PROTECTIVE EFFECTS OF COCCINIA GRANDIS LEAF EXTRACT: BEHAVIOURAL, ELECTROPHYSIOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL FEATURES OF DIABETIC NEUROPATHY

Bhaskar Nagilla and Pratap Reddy K*

Neurobiology lab, Department of Zoology, University College of Science, Osmania University, Hyderabad – 500 007, Andhra Pradesh, India

ABSTRACT: The aim of present study was to evaluate the nociceptive, neuro-muscular coordination, electrophysiological, biochemical and histological features of diabetic neuropathy in streptozotocin induced diabetic rats and the protective effects of Coccinia grandis leaf methanolic extract. Coccinia grandis (200mg/kg per day) was given to diabetic rats for 3 weeks. Metformin (150mg/kg body weight) was used as standard reference drug. Coccinia grandis showed its protection against allodynia test. NCV were also attenuated by treatment of Coccinia. The significant decrease AR shows its protection against diabetic complications. Histological alterations induced by diabetes in sciatic nerve were restored with Coccinia grandis leaf extract treatment. These results suggest that Coccinia grandis has attenuated progression diabetic neuropathy in STZ-induced diabetic rats.

INTRODUCTION: Diabetic neuropathic pain is one of the most recognized most difficult types of pain, which is a symptom of diabetic neuropathy (DN). DN is the microvascular complication associated with diabetes. Peripheral nerve injury is associated with neuropathic pain and is characterized by the sensory abnormalities such as unpleasant abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia), and pain in response to a stimulus that does not normally provoke pain (allodynia). The various proposed mechanism which lead to pathogenesis of diabetic neuropathy are activated polyol pathway, AGE’s formation, PKC activation and Hexosamine pathway.

Hyperglycemia is the primary culprit for diabetic neuropathy. There have been major advances in the control of hyperglycemia (diabetes), through dietary changes, hypoglycemic agents, insulin and islet transplantation, even though the long-term complication of diabetes, such as neuropathy remains serious problem.

Although the oxidative stress is a major determinant in diabetic complications including diabetic neuropathy which is a result of cross links between above pathways, which was proposed by several, studies.
Therefore, agents or compounds that exert multiple actions, such as antioxidant, antidiabetic/hypoglycemic, AR inhibitory, and antiglycation properties could be more effective than agents with a single action.

*Coccinia grandis*, the ivy guard, of the family Cucurbitaceae is distributed in tropical Asia, Africa and is commonly found in India, Bangladesh and Srilanka. Since long before the leaves are consumed to control of hyperglycemia as indigenous system of medicine. Aqueous fractions significantly inhibited inflammation which can be thought to possess antiproliferative and antiarthritic activities similar to cyclooxygenase inhibitor.\(^2\)

Every part of this plant is valuable in medicine and various preparations have been mention in indigenous system of medicine for various skin diseases, bronchial catarrh, bronchitis and Unani systems of medicine for ring worm, psoriasis, small pox, and scabies and other itchy skin eruptions and ulcers\(^3\). The leaves of this species are widely used in Indian folk medicine for reducing the amount of sugar in urine of patients suffering from diabetes mellitus. Literature suggests the use of this plant in the treatment of diabetes\(^4\).

The present study reports the possible protective effects of *Coccinia grandis* leaf extract on serum glucose, neuronal protein carbonyls, allodynia, motor coordination, nerve conduction velocity, Aldose reductase and Na"K" ATPase activity.

**MATERIAL AND METHODS:**

**Plant material and extraction** - The fresh leaves of *Coccinia grandis* were collected locally. Leaves were then shade dried at room temperature. Dry material was coarsely pulverized to powered form. The powder was extracted with boiling water and ethanol using rotary evaporator and the crude extract was used for experiment. A voucher specimen (No.018) was deposited at Department of Botany, University College of Science, Osmania University, Hyderabad-500007.

**Animals** – Adult male albino rats of Wistar strain (NIN) aged 11–12 weeks (100–200g wt.,) obtained from National centre for laboratory animal sciences, NIN, Hyderabad were used.

The animals were housed in plastic cages, which were maintained in the climate-controlled animal facility (Dept. of Zoology, Osmania University, Hyderabad) with a 12-h light/12 h dark cycle at a stable temperature 18-22 °C with standard pellet diet (NIN) and water ad libitum. All experiments involving animals were conducted according to guidelines of the Institutional Animal Ethics Committee (CPCSEA No: 383/01/a/CPCSE).

