+ ATPase)

It was estimated in the membrane sediments remain after centrifugation of the tissue homogenates. 1.0ml of tris-hydrochloride buffer and 0.2ml each of magnesium sulphate, sodium chloride, potassium chloride, EDTA, ATP were added to test tube containing 0.2ml of homogenate. The mixture was incubated at 36°C for 15 minutes. The reaction was arrested by addition of 1.0ml of 10% TCA, mixed well and centrifuged. The phosphorus content of the supernatant was estimated as described by Fiske and Subbarow (1925). The enzyme activity was expressed as µM of inorganic phosphorus liberated/mg protein/min [13].

Assay of nitric oxide (NO)

0.1ml of tissue homogenate (Supernatant) mixed with 1ml of Griess reagent and let stand for 15 min. Measured the absorbance of the solution against griess reagent at 540 nm using 10 mm cuvettes. Read on the standard curve the amount of nitrite corresponding to the actual absorbance [14].

Statistical analysis

All the values are expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Tukey's multiple comparison test as appropriate using computer based fitting program (Prism 5). Differences were considered to be statistically significant when P<0.05.

Results

Effect of Corosolic acid on serum glucose level and body weight

Rats injected with STZ (60mg/kg, i.p.), showed the significant (P<0.001) rise in serum glucose level and reduction in body weight as compared to normal control rats (Table 1).

Table 1. Effect of Corosolic acid on serum glucose level and body weight in DNP animals.

Groups	*Body weight (g)*	*Serum glucose(mg/dl) 72hrs*	*Serum glucose(mg/dl) 8th week*
Normal control	*248.22 ± 10.48*	*114.12± 1.90*	*118.30 ± 3.19*
STZ induced Control	*174.12± 13.53****	*291. 05 ± 5.42****	*390. 00 ± 5.42****
STZ + CA (2mg/kg)	*215.11 ± 10.09$^{\#\#}$*	*284.70 ± 1.51*	*246.50 ± 1.51$^{\#\#\#}$*
STZ + CA (4mg/kg)	*230.21 ±15.83$^{\#\#\#}$*	*278.20 ± 2.36*	*228.20 ± 2.36$^{\#\#\#}$*
STZ + PG (10mg/kg)	*219.32 ±11.14$^{\#\#\#}$*	*282.52 ± 1.58*	*319.72 ± 2.58*

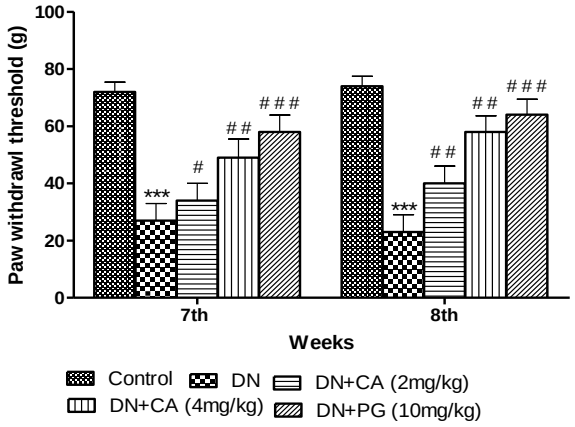
All values are presented as mean ± SEM, (n=6). P<0.05*, P<0.01**, P<0.001*** as compared to control group. P<0.05$^{\#}$, P<0.01$^{\#\#}$, P<0.001$^{\#\#\#}$as compared to disease group.

Serum glucose level was tested 72 hr. after the injection of STZ and at the end of experimentation. Rats whose glucose level was found to be more than 250 were consider as diabetic and included in the study, thereafter the glucose level was estimated every week up to the end of the study. Treatment with CA (2 and 4 mg/kg, p.o) for two weeks i.e. for the 7th and 8th week showed a significant (P<0.001) reduction in serum glucose level and improvement in body weight as compared to DN animals.

