Research Article

Combined effect of *Coccinia indica* leaf extract with acarbose in type ii diabetes induced neuropathy in rats

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Abstract

Uncontrolled diabetic can leads to various acute and chronic complications such as neuropathy, nephropathy, gastropathy, retinopathy etc. Combination of *Coccinia indica* with low dose of acarbose can be administered which would be useful in diabetic neuropathic pain by restoring blood glucose level and antioxidant status. Low dose of acarbose is given to avoid the hypoglycemic effect. Adult male *Spraque Dawley* rats weighing 200-250gm were selected for the study. They are divided into 6 groups with 6 animals each. Type 2 diabetes was induced by two weeks of HFD treatment followed by low dose 35mg/kg of STZ dissolved in 0.1M/l of citrate buffer through i.p route. Blood glucose estimation is done on every 15 days once. Single and multiple dose studies of ethanolic extract of *Coccinia indica* and acarbose was performed to assess the antihyperglycemic activity. Diabetic rats were treated with acarbose, quercetin, *coccinia indica* and its combination with acarbose for period of 7 weeks. Eddy's hot plate test and tail immersion test were performed to assess the antinociceptive activity of the drugs. At the end of the treatment antioxidant status in the sciatic nerve was performed to find out the antioxidant status. Diabetic rats treated with *Coccinia indica* alone and in combination with low dose of acarbose produced significant decrease in the blood glucose level after 7 weeks of treatment. Untreated diabetic rats showed significant hypersensitivity towards thermal stimuli when compared with normal control. Histopathological studies proved that there is no damage in the sciatic nerve of the groups treated with the ethanolic extract of *coccinia indica* and its combination with low dose of acarbose.

Key words : Diabetic neuropathy, ethanolic extract of *coccinia indica* leaf, Hyperalgesia, Sciatic nerve, nociception.

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1. Introduction

Diabetes is a major health problem today, as approximately 5% of the world's population suffers from diabetes. It is a group of diseases characterized by high blood glucose level, which results from defects in Insulin Secretion or action or

both [1]. Pre-diabetes' or better called 'potential diabetes' is a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. The development of peripheral neuropathy is the most common complication of Type2 DM and occurs in 50% of the subjects based on epidemiological data [2]. Diabetic neuropathy occurs through diverse pathogenic mechanisms most of them initiated by hyperglycaemia and oxidative stress [3]. *Coccinia indica* commonly known as Little gourd or *Rantondli* in Marathi, *Bimba* in Sanskrit and *Kandutikibel* in Hindi belonging to family *Cucurbitaceae* It is indigenous to Bengal and other parts of India.Also grows abundantly all over India, Tropical Africa, Australia, Fiji and throughout the oriental countries. The plant has also been used extensively in Ayurvedic and Unani practice in the Indian subcontinent (Wealth of India, 1992). Hypoglycaemic action of *Coccinia indica* could be due to potentiating the insulin effect of plasma by increasing the pancreatic secretion of insulin from the existing β-cells [4]. *Coccinia indica* is also believed to bring about its antidiabetic action by stimulating glucose transport [5]. Blood glucose lowering activity was may be due to the inhibition of intestinal glucose uptake, insulin secreting property, insulinotropic activity of the component present in the extract.

Figure 1. *Coccinia indica* plant.

The diabetic rats when treated with combination of *Coccinia indica* leaf with acarbose exhibited significant decrease in blood glucose level when compared to diabetic control without causing hypoglycemia. Increasing evidence indicates that a change in the inner mitochondrial membrane potential is associated with induction of reactive oxygen species (ROS) [6]. It has been documented that the degree and duration of hyperglycaemia are primary risk factors for diabetic neuropathy but at present, the therapeutic strategy available is strict glycemic control [7]. The strong glycemic control reduces the incidence of micro vascular complication [8]. Hence in the present study *Coccinia indica* and its combination with low dose Acarbose are used to obtain adequate glucose control.

Material and Methodology

Experimental Animals
Male *Sprague Dawley* Rats weighing 200-250 g were housed at 25°±5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (2013/ PCOL / 004)

Extraction of *Coccinia indica*
The extract of *Coccinia indica* (Batch No. CIL/12005) was collected from the Green Chem industry, Bangalore.

Induction of diabetes:
Single and multiple dose study in normal and diabetic rats [9].