**Chemicals** - STZ was obtained Sigma Chemical (USA). Metformin drug procured from Hetero drugs, INDIA. Other essential chemicals were obtained from SRL biochemical, INDIA.

**Experimental design** – The animals were divided in to five groups each containing six animals each.

1. Groups-I: This group served as untreated control and received physiological saline
2. Group-II: This group served as positive control (diabetic) treated with STZ (Streptozocin 50 mg/Kg body weight in 100mM Citrate buffer pH 4.5
3. Group-III: This group served as Metformin, wherein STZ induced diabetic animals treated with Metformin drug (150mg/kg body weight in RO water)
4. Group-IV: This group served as Coc+D, STZ induced diabetic animals treated with *Coccinia grandis* leaf extract (200mg/kg body weight in RO water)
5. Group-V: This group served as Coc+C, control animals were treated with *Coccinia grandis* leaf extract (200mg/kg body weight in RO water).

The experiments were carried out to see the short-term effect of diabetes on the sciatic nerve. The animals were sacrificed after 21 days and various biochemical and histological studies were conducted on sciatic nerve.

**Analgesic Test:**

1. **Allodynia test** – Allodynia test was conducted as described by Bennet and Xie, 1998\(^5\), to observe sensitivity of animals to cold.

2. **Neuro-muscular coordination** – Neuro-muscular coordination and balance was measured on Roto rod as described Carter *et al* 1999\(^6\), which were used to assess the ability of
an animal to balance on a rotating rod at specific speed of 24 RPM. The time taken was recorded in seconds. 

3. **Nerve conduction velocity** - In vivo nerve conduction experiments were performed in deep anaesthesia with 60 mg/kg body wt., of sodium pentabarbitone, supplemental pentobarbitone was provided during the entire experiment through i.p injection. Sciatic nerve about 4-6 mm at mid-thigh region was exposed and freed from adherent tissue. Animal body temperature maintained at 37°C with a warm blanket and provides the O₂, CO₂ supply with 90:10 ratios. Nerve perfused with mammalian ringer solution to prevent the drying of tissue. The electrodes place at two sites of sciatic nerve, one is placed at the site of injury (for recording) another placed near the ischium or just proximal to the injury site (stimulator). The setup is ready for recording. The nerve conduction velocity (NCV) was calculated by the ratio distance between the two sites of stimulation in mm divided by the difference between proximal and distal latencies in ms, giving a value for NCV in meters per second (m/s). 

**BIOCHEMICAL ESTIMATIONS:**

**Preparation of tissue extracts:** The animals were sacrificed by cervical dislocation after 21st day and sciatic nerves carefully dissected out avoiding extraneous tissue, washed with normal saline, blotted dry, the nerves were immediately transfer and kept at 80 °C to be used later.10% tissue (sciatic nerve) homogenate was prepared in 50mM potassium phosphate buffer pH 7.2 and centrifuged at 25,000g for 30 min at 4°C and the supernatant is used for Aldose reductase (AR) activity.

**Estimation of Aldose Reductase (AR, EC.1.1.1.21):** The oxidation of NADPH is measured as an index of Aldose reductase activity by Hayman and Kinoshita.

**Other estimations:** Blood glucose was estimated in the plasma using glucose measuring kit from (Beacon Diagnostics Pvt Ltd), New Delhi India., utilizing glucose oxidase-peroxidase (GOD-POD) method. The extent of protein oxidation was determined by measuring the protein carbonyl content of soluble protein of tissues (sciatic nerve) spectrophotometrically using 2, 4,-dinitro phenyl-hydrazine.

**Histological processing:** Fixation of nerves and staining was done as explain by Federica Di Scipio, et al., 2008.

**Statistical analysis:** Results are presented as mean ± S.E., six in each group. Statistical difference between control and various groups was determined by one-way ANOVA, followed by post Hoc test (Multiple comparisons). p-values less than 0.05 were considered significant.

**RESULTS:** The body weight, serum glucose levels and protein carbonyls of all the experimental groups with diabetes are shown in Fig 1, Fig. 2 and Fig. 3. The body weights of STZ induced diabetic rats were found to be reducing throughout of the study.