Effect of Corosolic acid on Mechano-tactile allodynia on Diabetes induced neuropathic pain

Mean paw withdrawal threshold of STZ diabetic control rats on the 8th week after induction of diabetes were significantly decreased (P < 0.001) as compared to normal non-diabetic control rats. Treatment with CA (2 and 4 mg/kg) for 2 weeks resulted in a significant increase (P < 0.001) in paw withdrawal threshold as compared to STZ diabetic neuropathy rats. This decrease in the mean paw withdrawal threshold was significantly increased by the treatment of Pregabalin (10mg/kg) for 2 weeks as compared to STZ diabetic control rats. There was no significant change in the mean paw withdrawal threshold of normal rats and per se group over the same time period (Figure 1).

Figure 1. Effect of Corosolic acid on Mechano-tactile allodynia in Diabetes induced neuropathic pain (Von Frey hair test).

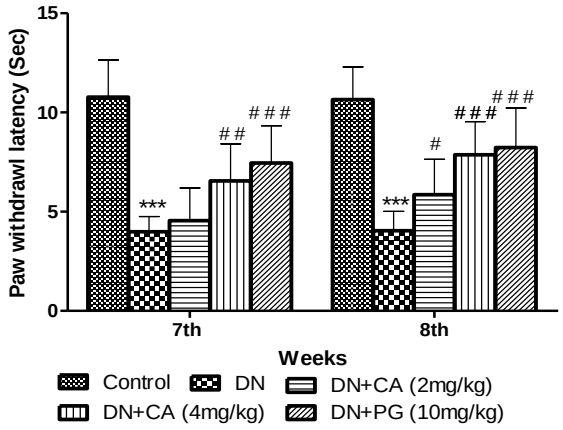


All values are presented as mean ± SEM, (n=6). P<0.05*, P<0.01**, P<0.001*** as compared to control group. P<0.05$^{\#}$, P<0.01$^{\#\#}$, P<0.001$^{\#\#\#}$as compared to disease group.

Effect of Corosolic acid on Cold allodynia in Diabetes induced neuropathic pain

As shown in figure 2, the test was performed using fixed volume of acetone. The acetone exposure showed significant pain to the animals with diabetes. Mean paw withdrawal threshold of STZ diabetic control rats on the 8th week after induction of diabetes was significantly decreased (P < 0.001) as compared to normal non-diabetic control rats. Treatment with CA (2 and 4 mg/kg) for 2 weeks resulted in a significant increase (P < 0.001) paw withdrawal threshold in cold allodynia as compared to STZ induced rats. Rats treated with pregabalin (10mg/kg) showed more significant inhibition (P < 0.001) in decrease in the mean paw withdrawal threshold than CA (2 and 4 mg/kg) treated rats. There was no significant change in the mean paw withdrawal threshold of normal rats and per se group over the same time period (Figure 2).

Figure 2. Effect of Corosolic acid on Cold allodynia in Diabetes induced neuropathic pain.

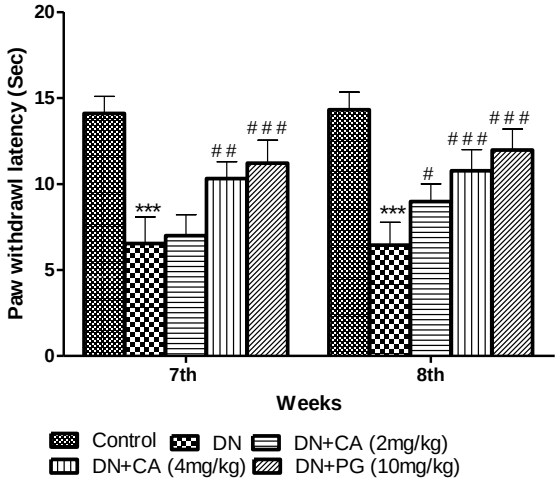


All values are presented as mean ± SEM, (n=6). P<0.05*, P<0.01**, P<0.001*** as compared to control group. P<0.05#, P<0.01##, P<0.001###as compared to disease group.

