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Research Article

AMELIORATIVE EFFECTS OF *TINOSPORA CORDIFOLIA* IN SCIATICA PAIN INDUCED RATS

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ABSTRACT
The present study was aimed at investigating the ameliorative effect of *Tinospora cordifolia* in sciatic nerve root Ligation -induced sciatica pain in rats. Adult male albino rats weighing 130-150gm were used for the study, and were divided into seven groups and ligation was performed on left sciatic nerve in group II to group VII. Tail cold-hyperalgesia, motor co-ordination tests, foot deformity, and total calcium levels were estimated to assess the extent of sciatica. Superoxide dismutase (SOD), catalase (CAT), and lipid peroxide (LPO) levels were estimated to evaluate the extent of oxidative stress. The alcoholic and aqueous extract of *Tinospora cordifolia* was administered at a dose of 100 and 200 mg/kg/p.o for 15 days. *Tinospora cordifolia* attenuated sciatic nerve root Ligation-induced motor in-coordination, foot deformity, tail cold hyperalgesia, reversed ligation-induced alterations in lipid peroxides, total calcium, superoxide dismutase, catalase levels in a dose-dependent manner. Ameliorative effects of *Tinospora cordifolia* in ligation-induced sciatica may be due to its foot deformity, antioxidant, and calcium attenuating actions.

KEYWORDS: *Tinospora cordifolia*, Free radicals, Sciatica pain, Sciatic nerve root irritation.

INTRODUCTION

Sciatic neuralgia is defined as 'pain in the distribution of the sciatic nerve due to the pathology of the nerve itself[1]. Low back pain is a common problem that will affect approximately two thirds of the adult population[2]. It is the second leading reason for ambulatory care in the United States and direct medical costs are estimated at over $20 billion per year[3].

Oxidative stress plays an important role in various neurological disorders, such as ischaemia, Alzheimer's disease, multiple sclerosis, epilepsy, spinal cord injury and pain.

To counteract free radical induced cell damage biological systems have evolved with endogenous mechanisms to protect themselves where as in pathological conditions, ROS leads to cellular damage[4]. The change in the injured afferents sensitize the nociceptive afferents that account for the characteristic symptoms of sciatica, neuropathic pain such as allodynia, hyperalgesia and ongoing pain[5]. Scavenging free radicals reduced the above effects in sciatica pain models[6]. Pain is a neural integrated physiological phenomenon which carries message from the injury to the spinal cord, and then from spinal cord to the brain gets disturbed due to excessive ROS[7] in sciatica or neuropathic pain condition. ROS regulates expression of apoptotic protease-activating factor-1 (apaf-1), caspase-9 in the dorsal horn, spinal cord[8]. Increased ROS in dorsal horn neurons contribute to central sensitization in sciatic rats[9].

This disease is of concern as it affects the quality of life and is usually treated with non-steroidal anti-inflammatory drugs (NSAID), which mainly act by blocking prostaglandin synthesis. Moreover, none of the medications assessed in randomized controlled studies are effective in sciatica pain[10]. NSAIDs are less than ideal as most of the NSAIDs are known to cause the gastric irritation, gastrointestinal ulceration, reduces renal blood flow, platelet dysfunction and exacerbates asthma, allergic reactions and skin rashes. Sciatica pain or inflammation requires chronic drug treatment and NSAIDs are not recommended for long-term administration. Globally currently there is greater interest in non-synthetic, natural drugs derived from plant/herbal sources due to better tolerance and decreased adverse drug reactions. The use of herbal medicines is based on traditional healing, and is also influenced by culture. In India, the medicines plants and herbal therapy is practiced long before recorded history. However, scientific knowledge concerning the use of medicinal plants in sciatica pain is very limited.

Tinospora cordifolia Miers (Menispermaceae) is widely used in the Indian system of medicine in the treatment of various ailments. Categorized as a "Rasayana" in Ayurveda, it is used for its general adaptogenic and prohost immunomodulatory activity in fighting infections. It is antiperiodic, antipyretic, alterative, diuretic and anti-inflammatory. It is used in fever, urinary disorders, dyspepsia, general debility, rheumatism, jaundice and urinary diseases. It is useful in burning sensation hyperdipsia, helminthiasis, dyspepsia, flatulence, gout, vomiting, skin diseases, leprosy, erysipelas, anemia, cough, asthma, seminal weakness, uropathy and splenopathy. Tribals living in chittoor district use the decoction of leaves for sciatica

The present study was designed to evaluate and validate the use of *Tinospora cordifolia* in sciatica pain.

MATERIALS AND METHODS

Collection of plant material

The plant material was collected from the natural sources of S.V. University region in Andhra Pradesh in India. The plant was authenticated from the botanist with specimen No.846. The Voucher Specimen was placed in the Herbarium of S.V. University. The fresh stems of *Tinospora cordifolia* were collected, shade dried, cut into small pieces and coarsely powdered.

Extraction procedure

Stem Powder of *Tinospora cordifolia* was extracted by maceration with alcohol. For aqueous extraction heat distillation process was used. The powdered material and water in the ratio of 1:5 refluxed at 50^0 C for 3 hrs and filtered through the 100#mesh. Again the marc was subjected to the refluxion and filterate was collected. All the filterates

were pooled together and dried. The crushed stem powder of *Tinospora cordifolia* was soaked in alcohol in a ratio of 1:5 solutes versus solvent in a conical flask. The entire setup was kept at room temperature for 24 h with intermittent shaking. After 24 h, the mixture was filtered through Whattman No.1 filter paper and the filterate was dried to evaporate the solvent[11, 12].