Single dose study

Normal and diabetic rats will be administered with a single dose of plant extract and the selected oral hypoglycemic agents (OHA). The blood glucose level will be estimated just prior to administration of plant extract and OHA and at 1, 2 and 4 h after administration. Glucose levels will be estimated as mentioned above.

Repeated dose study

The same groups (Single dose study) of normal animals will be continued with the same dose levels of plant extract and OHA once daily, for 11 days. The glucose levels of all the animals will be measured on 3, 5, 7, 9 and 11th day of the treatment period.

Development of high fat diet (HFD) fed / Low dose streptozotocin Treated type 2 diabetic rats: [10]

The animals were fed with HFD once a day for two weeks followed by I.P injection of streptozotocin (35mg/kg) dissolved in 0.5M/l citrate buffer (pH: 4.4) after over night fasting. STZ injected animals were then given 5% w/v glucose solution for 5-6 hours following the injection to prevent initial drug induced hypoglycemic mortality. The rats with non fasted plasma level ≥ 300mg/dl were considered diabetic. The blood Sample was collected from tail vein and blood glucose was checked using glucose diagnostic kit (Accucheck).

Standardization and selection of low dose for standard drugs:
Oral Glucose Tolerance Test [11]

After overnight fasting (18hrs), a 0-min blood sample was taken from the tip of the tail of each rat of different groups. Glucose solution (2 g/kg P.O) was given after 30 min after the administration of the drug. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration. All blood samples were checked with the help of accucheck glucometer.

Treatment Groups

Effect of *Coccinia indica* leaf extract alone and in combination with Acarbose in streptozotocin induced diabetic rats: n = 6

Group 1: Normal control, rats receive saline/vehicle.

Group 2: Diabetic control.

Group 3: Diabetic rats treated with *Coccinia indica* (200mg/kg) [12] leaf extract.

Group 4: Diabetic rats treated with Acarbose (Low dose) [13]

Group 5: Diabetic rats with *Coccinia indica* (200mg/kg) leaf extract + Acarbose (5mg/kg)

Group 6: Diabetic rats treated with Quercetin (50mg/kg p.o.).

At the end of the treatment period, the rats will be sacrificed by decapitation and the sciatic nerve will be excised and histopathological studies will be carried out.

Neuropathic pain models-
Thermal stimuli-
Hot-plate test (Eddy's) [14]

The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms. In this test, animals will be individually placed on a hot-plate (Eddy's hot-plate) with the temperature adjusted to $55\pm1^{\circ}C$. The latency to the first sign of paw licking or jump response to avoid the heat will be taken as an index of the pain threshold; the cut-off time will be 15 sec in order to avoid damage to the paw. The responses will be recorded one week post STZ injection.

Tail-Flick Latency Test [15]

The tail-flick measurement will be made using the Ugo Basile tail-flick unit (Ugo Basile, Comerio, Italy) based on a modification of a method described originally by D'Amour and Smith (1941). The tail-flick unit consisted of an infrared heat source (50-W bulb) of adjustable

intensity that will be set at 40 units (determined to elicit tail-flick latency of 2–4 s as baseline in naive animals). The infrared source will be focused to the base of the tail. The latency time (in seconds) required by the rat to reach the thermal threshold for pain and flick its tail will be recorded. Each rat will be given one test to determine baseline latency to tail-flick with a cut-off of 10 s set to avoid tissue damage. Animals will be given drug or vehicle and tested at varying time points after administration. Data will be calculated as %MPE (maximum possible effect) and expressed as means ± S.E.M. %MPE will be calculated using the following formula: %MPE = [(test latency – baseline latency)/(cut-off latency (10 s) – baseline latency)] × 100.

Statistical analysis [16]
Data were expressed as means ± S.E.M. the time course of the pharmacological effect was examined using analysis of variance (ANNOVA) followed by turkey t-test. P<0.05 was considered as significant. Student 't' test was used to compare the values from two groups.

Histopathological analysis [17]
Samples of sciatic nerve were kept in the fixative solution (10% formalin) and cut into 4um thickness. Staining was done by using hematoxylin and eosin. Nerve sections were analyzed under light microscope (400 x) for axonal degeneration and vascular defects.

Care of diabetic animals
Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding was changed frequently, usually every day and, in some circumstances, more than once per day. Diabetic rats should have sufficient food and water; hence six diabetic rats had been housed per cage.