Effect of Corosolic acid on mechanical hyperalgesia in Diabetes induced neuropathic pain

It was found that paw withdrawal latency in the diabetic control rats before the induction of diabetic neuropathy was not significantly different than that in normal control rats. Significant (P<0.001) decrease in mean paw withdrawal latency was produced in the diabetic neuropathic rats after 5 weeks of STZ injection as compare to normal control rats. Rats treated with CA and Pregabalin for 2 weeks significantly (P<0.001, P<0.01) and dose dependently increased the change in mean paw withdrawal latency as compared to diabetic control rats. CA 4mg/kg showed more significance (P<0.001). (Figure 3).

Figure 3. Effect of Corosolic acid on mechanical hyperalgesia (Pin prick) in Diabetes induced neuropathic pain.



All values are presented as mean ± SEM, (n=6). P<0.05*, P<0.01**, P<0.001*** as compared to control group. P<0.05#, P<0.01##, P<0.001###as compared to disease group.

Effect of Corosolic acid on endogenous antioxidants level in Diabetes induced neuropathic pain

LPO level in sciatic nerve of STZ treated diabetic control rats was significantly (P<0.001) elevated as compared to normal control rats. LPO level in CA treated rats at different doses (2, 4 mg/kg. p.o.) and Pregabalin (10mg/kg. p.o.) was significantly (P<0.05) decreased as compared to DNP rats. (Figure 4a). The level of GSH and SOD in sciatic nerve of STZ treated diabetic rats was significantly (P<0.01) reduced as compared to normal control rats. Treatment with CA (2 and 4mg/kg/p.o) and Pregabalin (10mg/kg.p.o.) showed significant (P<0.01, P<0.05) increase in the activity of GSH and SOD as compared to STZ induced DNP rats (Figure 4b, 4c). The level of Catalase was found to be decreased as compared to control animal but it was found to be non-significant (Figure 4d). Treatment with CA at different doses (2, 4 mg/kg. p.o.) and Pregabalin (10mg/kg.p.o.) showed no effects on the level of Catalase.

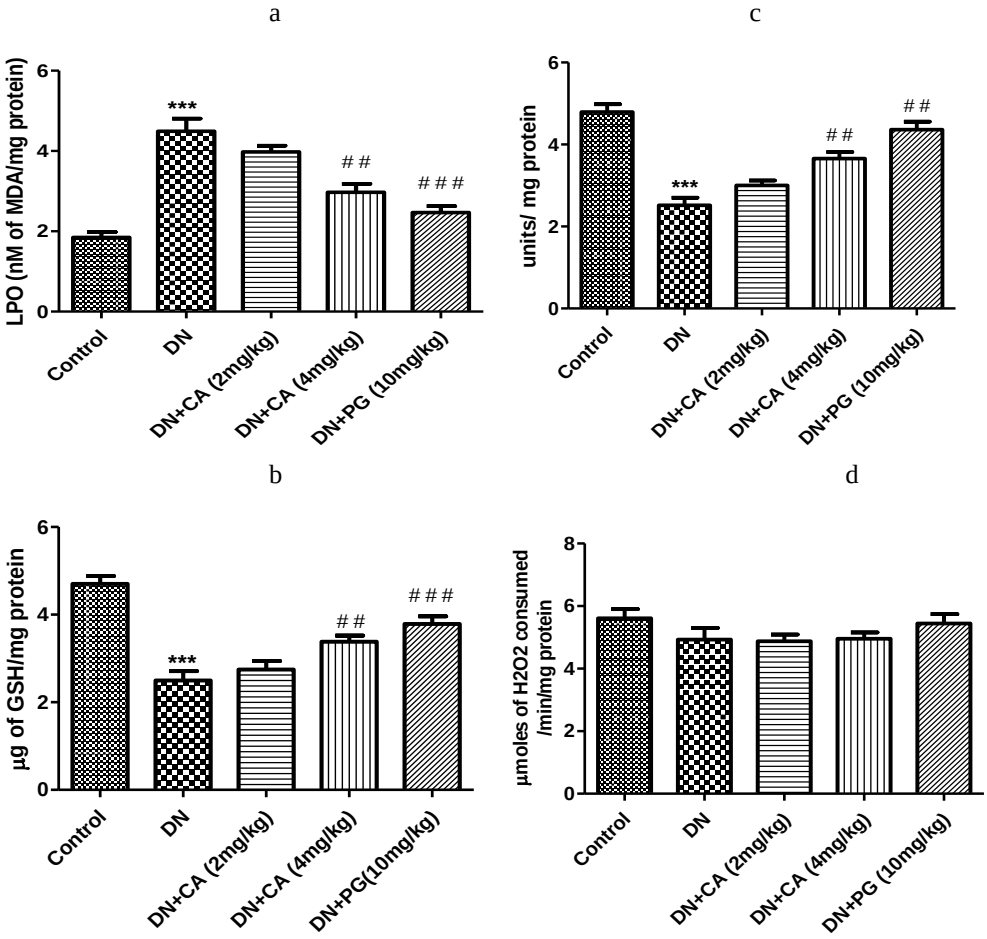
Effect of Corosolic acid on membrane bound Phosphates (ATPase) level in Diabetes induced neuropathic pain