Animals

Male albino rats weighing 150-200 gms were chosen to avoid fluctuations due to estrous cycle. The rats were housed in polypropelene cages under 12 hrs light /dark cycle, fed with standard laboratory chow (Hindustan Lever Limited, Mumbai) and water ad libitum. Animals were acclimatized to the laboratory conditions prior to experimentation, all the experiments were carried out according to the study protocol approved by the Institutional Animal Ethical Committee No. 1653/CPCSEA/2011.

Surgery

Rats were deeply anesthetized using thiopetone sodium (40 mg/kg). The radiculopathy/sciatica model has been previously described[13]. Briefly, the spinal root, dorsal root ganglia (DRG), and the adjacent dura mater on the left side at L5 were carefully exposed. Five 0.3 cm pieces of 4-0 chromic gut ligature were laid adjacent to the root and secured by two loose ligatures of 5-0 chromic gut. The muscle layers and incision were closed.

Animal protocol

A total of 78 Male albino rats were used. The rats were randomly divided into 7 groups comprising of 6 animals in each group. The first group served as sham control and received 2% gum acacia in water orally. The second group served as ligation control, received 2% gum acacia in water orally. The third group was treated with Diclofenac 10 mg/kg and the fourth, fifth groups were treated with aqueous extract of *Tinospora cordifolia* 100, 200 mg/kg/p.o; sixth, seventh groups were treated with alcoholic extract of *Tinospora cordifolia* 100, 200 mg/kg/p.o; respectively for 15 days after performing surgery to the rats. Behavioral tests were carried out to assess nociceptive threshold and motor coordination, at 5 day intervals up to 15th day. All the animals were sacrificed at the end of the 15th day and biochemical analysis were done for the estimation of protein, superoxide dismutase, catalase, lipid peroxide and total calcium levels.

Behavioral studies and Pharmacological studies

Assessment of foot deformation

The foot deformation in Ligation induced and drug treated groups were assessed by foot deformation score. The rat was placed on a plate with a neutral temperature and the posture of the foot was observed. The foot deformation was scored as follows[14]:

- Score 0 if the paw is in normal position with fanned toes
- Score 1 if the toe is ventroflexed
- Score 2 if the paw is everted so that only the internal edge of the paw touches the floor.

Tail immersion test (tail cold-hyperalgesia test)

Spinal thermal sensitivity was assessed by the tail immersion test as described by Necker and Hellon[15]. Briefly, the terminal part of the tail (1 cm) of the rat was immersed in cold noxious temperature (0–4 °C), until the tail was withdrawn. The reaction time for the tail withdrawal reflex was recorded and a cut-off time of 15 Sec was used.

Motor coordination test

Motor coordination was evaluated by a Rota-Rod device as described by Jones and Roberts[16]. Rats were placed for 2 min on the rotating rod, the time taken for falling from the roller, was recorded.

Assessment of inflammation

A mark was made at the lateral maleolous of the right paw and the foot was dipped to the same distance of the mark into the arm of Digital Plethysmograph (UGO Basile, Italy). Average edema volume in RF and Diclofenac treated rats was measured and expressed as percent edema inhibition, which is calculated using the formula and was compared statistically with those of the sham control and ligation control animals[17].

Assessment of nociception by Tail Flick method

Tail Flick method is used to study the analgesic effect of the drug in laboratory animals. Painful reaction in rats was produced by applying noxious stimuli supplied by analgesiometer; basal reaction time to heat was taken by placing the tip of the tail on heat source. A cut off period of 10sec was observed to prevent the damage to the tail. 3-5 basal reaction times for each rat were taken at a gap of 5 min to confirm normal behavior of the animal.

Assessment of Memory

It is used to assess learning and memory performance in laboratory animals. It is used to measure the cognitive performance, notably to evaluate the spatial long term memory in rats. The Elevated plus maze consists of two open and two enclosed arms. The animals were placed individually at the end of either of the open arm and the time the animals take to move from open to enclosed arm (transfer latency) was noted on the first day. The transfer latency is again recorded 24hrs after first exposure. The transfer latency on the first day trial serves as acquisition (learning) and the retention consolidation (memory) is examined 24hrs later. Each animal was used only once. One hour after administration of the drug, the test was carried out and the memory was assessed.

Exploratory behaviour (Hole Board Test)

This test was done using Hole Board. The Hole Board consisted of a $0.5m^3$ wooden board with 16 holes (3cm in diameter). The rat was placed at the corner of the board and allowed to move freely. First two minutes were allowed for adaptation and the number of head dippings in next four minutes was counted.

Biochemical estimation of markers of the Oxidative stress

After 15 days of surgery, animals were sacrificed by cervical dislocation and sciatic nerve was immediately isolated from the upper part of the axotomised nerve. The sciatic nerve was homogenized in ice cold 50mM phosphate buffer (p^H -7) containing 0.1mM EDTA to give 5% (w/v) homogenate. The homogenate was centrifuged at 10,000 rpm for and supernatant of homogenate was employed to estimate total protein, superoxide dismutase, lipid peroxide, catalase and total calcium content.

Estimation of tissue protein

Protein concentration was estimated according to the method of Lowry[18], using BSA (bovine serum albumin) as a standard.

Estimation of superoxide dismutase

SOD activity was estimated according to the method of Misra and Fridovich[19] at room temperature. 100µl of tissue extract was added to 880µl of carbonate buffer (0.05M, p^H -10.2, containing 0.1mM EDTA) and 20µl of 30mM epinephrine (in 0.05% acetic acid) was added to the mixture and the optical density values were measured at 480nM for 4 min on a UV-Vis Spectrophotometer. Activity is expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% is equal to 1 unit.