Results

Feed consumption and water consumption of diabetic rats increased significantly when compared to the normal control groups. (Figure 2 and Figure 3). Body weights of diabetic animals decreased significantly when compared to the normal control groups. (Figure 4). In Eddy's hot plate test and Tail immersion test, diabetic animals showed significant reduction in reaction time from 2nd week when compared to normal control group which signifies that that the hypersensitivity of diabetic animal towards thermal stimuli. The significance of hypersensitivity was seen until 7th week of diabetic animals. From 8th week diabetic animals shown loss of sensation. (Figure 5 & Figure 6). There is a significant decrease in the blood glucose level in treated rats when compared to diabetic control group. (Table 1).

Effect of *CI* and their combination with Acarbose on Hot Plate test –
After 7 weeks of treatment acarbose latency period was significantly increased. *CI* +AC showed significant reduction to hyperalgesia in 7 weeks. *CI* alone and in combination with acarbose showed the significant effect in 7 weeks respectively. The values were compared with diabetic control. (Table 2).
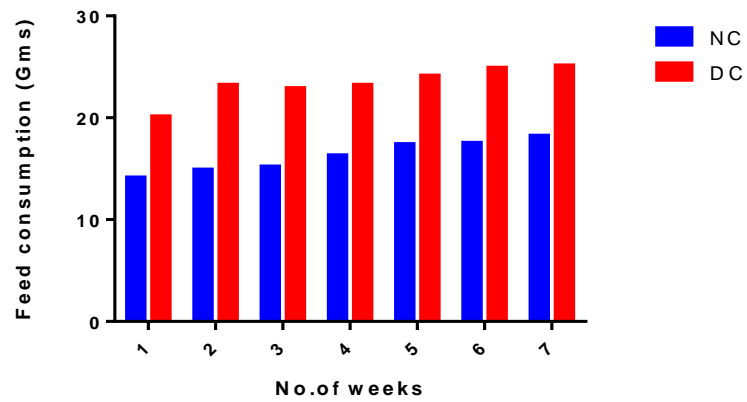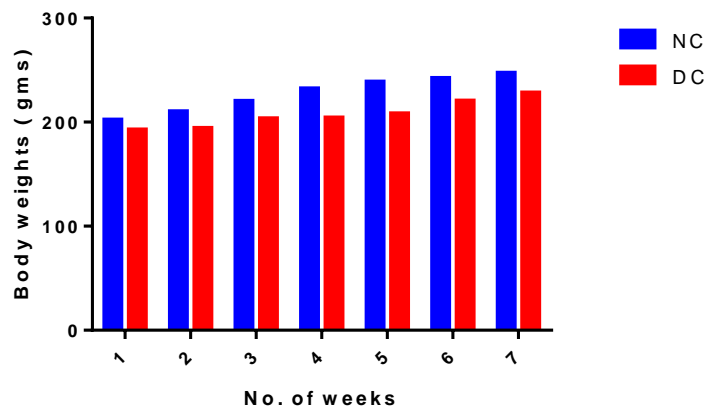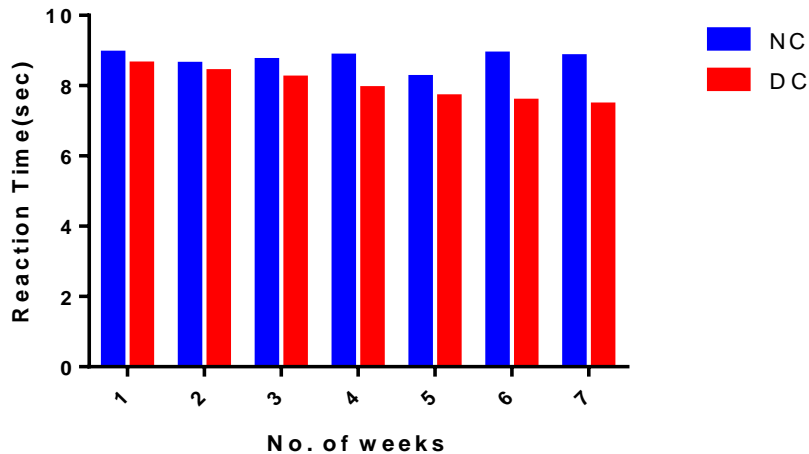
Figure 2. Feed consumption.
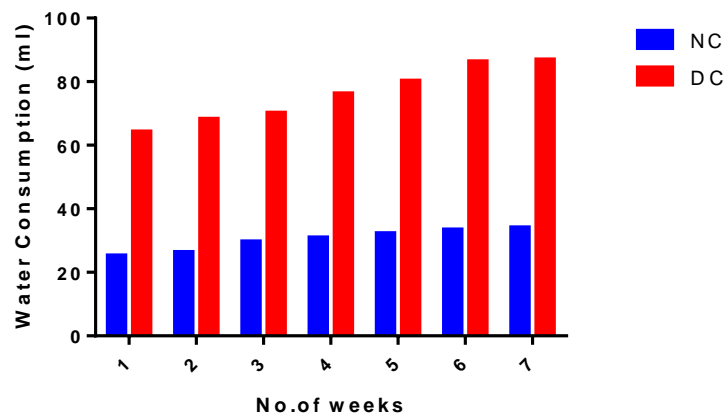
Figure 3. Water consumption.