Na^+-K^+-ATPase level in STZ diabetic control rat was significantly decreased (P < 0.05) after 8 weeks of induction of diabetic neuropathy as compared to the normal nondiabetic control rats. Treatment with Corosolic acid (2 and 4 mg/kg) for 2 weeks significantly (P < 0.001) and dose dependently elevated the decreased Na^+-K^+-ATPase level in sciatic nerve as compared to STZ diabetic control rats. CA 2 mg/kg didn't show any significant effects on ATPase level. Treatment with pregabalin didn't show any significant improvement in APTase level as compared to diseased animals (Figure 5).

Effect of Corosolic acid on Nitric oxide level in Diabetes induced neuropathic pain

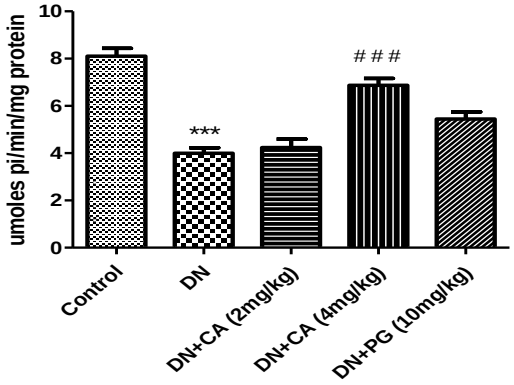
The level of Nitrite was significantly elevated (P < 0.001) in the sciatic nerve of STZ diabetic control rat on 7^{th} and 8^{th} week after induction of diabetes as compared to the normal nondiabetic control rats. Two-week treatment with corosolic acid (2 and 4 mg/kg) significantly (P < 0.05) inhibited this increased nitrite levels as compared to STZ diabetic control rats. Rats treated with pregabalin (10 mg/kg) also showed the significant attenuation (P < 0.05) in these elevated levels of neural nitrite as compared to STZ diabetic control rat. Normal rats and per se group did not show any change in the levels of neural nitrite over the same period of time (Figure 6).

Figure 4. Effect of Corosolic acid on (a) Lipid Peroxidation, (b) reduced glutathione, (c) superoxide dismutase and (d) catalase in diabetes induced neuropathic pain.