Estimation of lipid peroxidation

200µl of the tissue extract was added to 50µl of 8.1% sodium dodecyl sulphate, vortexed and incubated for 10min at room temperature. 375µl of thiobarbituric acid (0.6%) was added and placed in a boiling water bath for 60min and then the samples were allowed to cool at room temperature. A mixture of 1.25ml of butanol : pyridine (1.5 : 1) , was added, vortexed and centrifuged at 1000rpm for 5mins. The coloured layer (500µl) was measured at 532nm on a Systronics UV Spectrophotometer, using 1, 1, 3, 3-tetraethoxypropane as standard. The values were expressed as mmoles of MDA formed/mg protein/hr[20].

Estimation of Catalase

Catalase activity was measured by a slightly modified version of Aebi[21], at room temperature. Catalase activity was expressed in moles of hydrogen peroxide degraded/mg protein/min.

Estimation of total calcium

To 4.5ml of deproteinated buffer in a glass centrifuge tube, 0.5ml of the sample was added and was placed in water bath for 3minutes. Tubes were centrifuged while they were still hot, 0.5ml of each supernatant was transferred into clean test tubes, and 0.5ml of each standard was pipetted into test tubes. For the reagent blank, 0.5ml of blank solution was prepared by mixing 9 volumes of deproteination buffer with one volume of water. 5ml of working colouring reagent was added to each tube, mixed well and then read at 570nm[22].

Statistical analysis

All the results were expressed as mean ± S.E.M. Data was analyzed using one-way ANOVA, followed by Dunnet's T test using Instat. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Effect of *Tinospora cordifolia* on Foot deformation

Upon ligation the foot deformation occurred in all animals. The rats with induced sciatica developed abnormal gait and posture. The foot was ventroflexed, with the toes held tightly together and rats were unwilling to place weight on the foot of the injured side. Foot positioning and toe spread rating was significantly different between control and experimental groups. Treatment with alcoholic and aqueous extract of *Tinospora cordifolia* (200mg/kg) (Table 1) and Diclofenac significantly reduced the foot deformation ($P<0.001$).

Effect of *Tinospora cordifolia* on cold-hyperalgesia in tail

Administration of *Tinospora cordifolia* significantly attenuated sciatic nerve root ligation-induced increase in spinal cold sensitivity, assessed by tail withdrawal latency time, in a dose-dependent manner. Tail withdrawal latency time was prolonged in rats treated with alcohol and aqueous extract of *Tinospora cordifolia* (100, 200 mg/kg) when compared to ligation control ($P<0.001$) (Table 2) and significantly decreased when compared to sham control ($P<0.001$). Comparable significant increase was also observed in Diclofenac (10 mg/kg) treated rats ($P<0.001$).

Effect of *Tinospora cordifolia* on motor coordination test

Administration of *Tinospora cordifolia* significantly attenuated sciatic nerve root ligation-induced decrease in motor performance as assessed by time spent on rota rod in a dose-dependent manner (Table 1). The rats treated with aqueous extract of *Tinospora cordifolia* (100mg/kg) had no significant effect on motor performance when compared to ligation control and significantly decreased when compared to sham control rats ($P<0.001$) (Table 3). Whereas the rats treated with alcohol extract of *Tinospora cordifolia* (100, 200mg/kg) and Diclofenac significantly increased time spent on rota rod when compared to ligation control ($P<0.001$).

Effect of *Tinospora cordifolia* on inflammation

Administration of the *Tinospora cordifolia* significantly attenuated sciatic nerve root ligation-induced inflammation as assessed by the paw oedema volume by Digital Plethysmograph in a dose-dependent manner. The percentage inhibition of paw oedema in alcohol and aqueous extract of *Tinospora cordifolia* (100, 200mg/kg) treated rats showed 69.3%, 78.8% and 55.08%, 58.55% respectively and is significant when compared to sham control ($P<0.001$) and axotomy control ($P<0.001$). Whereas Diclofenac treated rats showed 87% inhibition ($P<0.001$) (Table 4).

Tail flick method

Administration of *Tinospora cordifolia* significantly attenuated sciatic nerve root ligation-induced increase in spinal cold sensitivity, assessed by tail withdrawal latency time (Fig 3), in a dose-dependent manner. Tail withdrawal latency time was decreased in ligation control group on 5^{th}, 10^{th} and 15^{th} day ($p<0.001$) when compared to sham control group. Rats treated with aqueous and alcoholic extract of *Tinospora cordifolia* (100, 200 mg/kg) increased the tail withdrawal latency time on 5^{th}, 10^{th} and 15^{th} day ($p<0.001$) when compared to ligation control ($P<0.001$) and significantly decreased when compared to sham control ($P<0.001$). Comparable significant increase was also observed in Diclofenac (10 mg/kg) treated rats ($P<0.001$) (Table 5). Both the extracts showed significant increase in tail withdrawal latency time when compared to ligation control group ($p<0.001$).

Assessment of memory

Administration of *Tinospora cordifolia* significantly attenuated sciatic nerve root ligation-induced increase in by transfer latency time (Fig 3), in a dose-dependent manner. Transfer latency time was decreased in ligation control group on 5^{th}, 10^{th} and 15^{th} day ($p<0.001$) when compared to sham control group. Rats treated with aqueous and alcoholic extract of *Tinospora cordifolia* (100, 200 mg/kg) increased the transfer latency time on 5^{th}, 10^{th} and 15^{th} day ($p<0.001$) when compared to ligation control ($P<0.001$) and significantly decreased when compared to sham control ($P<0.001$). Comparable significant increase was also observed in Diclofenac (10 mg/kg) treated rats ($P<0.001$) (Table 6). Both the extracts showed significant increase in transfer latency time when compared to ligation control group ($p<0.001$).