All values are mean ± SEM, n=6, P<0.001 when compared to normal control group.
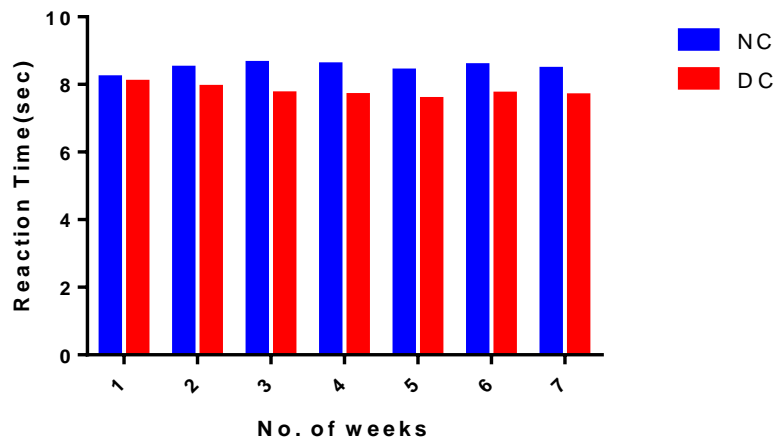NC- Normal control , DC- Diabetic control.

Figure. 4 Body weight.

Figure 5. Eddy-s hot plate.

All values are mean ± SEM, n=6, P<0.001 when compared to normal control group.

Figure 6. Tail immersion test.

All values are mean ± SEM, n=6, P<0.05 when compared to normal control group.

Table 1. Blood glucose level in treated group.

GROUPS	INITIAL	2ND WEEK	4ND WEEK	6TH WEEK	8TH WEEK
NC	130.12±6.78[a]	138.2±7.2[a]	143.11±7.46[a]	139.22±7.2[a]	133.54±6.9[a]
DC	267.23±13.93	284.14±14.2	298.14±15.51	338.06±17.62	345.22±18
CI	248.21±12.94[c]	232.12±12.1[c]	219.68±11.46[a]	198.66±10.36[a]	166.12±8.66[a]
AC	258.51±13.48[c]	251.12±13.09[c]	226.33±11.8[a]	202.11±10.57[a]	187.21±9.76[a]
CI+AC	233.22±12.16[c]	218.09±11.37[b]	204.14±10.65[a]	185.44±9.66[a]	161.61±9.23[a]

All values are mean ± SEM, n=6, [c]P<0.001, [a]P<0.05, [b]P<0.01 when compared to Diabetic control group. AC-Acarbose.

Table 2. Effect of *CI,* Acarbose and their combination in Hot Plate test.

GROUPS	INITIAL	2ND WEEK	4TH WEEK	6TH WEEEK	8TH WEEK
NC	8.92±0.46[a]	8.61±0.44[a]	8.72±0.44[a]	8.84±0.45[a]	8.23±0.42[a]
DC	8.61±0.44	8.4±0.43	8.21±0.42	7.91±0.41	7.68±0.40
AC	8.72±0.45[c]	8.45±0.42	8.31±0.43[a]	8.21±0.42[a]	7.86±0.40
CI	8.79±0.45[c]	8.52±0.44c	8.38±0.43[a]	8.75±0.49[a]	8.02±0.41*
CI+AC	8.86±0.45[a]	8.62±0.44*	8.54±0.44[a]	8.8±0.45[a]	8.11±0.42[b]

All values are mean ± SEM, n=6, [c]$P<0.001$, [a]$P<0.05$, [b]$P<0.01$ when compared to Diabetic control group.