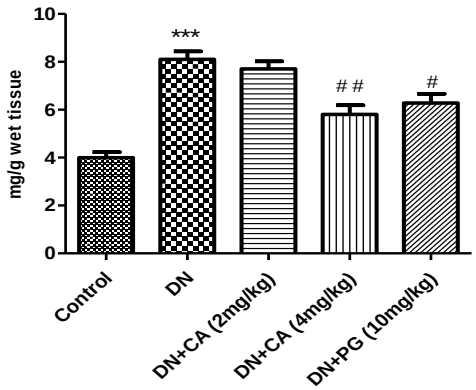
All values are presented as mean ± SEM, (n=6). P<0.05*, P<0.01**, P<0.001*** as compared to control group. P<0.05#, P<0.01##, P<0.001###as compared to disease group.

Figure 5. Effect of Corosolic acid on Na^+/K^+ATPase activity in diabetes induced neuropathic pain.



All values are presented as mean ± SEM, (n=6). P<0.05*, P<0.01**, P<0.001*** as compared to control group. P<0.05#, P<0.01##, P<0.001###as compared to disease group.

Figure 6. Effect of Corosolic acid on Nitric oxide levels in diabetes induced neuropathic pain.



All values are presented as mean ± SEM, (n=6). P<0.05*, P<0.01**, P<0.001*** as compared to control group. P<0.05#, P<0.01##, P<0.001###as compared to disease group.

Discussion

STZ is a glucosamine nitrosourea compound which is toxic to pancreatic β-cell. The STZ-induced diabetic rat model is widely used in the study of DNP. It is reported that STZ at a dosage of 50-60 mg/kg is able to induce a hyperglycemic condition in rats by destroying the pancreatic islet cells.

In the present study STZ at 60mg/kg showed persistent hyperglycemia in rats, and reduction of body weight. This incidence is in line with previous reports [15, 16].

Neuropathic pain comprising of mechanical allodynia and thermal hyperalgesia represents the peripheral and central component of nociception. In animal models this features

can be measured by tail withdrawal latency and paw withdrawal threshold. Chronic hyperglycemia leads to reduced threshold of pain due to increased oxidative stress, advance glycated end product, voltage gated Na^+ channels, and alteration of inflammatory mediators. The change in nociception occurred due to ischemia, peripheral receptors sensitization, ectopic activity in sprouting fibers and alterations in dorsal root ganglia cells [17,18]. In the present investigation, painful neuropathy was ameliorated and justified its previous pharmacological profile. There was a significant decrease in the paw withdrawal pressure and tail withdrawal latency in diabetic rats which are in line with study conducted by Sharma *et al*, [19]. In our study, treatment with Corosolic acid showed a significant increase in paw withdrawal pressure as well as tail withdrawal latency dose-dependently. Corosolic acid has been proved as a potent antidiabetic agent who has antioxidant and anti-inflammatory profile [16, 20]. The reduction in blood glucose level might be the reason for a decrease in pain threshold in rats.

In the present investigation, the level of endogenous antioxidants like SOD and GSH was significantly decreased in the STZ control rats. The diabetogenic drug STZ has been reported to give rise to superoxide (O_2-) whereby the drug destroys the islet of langerhans of the pancreas and precipitate diabetes mellitus. The redox balance in the neuron was regulated by the SOD enzyme. The SOD is responsible for conversion of superoxide anions to H_2O_2 and thus preventing oxidative stress. The activation of aldose reductase and protein kinase C due to elevated levels of SOD in neurons resulted in pain perception [21, 22]. GSH is responsible for the quencher of this free electron. Intraperitoneal administration of STZ resulted in deprivation in level of GSH which caused cell death and thus give rise to hyperalgesia [22, 23]. Chronic treatment with corosolic acid for 2 weeks was able to elevate the reduced levels of SOD and GSH reiterating its antioxidant profile. In the present study Lipid peroxidation content was found to significantly increase in DPN rats. The increase in production of oxidative stress in diabetic condition leading to structural destruction of unsaturated fatty acids in lipid membrane resulted in the elevated levels of malondialdehyde leads to lipid peroxidation [24]. Treatment with Corosolic acid significantly attenuated this elevated level of lipid peroxidation in DPN in rats. Ionic changes are responsible for the conduction of nerve impulse. Na^+/K^+ ATPase are an endogenous enzyme modulating the entry and expulsion of cellular cations like Na^+ and K^+ [25]. Hence, the level of Na^+/K^+ ATPase was determined in the present study. DNP leads to a decrease in the level of Na^+/K^+ ATPase in sciatic nerve which indicated failure of the Na^+-K^+ pump. In the present study, treatment with corosolic acid significantly increased the neuronal level of Na^+/K^+ ATPase suggesting its role in the restoration of nerve conduction.