Exploratory behavior

Administration of aqueous and alcoholic extract of *Tinospora cordifolia* significantly attenuated sciatic nerve root ligation induced abnormality in exploratory behavior in a dose dependent manner as assessed by number of head dippings. The number of exploratory movements was decreased in ligation control group on 5^{th}, 10^{th} and 15^{th} day ($p<0.001$) when compared to sham control group. Rats treated with aqueous and alcoholic extract of *Tinospora cordifolia* (Table 7) (100, 200 mg/kg) increased the number of exploratory movements on 5^{th}, 10^{th} and 15^{th} day ($p<0.001$) when compared to ligation control ($P<0.001$) and significantly decreased when compared to sham control ($P<0.001$). Comparable significant increase was also observed in Diclofenac (10 mg/kg) treated rats ($P<0.001$).

Effect of *Tinospora cordifolia* on SOD, Catalase, MDA and Calcium levels of Sciatic nerve

Sciatic nerve root ligation resulted in significant rise in MDA and total calcium levels and decreased SOD and Catalase levels ($P<0.001$) as compared to sham control

group. Administration of the alcoholic and aqueous extracts of *Tinospora cordifolia* attenuated ligation-induced alterations in sciatic nerve malondialdehyde, total calcium, SOD and catalase levels in a dose-dependent manner.

The levels of SOD in the sciatic nerve were significantly decreased (P<0.001) in ligation control group as compared to the sham control group. Treatment with aqueous (Fig 1) and alcoholic extract of *Tinospora cordifolia* (Fig 2) (100, 200mg/kg/p.o) significantly increased the SOD levels as compared to ligation control group. Diclofenac treatment significantly increased SOD levels as compared to the ligation control group (p<0.001).

The levels of Catalase in the sciatic nerve was significantly decreased (P<0.001) in ligation control group as compared to the sham control group. Treatment with aqueous (Fig 3) and alcoholic extract of *Tinospora cordifolia* (Fig 4) (100, 200mg/kg/p.o) significantly increased the Catalase levels as compared to ligation control group (p<0.001). Diclofenac treatment significantly increased Catalase levels as compared to the ligation control group (p<0.001).

The levels of MDA in the sciatic nerve was significantly increased (P<0.001) in ligation control group as compared to the sham control group. Treatment with aqueous (Fig 5) and alcoholic extract of *Tinospora cordifolia* (Fig 6) (100, 200mg/kg/p.o) significantly increased the MDA levels as compared to ligation control group. Diclofenac treatment significantly increased MDA levels as compared to the ligation control group (p<0.001).

The levels of Calcium in the sciatic nerve was significantly increased (P<0.001) in ligation control group as compared to the sham control group. Treatment with aqueous (Fig 7) and alcoholic extract of *Tinospora cordifolia* (Fig 8) (100, 200mg/kg/p.o) significantly decreased the Calcium levels as compared to ligation control group. Diclofenac treatment significantly decreased Calcium levels as compared to the ligation control group (p<0.001).

Both the extracts showed significant increase in the ameliorating the sciatica pain induced alterations in the sciatic nerve as compared to the ligation control. The alcoholic extract showed better activity than the aqueous extract in ameliorating the sciatica pain induced alterations in the sciatic nerve.

DISCUSSION

In the present study, sciatic nerve root ligation increased spinal nociceptive sensation, hyperalgesia, foot deformation, motor in-coordination and reversed lipid peroxides, total calcium, superoxide dismutase, catalase levels in a dose-dependent manner with the treatment of *Tinospora cordifolia*.

Peripheral nerve injury is studied by electrophysiological and histological methods but functional evaluation is important to know the degree of injury and recovery[23, 24]. Hence the most reliable, quantitative and reproducible method is sciatic functional index[25], which takes into account the relation between toes and feet of hind limbs. Foot positioning and toe spread are useful in assessing not only the locomotory and behavioural movements but also the degree of injury. Rats treated with low and high doses of aqueous and alcoholic extract of *Tinospora cordifolia* showed significant protective effect against foot deformation. Rats treated with alcoholic extract of *Tinospora cordifolia* and Diclofenac showed least score for foot deformation, indicating its protective effect against foot deformation.

In the present study, sciatic nerve root ligation resulted in a significant development of cold hyperalgesia indicating the induction of sciatica pain. Rats treated with low and high doses of alcoholic extract of *Tinospora cordifolia* showed significant prolongation of tail withdrawal time indicating its analgesic effect. However, alcoholic extract of *Tinospora cordifolia* (200 mg/kg) treatment attenuated sciatic nerve root ligation-induced cold hyperalgesia to a greater suggesting its potential in ameliorating the state of sciatica pain induced by nerve root ligation.

Results of this study showed that ligation of sciatic nerve root impaired the Rota-rod performance of rats, and *Tinospora cordifolia* attenuated sciatic nerve root ligation, i.e. ligation induced alterations in motor in-coordination and the results are comparable to the standard drug. The Rota-rod technique is of great value in research involving screening of drugs that are potentially active on motor co-ordination[26]. Rota-rod is used to find the muscle grip strength of the rats and mice. Rats treated with high dose of alcoholic extract of *Tinospora cordifolia* had improved motor function.

Rats treated with Diclofenac showed significant decrease in the paw oedema volume (85%). Rats treated with low and high doses of aqueous and alcoholic extract of *Tinospora cordifolia* showed significant decrease in the paw oedema volume comparable to the standard drug, due to its anti-inflammatory effect. The inflammation plays a critical role in the chronic constriction injury, the partial sciatic nerve ligation -induced neuropathic pain[27]. The recent studies have indicated that in nerve injury conditions, pro-inflammatory mediators such as bradykinin, prostaglandin, TNF-α, interleukin-1 play a crucial role in the development and maintenance of sciatica pain[28].