Table 3. Effect of *CI,* Acarbose and their combination in Tail immersion test.

GROUPS	INITIAL	2ND WEEK	4TH WEEK	6THWEEEK	8TH WEEK
NC	8.2± 0.4	8.48±0.59[a]	8.62±0.44[a]	8.58±0.44[a]	8.5± 0.39[a]
DC	8.06±0.41	7.91±0.41	7.72±0.40	7.67±0.40	7.55± 0.39
CI	8.09±0.42[a]	8.02±0.41[c]	7.8±0.41[c]	8.15±0.42[a]	7.8±0.41
AC	8.21±0.42[c]	7.97±0.41	7.79±0.40[c]	7.9±0.41[a]	7.75±0.40*
CI+AC	8.01±0.41[c]	8.31±0.43[b]	8.41±0.43[a]	8.20±0.42[a]	8.28±0.43[a]

All values are mean ± SEM, n=6, [c]$P<0.001$, [a]$P<0.05$, [b]$P<0.01$ when compared to Diabetic control group.

Table 4. Standardization of biochemical parameters in diabetic and normal control rats.

GROUPS	SOD (U/mg of protein)	Catalase (U/ng of protein	TBARS (Mm/mg of protein)	GSH (Mm/mg of protein)
NC	19.85 ± 0.86[a]	16.67± 0.76[a]	0.17± 0.008[a]	81.62± 3.54[a]
DC	8.93± 0.38	6.14± 0.26	0.38± 0.01	30.75± 1.33
CI	12.21±0.63[b]	11.62±0.85[a]	0.32±0.013[b]	83.91±4.43[a]
AC	10.2± 0.44[c]	7.8± 0.34[c]	0.35± 0.01[c]	58.9±5.93[b]
CI+AC	14.41±0.75[a]	13.25±0.68[a]	0.28±0.013[a]	85.61±4.46[a]
QC	12.22±0.84*	10.14±0.70[a]	0.27±0.01[c]	56.88±3.95[b]

All values are mean ± SEM, n=6, [c]$P<0.001$, [b]$P<0.01$, [a]$P<0.05$ when compared to diabetic control group.

Effect of *CI* and their combination with Acarbose on tail immersion test

The diabetic groups that were treated with acarbose showed significant increase in tail withdrawal latency period at 8 week. *CI*+AC showed significant increase in tail withdrawal latency period on 8th week of treatment.*CI* alone showed significant increase in tail withdrawal latency period on 8th week when compared to diabetic control. (Table 3).

There was a significant decreased in a level of SOD (P<0.001), Catalase (P<0.001) and GSH (P<0.001) in the sciatic nerve homogenate of diabetic groups when compared to normal control groups and TBARS level in the sciatic nerve homogenate was significantly increased when compared to normal control group. (Table 4).

Effect of *CI* and Acarbose and their combination on sciatic nerve TBARS

CI (0.32Mm/mg, P<0.001), *CI*+AC (0.28Mm/mg, P<0.001) showed significant decrease in the TBARS level in sciatic nerve when compared to diabetic control (0.38Mm/mg). Quercetin (0.27 Mm/mg, P< 0.001) treated group showed significant decrease in TBARS when compared to diabetic control group (0.39Mm/mg).

Effect of *CI* and Acarbose and their combination on sciatic nerve catalase

CI (11.62U/ ng, P< 0.001), *CI*+AC (13.25U/ ng, P<0.001) significantly increase sciatic nerve catalase when compared to diabetic control (6.14U/ ng). Quercetin (10.14 U/ng, P< 0.001) treated group showed significant decrease in TBARS when compared to diabetic control group (6.11U/ng).

Effect of *CI* and Acarbose and their combination on sciatic nerve SOD

*CI* (12.21U/mg, P<0.001), *CI*+AC (14.41U/mg, P<0.001) treated group showed significant increase in sciatic nerve SOD level when compared to diabetic control group (8.93U/mg). Quercetin (12.22 U/ng, P< 0.001) treated group showed significant decrease in TBARS when compared to diabetic control group (8.89 U/ng).