![FIG. 1: EFFECT OF COCCINIA GRANDIS LEAF EXTRACT ON BODY WEIGHT IN RATS. (Body weight in grams)](image-url)
However, the percentage changes in body weights of metformin treated and Coccinia grandis leaf extract treated diabetic rats were not significantly different compared to the diabetic control rats. Blood glucose levels were persistently high STZ-induced diabetes in rats (169%) in comparison to the control group, which was restored to 43% in metformin treated animals and 43% in Coccinia grandis leaf extract treated animals. Levels of protein carbonyls an important marker of the long-term AGE’s state was found to be significantly elevated in short-term (p < 0.05) diabetic animals compared to control. The protein carbonyls levels in the Met group were found significantly nearer to the control group and that of Coc+D is -42% compared to control group.

Measurement of antinociceptive activity: At the end of the 3rd week the nociceptive threshold was significantly lower in diabetic rats from non-noxious stimuli as compared to control (Fig. 4).

The STZ induced diabetic rats has shown progressive decrease in coordination with the time periods. The decrease in coordination was moderate on 14th day with thereupon it has shown further decline in the coordination indicating neuropathy on 21st day. The subsequent treatment
with *Coccinia grandis* leaf extract was found reverse the coordination on 14th day, thereafter the coordination almost started to decline on 21st day respectively.

**FIG. 5:** EFFECT OF *COCCINIA GRANDIS* LEAF EXTRACT ON NEUROMUSCULAR COORDINATION TEST IN RATS ON GIVEN WEEKS. (Coordination test is expressed in seconds) (Values are given as mean ± Std.E for groups of six animals each. Values are statistically significant at p<0.05. Significance Control Vs Met is < 0.01; Control Vs Coc+C is < 0.4; Diabetes Vs Coc+D is <0.003; Met vs Coc+C is < 0.004; respectively)

**FIG. 6:** EFFECT OF *COCCINIA GRANDIS* LEAF EXTRACT ON NERVE CONDUCTION VELOCITY IN RATS ON 21ST DAY. (NCV expressed in meters/sec) (Values are given as mean ± Std.E for groups of six animals each. Values are statistically significant at p<0.05)

Coccinia grandis leaf extract treatment of diabetic rats (Coc+D) resulted in -14% lower NCV compared to control. However, the NCV of diabetic rats treated with metformin (met) was -22% lower than control.

**Aldose reductase and Na⁺-K⁺ ATPase activity of sciatic nerve** - There was significant increase in aldose reductase enzyme activity in sciatic nerve of diabetic animals (+83%) as compared to normal animals. Diabetic rats treated with *Coccinia grandis* leaf extract showed decrease in aldose reductase activity by +50% (Fig. 7).

**FIG. 7:** EFFECT OF *COCCINIA GRANDIS* LEAF EXTRACT ON AR ACTIVITY OF SCIATIC NERVE IN RATS ON 21ST DAY. (Expressed as µ moles of NADPH oxidized/ hour/100 mg of protein) (Values are given as mean ± Std.E for groups of six animals each. Values are statistically significant at p<0.05 Significance Met Vs Coc+C is < 0.015 respectively)

**FIG. 8:** EFFECT OF *COCCINIA GRANDIS* LEAF EXTRACT ON Na⁺K⁺ ATPASE ACTIVITY OF SCIATIC NERVE IN RATS ON 21ST DAY. (Expressed as µ moles of pi/ hour/mg wt. of tissue) (Values are given as mean ± Std.E for groups of six animals each. Values are statistically significant at p<0.05 Significance control vs diabetes is ns, con Vs met is ns; con vs Coc+D is ns; con vs Coc+C is ns; diabetes vs met is ns; diabetes vs Coc+D is ns; diabetes vs Coc+C is ns respectively)
Percentage of variation of metformin treated diabetic was +61% and that of control animals treated with *Coccinia grandis* leaf extract was +1%. Sciatic nerve Na⁺-K⁺ ATPase activity (**Fig. 8**) was significantly reduced in diabetic rats (-77%) as compared to normal control. This was largely corrected by *Coccinia grandis* leaf extract treatment by +8%. Percentage of variation of metformin treated diabetic was -46% and that of control animals treated with *Coccinia grandis* leaf extract was -6%.