In the present study ligation control decreased the SOD levels. Superoxide is produced at a relatively high rate by cells during normal metabolism / its low intra cellular level is maintained by spontaneous dismutation and/or catalytic breakdown by the enzyme superoxide dismutase (SOD)[29]. This enzyme is implicated as an essential defense against the potential toxicity of oxygen[30]. The radical scavenging activity of SOD is effective only when it is followed by actions of Catalase and glutathione peroxidase as SOD generates hydrogen peroxide as a metabolite, which is more toxic than oxygen radical and requires to be scavenged by, Catalase / glutathione peroxidase[31]. Hydrogen peroxide in the presence of transition metals like iron leads to the generation of highly toxic hydroxyl ion which is known to induce lipid peroxidation[31]. The decreased SOD levels found in the present study is in accordance with Varija et al[24].

In the present study ligation decreased the Catalase levels, the decrease in the Catalase levels suggests the increased production of H_2O_2 and peroxides. Catalase is a heam containing enzyme that catalyzes the dismutation of hydrogen peroxide to water and oxygen. The enzyme is found in all aerobic eukaryotes and important in the removal of hydrogen peroxide generated in peroxisomes by oxidases, involved in oxidation of fatty acids.

Ligation led to an increase in calcium levels in sciatic nerve suggesting the key role of calcium in sciatic nerve root ligation-induced radiculopathy. The results of the present study demonstrating the elevation in calcium levels are in consonance with the earlier reports[32]. Increase in calcium levels are demonstrated to induce series of biochemical changes leading to degradation of the axonal cytoskeleton and thus, axonal degeneration[33, 34]. Free radicals are well documented to increase calcium levels[35-37]. Therefore, the observed decrease in calcium levels with aqueous and alcoholic extract of *Tinospora cordifolia* may possibly be attributed to its anti-oxidant effects. The rats treated with

alcoholic extract of *Tinospora cordifolia* showed significant decrease in the calcium levels than the rats treated with the aqueous extract, representing the alcoholic extract is more effective than the aqueous extract.

Tinospora cordifolia, in the present study restored the levels of SOD and Catalase due to its anti-oxidant potential against free radicals. Therefore, the administration of *Tinospora cordifolia* in ligated rats showed an increase in the anti-oxidant enzyme levels. The antioxidant property of *Tinospora cordifolia* decreased the oxidative stress induced by Ligation.

Recently, there has been rapid progress in understanding the pathogenesis of sciatica pain. Most of the studies focus on newer and better drug therapy, over conventional drugs like NSAIDs, epidural steroids and periradicular infiltration. This has been the rational for the development of new drugs for sciatica pain including herbal drugs. Indian medicinal plants and their derivatives have been invaluable source of therapeutic agents to treat various disorders including sciatica pain. An indigenous drug possessing fewer side effects is the major thrust area of the present day research, aiming for a better and safer approach for the management of sciatica pain. The present study aims to verify the claim for *Tinospora cordifolia* for its analgesic and anti-inflammatory activities. In the present study NSAID is used as standard drug, it acts by inhibiting the cycloxygenase enzyme.

Tinospora cordifolia exhibited analgesic, antipyretic and anti-inflammatory activities in various in vitro and in vivo studies[38]. Leucocytes phagocytocize an inflammatory agent, by releasing lysosomal hydrolase, which damages the surrounding tissues. Anti-inflammatory drugs stabilize lysosomes, and prevent lysosomal hydrolase induced tissue damage. Dry barks of *Tinospora cordifolia* have antispasmodic, antipyretic[39], anti-allergic[40], anti-inflammatory[41, 42] and anti-leprotic properties.

Administration of aqueous and alcoholic extract of *Tinospora cordifolia* attenuated sciatic nerve root ligation-induced foot deformation, cold-hyperalgesia, motor in-coordination, inflammation and reversed the alterations in levels of superoxide dismutase (SOD), Catalase, MDA and calcium levels. Therefore, it may be tentatively suggested that anti-oxidant and calcium attenuating actions of aqueous and alcoholic extract of *Tinospora cordifolia* is contributing for attenuating sciatica pain associated with sciatic nerve root ligation.

This study showed the free radical activity increased at the site chromic gut ligature looping and contributed to the maintenance of cold allodynia and thermal hyperalgesia.

In our study, rats treated with alcoholic extract of *Tinospora cordifolia* attenuated sciatic nerve root ligation induced pain significantly as compared to aqueous extract of *Tinospora cordifolia*.

CONCLUSION

Tinospora cordifolia is claimed to be useful in the treatment of sciatica pain. The present study was designed to evaluate the analgesic and anti-inflammatory activities of *Tinospora cordifolia* in sciatica pain.

Foot deformation, motor coordination, tail cold hyperalgesia, tail flick, calcium levels were estimated to assess the extent of sciatica pain. Superoxide dismutase, Catalase, Lipid peroxide levels were estimated to assess the extent of oxidative stress.

Administration of *Tinospora cordifolia* attenuated sciatic nerve root ligation induced foot deformation, cold hyperalgesia, motor in-coordination, inflammation and reversed the ligation induced alterations in SOD, Catalase, lipid peroxide and total calcium levels. The rats treated with alcoholic extract showed better activity than the rats treated with aqueous extract of *Tinospora cordifolia*.

The administration of *Tinospora cordifolia* attenuated sciatica pain associated with nerve root ligation which may attributed to analgesic, anti-inflammatory, antioxidant and calcium attenuating actions, which supports the Ethnopharmacological activity of the Plant, *Tinospora cordifolia*.