Effect of *CI* and Acarbose and their combination on sciatic nerve GSH

CI (83.91Mm/mg, P<0.001), *CI*+AC (85.61Mm/mg, P<0.001) treated group showed significant increase in sciatic nerve SOD level when compared to diabetic control group (30.75Mm/mg). Quercetin (56.88 Mm/mg, P< 0.001) treated group showed significant decrease in TBARS when compared to diabetic control group (30.56 Mm/mg).

Histopathological study of sciatic nerve was carried out which showed effect of Acarbose and *Coccinia indica* on pathological changes in sciatic nerve. The section of 5 μm in thickness were made and stained with hemotoxylin and eosin to assess the pathological change of sciatic nerve using the light microscopy (10X). The sciatic nerve of diabetic rat developed pathological changes when compared to the sciatic nerve of the normal rat. (Figure 7 Figure 8). The concurrent administration of *Coccinia indica* and low dose of Acarbose markedly reduced these pathological changes in the sciatic nerve as compare to treatment with either drug alone. (Figure 9, Figure 10, Figure 11).
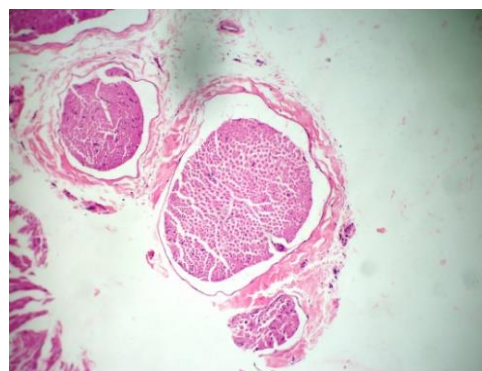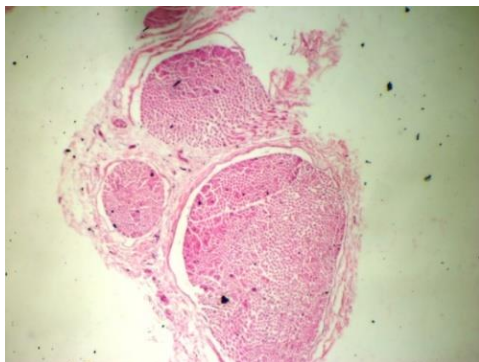

Figure 7. Normal control.
Fibers are completely arranged. Myelinated fiber density is well preserved.
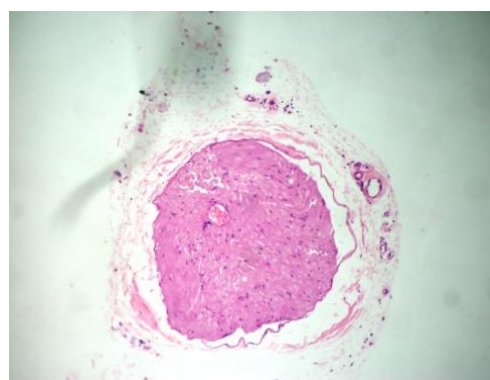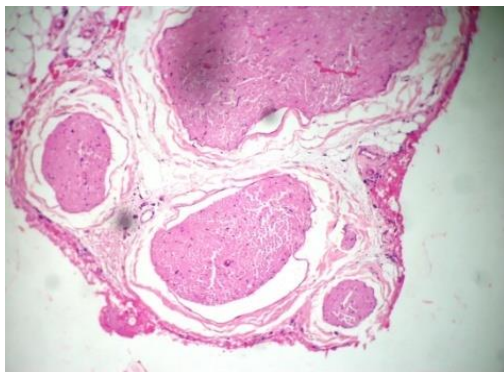

Figure 8. Diabetic control.
Multifocal loss of both large and small myelinated fibers. Small myelinated fiber loss is more prominent than large diameter fiber loss. Thickened and hyalinisedendoneurial vessel.
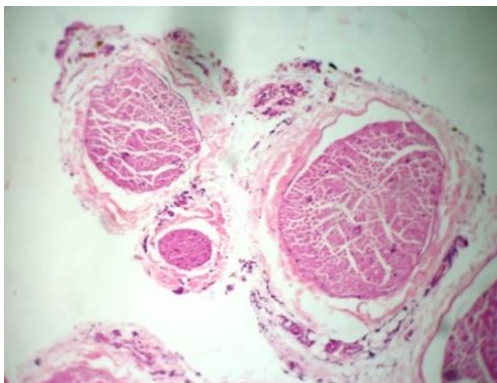
Figure 9. *Coccinia indica*
Myelination seen in larger nerves, partial loss of myelination in medium sized nerves

Figure 10. Acarbose.
Myelinated fibre density is well preserved, there is no axonal degeneration and the fibers are compactly arranged. Some regenerating clusters are seen.