NO combines with superoxide to form peroxynitrite, which rapidly causes protein nitration or nitrosylation, DNA damage, lipid peroxidation, and cell death and has direct toxic effects on the nerve tissue leading to neuropathic pain [26]. Nitrosative stress is inevitably enhanced when free radicals are generated due to diabetic neuropathy. This is represented by elevated levels of nitric oxide in our findings. Corosolic acid restores the level of nitric oxide and prevents further damage due to the formation of peroxynitrite radicals. This indicates the strong antioxidant activity of corosolic acid. Corosolic acid, is one of the major active constituents of some plants helping to reduced blood glucose level by activating the transport of glucose across the cell membranes [27, 28]. Due to the potent antidiabetic and antioxidant activity of corosolic acid it showed protection of neuropathic pain induced by diabetes.

Treatment with Corosolic acid in diabetes induced neuropathic pain significantly reversed mechanical allodynia, mechanical hyperalgesia and cold-allodynia in rats. Treatment also prevents the altered generation of free radicals and oxidative stress by maintaining the levels of endogenous antioxidant level. The neuroprotective effects of Corosolic acid in the present study might be due to its strong antidiabetic and antioxidant activities. This is the first report of Corosolic acid which showed strong protective effect in diabetic nephropathy. Further in depth study at molecular level is required to understand exact mechanism of action of corosolic acid.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgements

Author is thankful to Board of Colleges and University Development (BCUD), Savitribai Phule Pune University (SPPU), Pune, for providing financial assistance in the form of minor research project. Author is also thankful to

the management and Principal Dr. C. D. Upasani, SSDJ College of Pharmacy, Chandwad for providing necessary facilities for the project.