REFERENCES

1. Frymoyer J. Lumbar disc disease: epidemiology.Instr Course Lect 1992 : 41:217-28.
2. Deyo RA, Tsui-Wu YJ. Descriptive epidemiology of low back pain and its related medical costs in the United States. Spine 1987: 12: 264-268.
3. Frymoyer JW, Cats-Baril WL. An overview of the incidences and costs of low back pain. Orthop Clin North Am 1991: 22:263-271.
4. Naik AK, Tandan SK, Dudgaonkar SP, Jadhav SH, Kataria M, Prakash VR. Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-L-cysteine in rats. Eur J Pain 2006: 10: 573-9.
5. Shim B, Kim DW, Kim BH, Nam TS, Leem JW, Chung JM. Mechanical and heat sensitization of cutaneous nociceptors in rats with experimental peripheral neuropathy. Neuroscience 2005:132: 193-201.
6. Ziemmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983:16: 109-110.
7. Kim HK, Kim JH, Gao X, Zhou JL, Lee I, Chung K. Analgesic effect of vitamin E is mediated by reducing central sensitization in neuropathic pain. Pain 2006: 122: 53-62.
8. Siniscalco D, Fuccio C, Giordano C, Ferraraccio F, Palazzo E, Luongo L. Role of reactive oxygen species and spinal cord apoptotic genes in the development of neuropathic pain. Pharmacol Res 2007:55: 158-66.
9. Park ES, Gao X, Chung JM, Chung K. Levels of mitochondrial reactive oxygen species increase in rat neuropathic spinal dorsal horn neurons. Neurosci Lett 2006: 391: 108-11.
10. Kalita J, Vajpayee A, Misra UK. Comparison of prednisolone with piroxicam in complex regional pain syndrome following stroke: a randomized controlled trial. Journal of Medicine 2006: 99: 89–95.
11. Ranjith MS, Ranjithsingh AJA, Gokul Shankar S, Vijayalakshmi GS, Deepa K, Harcharan Singh Sindhu. Enhanced phagocytosis and antibody production by *Tinospora cordifolia* – A new dimension in immunomodulaion. African J of Biotech 2007: 7: 81-85.
12. Sonia RN, Igna'cio Jose' Luiz P, Ferreira MBA, Claire FK. Nitric oxide production by murine peritoneal macrophages in vitro and in vivo treated with Phyllanthus tenellus extracts. J Ethnopharmacol 2001: 74: 181-187.
13. Hashizume H, DeLeo JA, Colburn RW, Weinstein JN. Spinal glial activation and cytokine expression following lumbar root injury in the rat. Spine 2000: 25(10).
14. Etsuko NI, Yoichi K. Pharmacological characteristics of the hind paw weight bearing difference induced by chronic constriction injury of the sciatic nerve in rats. Life Sciences 2009: 84: 622–626.
15. Necker R, Hellon RF. Noxious thermal input from the rat tail: modulation by descending inhibitory influences. Pain 1978: 4: 231–242.
16. Jones BJ, Roberts DJ. The quantitative measurement of motor incoordination in naive mice using an accelerating rotarod. The Journal of Pharmacy and Pharmacology 1968: 20: 302–304.
17. Otterness IG, Bliven ML. "Laboratory Models for testing Nonsteroidal anti-inflammatory drugs", Chapter 3 in Nonsteroidal anti-inflammatory drugs by Lombardino J. G, John Wiley & Sons, Newyork 1985: 112-130.
18. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. Journal of Biological Chemistry 1951: 193: 265–275.
19. Misra HP, Fridovich J. The role of Superoxide anion in the auto oxidation of epinephrine and simple assay for superoxide dismutase. Journal of Biological chemistry 1972: 247: 3170-3175.
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animals and tissue by thiobarbituric acid reaction. Analatical Biochemistry 1979: 95: 351-358.
21. Aebi H. Catalase. Methods Enzymology 1984: 105: 125-12.
22. Lorentz K. Improved determination of serum calcium with Orthocresolpthalein complexone. Clinical Chemical Acta 1982: 126: 327-333.
23. Cornefjord M, Sato K, Olmarker K, Rydevik B, Nordborg C. A model for chronic nerve root compression studies: presentation of a porcine model for controlled, slow onset compression with analyses of anatomic