Figure 11. Acarbose + *C. indica*
The fibers are compactly arranged with no axonal degeneration. Density of myelinated fibers is well preserved. Endoneurinal vessel is not thickened.

Discussion

Cardiovascular disease causes most of the excess morbidity and mortality in diabetes mellitus. Cardiovascular disease accounts for up to 80% of premature excess mortality in diabetic patients [18]. Vascular complications can be caused by micro- and macro-angiopathy. Hyperglycaemia plays an important role in the pathogenesis of microvascular complications [19]. Hypoglycaemic action of *Coccinia indica* could be due to potentiating the insulin effect of plasma by increasing the pancreatic secretion of insulin from the existing β-cells [20]. *Coccinia indica* is also believed to bring about its antidiabetic action by stimulating glucose transport [21]. Lowered blood glucose by depressing its synthesis, on the one hand through depression of the key gluconeogenic enzymes glucose-6-phosphatase and Fructose-1, 6- biphosphatase and on the other by enhancing glucose oxidation by the shunt pathway through activation of its principal enzymes G6PDH.

This increase in insulin and consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this regard, some plants have also been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects. Hence In our present study it has been reported that combination of standard low dose acarbose with *CI* lower blood glucose level and beneficial in diabetic neuropathy. In any form of management of diabetic with insulin or drug, diet is a common factor. In the present study the groups treated with combination of standard drugs had produced significant decrease in blood glucose but on prolonged treatment caused hypoglycemia. OGGT showed significant increase in tolerance in the groups treated with low dose of OHA plus *coccinia indica*. Single and multiple dose study showed

significant hypoglycemic activity of ethanolic extract of leaf in diabetic rats at the 14th day study. Blood glucose lowering activity was may be due to the inhibition of intestinal glucose uptake, insulin secreting property, insulinotropic activity of the component present in the extract. The diabetic rats when treated with combination of *Coccinia indica* leaf with acarbose exhibited significant decrease in blood glucose level which shows herbal drug overcomes side effect hypoglycemia. Oxidative induced damage to the B cell can be prevented by herbal therapy due to potential antioxidant property. It has been reported that saponins, cardenolides, flavonoids and polyphenols present in *Coccinia indica* leaf posses the antioxidant, anti-inflammatory properties [22]. *Coccinia indica* leaf extract decrease the TBARS level and increase the SOD and CAT level in the diabetes neuropathic rats. Quercetin proved to stimulate B cell and is capable of inducing insulin secretion. Terpenoids are found to be responsible for antidiabetic activity of the *Coccinia indica* leaf extract.

In the present study diabetic rats exhibited significant hyperalgesia as compared to control rats. In preventive therapy the OHA, *CI* and combinations produced increase tail flick latency in tail immersion test and paw withdrawal in hot plate test. The increased tail flick latency of OHA and their combination may be due to their property to control blood glucose level and its analgesic and inflammatory property [23]. Hence combination of *CI* and AC (low dose) may be useful in preventing secondary complication when treated for prolong period.

Conclusion
Oxidative stress and hyperglycemia are very common to both types of diabetes mellitus and leads to development of secondary complications of diabetes which could be fatal also. Herbs possessing antidiabetic and antioxidant properties can be vital in prevention of Diabetic complications. Since there is a destruction of B cell in pre diabetic state, the therapy should focus on prevention of B cell destruction and regeneration of more and more B cells. Ethanolic extract of *coccinia indica* posses antidiabetic and antioxidant properties. Regular consumption of *coccinia indica* in diet could prevent the diabetes when consumed during pre-diabetic state.

Hence based on the result it is concluded that ethanolic extract of *coccinia indica* leaf with low dose of acarbose can be used as antinociceptive agent due to its antidiabetic and antioxidant property. Further studies can be carried out to determine the efficacy *of coccinia indica* with OHA on long term treatment.

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