References

1 Bril V. Treatment of Diabetic Neuropathy. J Periphera Nerv Sys. 2012; 2 (1), 22-27.
2 Hosseini A, Mahammad M. Diabetic Neuropathy and Oxidative stress: Therapeutic Perspective. Oxid Med Cell Longev. 2013,1-15.
3 Thiticornpong W. Pharmacognostic evaluations of Lagerstroemia speciosa leave. J Med Plants Res. 2011, 5(8), 1330-37.
4 Judy WV, Hari SP, Stogsdill WW, Judy JS, Naguib YM, Passwater R. Antidiabetic activity of a standardized extract (glucosol) from Lagerstroemia speciosa leaves in type II diabetics. A dose-dependence study. J Ethnopharmacol. 2003, 87,115–117.
5 Yamaguchi YU, Yamada K, Yoshikawa N, Nakamura K,Haginaka J,Kunitoma M. Corosolic acid prevents oxidative stress, inflammation and hypertension in SHR/NDmcr-cp rats, a model of metabolic syndrome. Life Sci. 2006, 79, 2474-79, 2006.
6 Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksha TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Meth. 1994, 53, 55-63.
7 Shao-rui C, Samoriski G, Hui-lin P. Antinociceptive effect of chronic administration of uncompetitive NMDA receptor antagonists in a rat model of diabetic neuropathic pain. Neuropharmacol. 2009, 57,121-126.
8 Naik AK, Tandan SK, Dudhgaonkar SP, Jadhav SH, Kataria M, Prakash VR, et al. Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-l-cysteine in rats. Eur J Pain.2006, 10 (7), 573-9.
9 Slater TF, Sawyer BC. The stimulatory effect of carbon tetrachloride and other halogenalkane or peroxidative reaction in the rat liver functions in vitro. Biochem J. 1971, 123, 805-815.
10 Moron MS, Depierre JW. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochimbiophis Acta. 1979, 582, 67-78.
11 Misera HP, Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for SOD. The J Biol Chem .1972, 247,3170–5.
12 Aebi H. Catalase in vivo. Methods Enzymol. 1984,105,121-126.
13 Bonting SL. Presence of enzyme system in mammalian tissues. Membrane and ion transport. Wiley Int Sci. 1970, 257–63.
14 I. Guevara, J, Iwanejko A, Dembinska-Kiec J, Pankiewicz A, Wanat PA et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clinica Chimica Acta. 1998, 274, 177-88.
15 Kandhare AD, Raygude KS, Ghosh P, Bodhankar SL. The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. Int J Green Pharm. 2011, 5, 236–43.
16 Negi G, Kumar A, Kundal RK, Gulati A, Sharma SS. Functional and biochemical evidence indicating beneficial effect of melatonin and Nicotinamide alone and in combination in experimental diabetic neuropathy. Neuropharmacol.2010,58, 585-592.
17 Anjaneyulu M, Chopra K. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol. 2004, 31, 244–8.
18 Mahesh T, Menon VP. Quercetin allievates oxidative stress in streptozotocin induced diabetic rats. Phytother Res. 2004, 18(2), 123–7.
19 Sharma S, Kulkarni SK, Chopra K. Resveratrol, a polyphenolic phytoalexin attenuates thermal hyperalgesia and cold allodynia in STZ induced diabetic rats. Ind J Exp Biol. 2006, 44,566–9.
20 Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. Antidiabetic and anti-obesity activity of Lagerstroemia speciosa. Evid Based Complement Alternat Med. 2007, 4,401–407.
21 Kamei J, Mizoguchi H, Narita M, Tseng LF. Therapeutic potential of PKC inhibitors in painful diabetic neuropathy. Expert Opin Investig Drugs. 2011, 10, 1653–64.
22 Jain D, Bansal MK, Dalvi R, Upaganlawar A, Somani R. Protective effect of diosmin against diabetic neuropathy in experimental rats. J Int Med. 2004, 12(1),35-41.
23 Patil MK, Kandhare AD, Bhise SD. Anti-inflammatory effect of Daucus carota root on experimental colitis in rats. Int J Phar Pharmaceutical Sci. 2012, 4(1), 337–43.
24 Upaganlawar A, Balaraman R. Protective effects of Pomegranates fruit juice on electrocardiographic, biochemical and Histoarchitectural changes in isoproterenol induced myocardial infarction in rats. J Int Health Sci. 2013, 1(1), 20-27.
25 Dixon IMC, Kaneko M, Hata T, Panagia V, Dhalla NS. Alteration in cardiac membrane Ca2+ transport during oxidative stress. Mol Cell Biochem. 1990, 99,125-133.
26 Kim SY, Lee JH, Yang ES, Kil IS, Park JW. Human sensitive to apoptosis gene protein inhibits peroxynitrite-induced DNA damage. Biochem Biophy Res Commun. 2008, 301 (3), 671-674.
27 Miura T, Ueda N, Yamada K, Fukushima M, Ishida T, Kaneko T et al. Antidiabetic effects of corosolic acid in KKAy diabetic mice. Biol Pharm Bull. 2006, 29, 585–587.
28 Fukushima M, Mastuyama F, Ueda N, Egawa K, Takemoto J, Kajimoto Y, et al. Effect of corosolic acid on post challenge plasma glucose levels. Diabetes Res Clin Pract. 2006, 73, 174-177.