aspects, compression onset rate, and morphologic and neurophysiologic effects. Spine 1997: 22: 946-957.
24. Varija D, Kumar KP, Reddy KP, Reddy VK. Prolonged constriction of sciatic nerve affecting oxidative stressors and antioxidant enzymes in rat, Indian Journal of Medical Research 2009: 129: 587-592.
25. Monte Raso VV, Barbieri CH, Mazzer N. Sciatic functional index in smashing injuries of rats sciatic nerves. Evaluation of method reproducibility among examiners. Acta Ortop Bras 2006: 14: 133-136.
26. Kulakarni SK: Hand book of experimental pharmacology, Vallabh Prakashan. Delhi-110088 1999 3rd edition, Reprint 2003:PP 122-123.
27. Daemen M, Kurvers H, Bullens P, Barendse G, Van Kleef M, Vanden Wildenberg F. Neurogenic inflammation and reflex sympathetic dystrophy in vivo and in vitro assessment in an experimental model. Acta Orthop Belg 1998: 64(4): 441-447.
28. Stafford MA, Peng P and Hill DA. Sciatica: a review of history, epidemiology, pathogenesis, and the role of epidural steroid injection in management; British Journal of Anaesthesia 2007: 99(4): 461–473.
29. Matkovics B, Varga SI, Szabo L, Witas H. The effect of diabetes on the activities of peroxide metabolism enzymes. Horm Metab Res 1982: 14: 77-9.
30. Manuel R and Francisco JO. Eight week moderate exercise training program increases plasmatic total antioxidant status in rats stressed by immobilization. Haema 2006: 9(1): 124-126.
31. Harman D. The aging process; major risk factor for health and disease. Proceedings of National Academy of Sciences 1991: 88: 5360-5363.
32. Young W. Role of calcium in central nervous system injuries. Journal of Neurotrauma 1992: 9: 9–25.
33. Xie X, Barrett JN. Membrane resealing in cultured rat septal neurons after neurite transection: evidence for enhancement by $Ca2^{+}$-triggered protease activity and cytoskeletal disassembly. Journal of Neuroscience 1991: 11: 3257–3267
34. Glass JD, Culver DG, Levey AI, Nash NR. Very early activation of m-calpain in peripheral nerve during Wallerian degeneration. Journal of Neurological Sciences 2002:196: 9-20.
35. Okabe E, Odajima C, Taga R, Kukreja RC, Hess ML, Ito H. The effect of oxygen free radicals on calcium permeability and calcium loading at steady state in cardiac sarcoplasmic reticulum. Molecular Pharmacology 1988: 34: 388–394.
36. Doan TN, Gentry DL, Tylor AA, Elliott SJ. Hydrogen peroxide activates agonist-sensitive $Ca2^{+}$ influx pathways in canine venous endothelial cells. The Biochemical Journal 1994: 297: 209–215.
37. Kawakami M., Okabe, E. Superoxide anion radical-triggered $Ca2^{+}$ release from cardiac sarcoplasmic reticulum through ryanodine receptor $Ca2^{+}$ channel. Molecular Pharmacology 1998: 53: 497–503.
38. Upadhyay L, Tripathi K, Kolhapure SA. Clinical evaluation of the efficacy of JT-2000 in the management of osteoarthritis: A double- blind placebo-controlled trial. Medicine Update 2004: 11, 10: 31-36.
39. Ikram M, Khattak SG, Gilani SN. Antipyretic studies on some indeginous Pakistani medicinal plants: II. Journal of Ethnopharmacology 1987: 19: 185-192.
40. Nayampalli SS, Desai NK, Ainapure SS. Anti-allergic properties of Tinospora cardifolia in animal models. Indian Jornal of Pharmacology 1986: 18: 250-252
41. Rai M, Gupta SS. The deposition of the secondary salts over the five pellets in rats was inhibited by the aqueous extract of T. Cordifolia. Journal of Research in Indian Medicine 1996:10: 113-116.
42. Pendse VK, Dadhich AP, Mathur PN, Bal MS, Madam BR. Anti-inflammatory, immunosuppressive and some related pharmacological actions of the water extract of Neem Giloe (*Tinospora cordifolia*)-A preliminary report. Indian Journal of Pharmacology 1997: 9: 221-224.

Table.1 Effect of aqueous and alcoholic extracts of *Tinospora cordifolia* on foot deformation in sciatica pain induced rats

Groups	Foot deformation score
Ligation control	2.017±0.01687
Diclofenac	$1.083\pm0.04773^{+++}$
TCAE-I	$1.450\pm0.04282^{+++}$
TCAE-II	$1.133\pm0.06146^{+++}$
TCALE-I	$1.6\pm0.06831^{+++}$
TCALE-II	$1.083\pm0.03073^{+++}$

Values are expressed as Mean ± SEM [n=6]
+,++,+++(P<0.05),(P<0.01),(P<0.001) Vs Ligation control Group
ABBR: TCAE-I : *Tinospora cordifolia* aqueous extract 100mg/kg
TCAE-II : *Tinospora cordifolia* aqueous extract 200mg/kg
TCALE-I : *Tinospora cordifolia* alcoholic extract 100mg/kg
TCALE-II : *Tinospora cordifolia* alcoholic extract 200mg/kg

Table.2 Effect of aqueous and alcoholic extracts of *Tinospora cordifolia* on cold allodyna in sciatica pain induced rats

Groups	Transfer Latency Time in Sec			
	0th Day	5th Day	10th Day	15th Day
Sham Control	12.100±0.1732	12.100±0.1732	12.100±0.1732	12.100±0.1732
Ligation Control	12.050±0.1478	$2.8\pm0.0816^{***}$	$4.250\pm0.1335^{***}$	$4,9\pm0.1571^{***}$
Diclofenac	12.100±0.1461	$4.816\pm0.0703^{***+++}$	$7.030\pm0.0988^{***+++}$	$10.589\pm0.1249^{**+++}$
TCAE-I	12.150±0.1176	$3.21\pm0.0840^{***+}$	$4.55\pm0.0703^{***++}$	$5.4\pm0.1046^{***+++}$
TCAE-II	12.050±0.1668	$3.8\pm0.1335^{***++}$	$5.38\pm0.0988^{***+++}$	$7.5\pm0.2257^{***+++}$
TCALE-I	12.150±0.1176	$3.77\pm0.05333^{***++}$	$5.75\pm0.0703^{***++}$	$8.133\pm0.0843^{**+++}$
TCALE-II	12.050±0.1668	$5.05\pm0.099^{***++}$	$7.616\pm0.07030^{***+++}$	$10.9\pm0.0365^{*+++}$

Values are expressed as Mean ± SEM[n=6]
*,**,*** (P<0.05),(P<0.01),(P<0.001) Vs Sham control Group
+,++,+++(P<0.05),(P<0.01),(P<0.001) Vs Ligation control Group

Table 3 Effect of aqueous and alcoholic extracts of *Tinospora cordifolia* on motor coordination in sciatica pain induced rats