**Histological changes in Sciatic nerve:**

**FIG. 9:** PHOTOMICROGRAPHS SHOWING THE TRANSVERSE SECTIONS OF THE SCIATIC NERVES. Fig. A is of Control rat (5µm thick, 100X+Immersion oil) showing normal histological features with thick myelin membrane. B is the photomicrograph of T.S of Sciatic nerve of STZ-induced diabetic rat, showing axonal atrophy (swelling), and increased extra-axonal space among nerve fibres (↑), deposition of electron dense material within the axons. In Fig.C STZ-induced diabetic rats treated with metformin showed onion bulb formation (→) and also segmental demyelination. STZ-induced diabetic rats treated with Curcumin (Cur+D) are shown in Fig.D, no demyelination and no onion bulb formation is seen and also myelin membrane is intact (↑). Control animals treated with Curcumin have not shown any significant histological changes (Fig not shown).

**DISCUSSION:** The present work is undertaken to assess the alterations in serum glucose levels and nociceptive tests after systematic treatment with *Coccinia grandis* leaf extract. The STZ induced diabetic rats has shown a marked increase in the serum glucose levels after 7th, 14th and 21st day indicating the hyperglycemia, when compared with controls. The increase in serum glucose levels were observed due to diabetogenic action of STZ in diabetic rats (Diabetic group) on all the time periods 22.

After simultaneous treatment *Coccinia grandis* leaf extract the serum glucose levels were decreased significantly. And the antidiabetic activity of *Coccinia grandis* may be due to various steps which results in the increased glucose tolerance. Reduction of sugar absorption from the gut, increased insulin production from the pancreas, reduction of release of glucose from the liver, increasing glucose uptake by fat and muscle cells are probable mechanisms which may be involved.
Neuropathic pain associated with peripheral nerve injury is characterized by the sensory abnormalities such as unpleasant abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia), and pain in response to a stimulus that does not normally provoke pain (allodynia) 25. Spontaneously diabetic mice with hyperglycemia have shown a decreased sensitivity to the antinociceptive effects of morphine 26. The behavioural alterations start on 3rd day after the STZ-induced diabetes in rats and lasts throughout the experimental period showing hyperalgesia. These observations were reported earlier findings 27, 28.

In the present study, STZ-induced diabetic rat group has shown, a significant increase in hind paw cumulative withdrawal latencies in allodynia response showing hyperalgesia. Administration of Metformin and Coccinia grandis resulted in consequent decrease was observed in allodynia response, compared to diabetic rats suggesting reduction of hyperalgesic condition. The antinociceptive effect of Coccinia grandis may be attributed to its powerful antioxidant activity. Coccinia grandis leaf extract treatments, was efficacious in reversing and/or preventing impairment of normal motor function as assessed on the Roto rod. These behavioural abnormalities are hypothesized to be a consequence of the irregularities and variability in muscle spindle group Ia innervation.

Neuropathic pain is characterised by decreased nerve conduction velocities. The change in microenvironment of the nerve involving metabolic and vascular processes have been proposed, that is leading to early reductions in nerve conduction velocities 29. Further slowing of nerve conduction velocity may be due to structural changes. The key findings of the present study were to report that Coccinia grandis leaf extract have prevented a diabetes induced deficit in NCV. This protection occurred in line with that of obvious alteration in the biochemical indexes measured, which included Na⁺-K⁺-ATPase activity and polyol pathway enzyme such as AR. Altered polyol metabolism and an associated decrease in neuronal myo-inositol content were reported to be associated with diabetes 30.

This finding suggests that lower tissue myo-inositol concentrations may result in abnormal neuronal Na⁺-K⁺-ATPase activity through a reduction in the formation of diacylglycerols and inositol trisphosphate and incomplete activation of the sodium pump by activated protein kinase C 31.

Lower Na⁺-K⁺ ATPase activity may underpin the depressed NCV in diabetes. In addition, inhibition of aldose reductase has been reported to restore neuronal polyol metabolite content (principally the myo-inositol) and the nerve conduction deficit in diabetes.

In the present study, Coccinia grandis leaf extract treatment ameliorates diabetes-associated changes in Na⁺-K⁺ ATPase and NCV. These effects were associated with attenuated anatomical damage and preventive effect on the bimodal distribution of myelinated fiber diameters. The improved NCV was strongly correlated with Na⁺-K⁺ ATPase.

**CONCLUSION:** In conclusion, data from the present study show that Coccinia exhibited antinociceptive action. There is significant decrease in levels of AR, Na⁺-K⁺ ATPase treated groups indicating the protective role of Coccinia against STZ induced alignments, could be beneficial in preventing the progression of diabetic neuropathy. Further studies are anticipated for phytochemical investigations to isolated the active compounds and lead to their further clinical use.

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**REFERENCES:**


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