Groups	Time in Secs			
	0th Day	5th Day	10th Day	15th Day
Sham Control	127.5±0.7638	127.5±0.7638	127.5±0.7638	127.5±0.7638
Ligation Control	129.33±0.8819	$33.33\pm0.666^{***}$	$43.66\pm0.9189^{***}$	$53.66\pm0.8819^{***}$
Diclofenac	126.33±1.4760	$57.50\pm0.6191^{***+++}$	$95.5\pm0.6708^{***+++}$	$121.10\pm0.7724^{***+++}$
TCAE-I	127.33±0.988	$38.16\pm0.6009^{***+}$	$53.1\pm0.6009^{***++}$	$85.6\pm0.4940^{***+++}$
TCAE-II	127.5±1.1760	$55\pm0.6325^{***++}$	$86.5\pm0.6708^{***+++}$	$110.6\pm0.666^{**+++}$
TCALE-I	127.33±0.988	$40.66\pm0.8028^{***+++}$	$57.66\pm0.4944^{***+++}$	$95.33\pm0.5578^{**+++}$
TCALE-II	127.5±1.1760	$60.16\pm0.4773^{***+++}$	$96.83\pm0.7032^{**+++}$	$121.16\pm0.8724^{+++}$

Values are expressed as Mean ± SEM [n=6]
*,**,*** (P<0.05),(P<0.01),(P<0.001) Vs Sham control Group
+,++,+++(P<0.05),(P<0.01),(P<0.001) Vs Ligation control Group

Table.4 Effect of aqueous and alcoholic extracts of *Tinospora cordifolia* on % reduction in edema in sciatica pain induced rats

GROUPS	% Reduction in edema (Mean with SEM)
Sham control	23.02±1.365
Ligation control	44.39±2.373***
Diclofenac	89.44±2.222***+++
TCAE-I	55.08±1.008***+
TCAE-II	58.55±4.478***+++
TCALE-I	63.82±3.961***+++
TCALE-II	86.26±1.887***+++

Values are expressed as Mean±SEM[n=6]
*,**,*** (P<0.05),(P<0.01),(P<0.001) Vs Sham control Group
+,++,+++(P<0.05),(P<0.01),(P<0.001) Vs Ligation control Group

Table.5 Effect of aqueous and alcoholic extracts of *Tinospora cordifolia* on tail flick in sciatica pain induced rats

Groups	Transfer Latency Time in Secs			
	0th Day	5th Day	10th Day	15th Day
Sham Control	13.03±0.1202	13.03±0.1202	13.03±0.1202	13.03±0.1202
Ligation Control	12.933±0.1456	3.01±0.266***	4.3±0.0632***	5.06±0.0714***
Diclofenac	13.016±0.1621	4.76±0.0744***++	8.58±0.1078***+++	12.21±0.094*+++
TCAE-I	12.966±0.1493	3.65±0.0562***	5.61±0.065***++	7.73±0.42***+++
TCAE-II	13.066±0.1308	4.7±0.0730***++	7.93±0.057***+++	9.63±0.1308**+++
TCALE-I	12.966±0.1493	3.73±0.068***++	6.3±0.073***+++	8.5±0.447***+++
TCALE-II	13.066±0.1308	4.93±0.088***+++	8.66±0.066***+++	12.45±0.228*+++

Values are expressed as Mean ± SEM [n=6]
*,**,*** (P<0.05),(P<0.01),(P<0.001) Vs Sham control Group
+,++,+++(P<0.05),(P<0.01),(P<0.001) Vs Ligation control Group

Table.6 Effect of aqueous and alcoholic extracts of *Tinospora cordifolia* on memory impairment in sciatica pain induced rats

Groups	Transfer Latency Time in Secs			
	0th Day	5th Day	10th Day	15th Day
Sham Control	11.66±0.988	11.66±0.988	11.66±0.988	11.66±0.988
Ligation Control	12.10±1.056	45.60±1.6470***	40.60±1.050***	36.16±0.8724***
Diclofenac	12.5±1.0570	25.50±0.768***++	20.30±0.666***+++	11.16±1.249+++
TCAE-I	12±1.033	39.66±1.116***++	34.16±0.477***++	24.66±0.8819***+++
TCAE-II	11.6±0.7032	35±0.6831***++	29.6±0.666***+++	20.66±1.1366**+++
TCALE-I	11.5±0.8466	31.5±0.619***+++	26.16±0.703***+++	19.8±0.477**+++
TCALE-II	12.5±1.057	25.30±0.8819***+++	20±0.5774**+++	11.16±0.8724+++

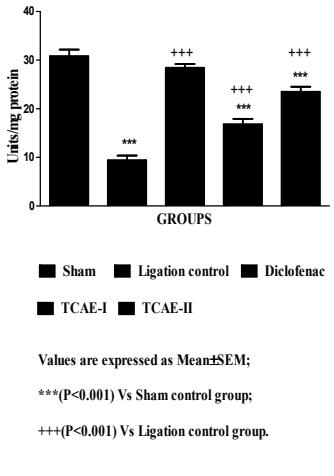
Values are expressed as Mean ± SEM [n=6]
*,**,*** (P<0.05),(P<0.01),(P<0.001) Vs Sham control Group
+,++,+++(P<0.05),(P<0.01),(P<0.001) Vs Ligation control Group

Table.7 Effect of aqueous and alcoholic extracts of *Tinospora cordifolia* on exploratory behavior in sciatica pain induced rats

Groups	Number of Head dippings			
	0th Day	5th Day	10th Day	15th Day
Sham Control	26.33±0.6667	26.33±0.6667	26.33±0.6667	26.33±0.6667
Ligation Control	26.33±0.8819	4.83±0.4014***	8±0.3651***	11.8±0.6009***
Diclofenac	25.16±1.078	11.5±0.4282***++	17.83±0.4010**+++	24.16±0.5426*+++
TCAE-I	26.1±0.6009	6.16±0.4014***++	7.93±0.057***+++	14.63±0.1308***+++
TCAE-II	26.11±0.8720	7.45±0.5426***++	10.03±0.333***+++	18.66±0.7603**+++
TCALE-I	26.1±0.6009	7.30±0.4216***++	11.83±0.3070***+++	19.50±0.4280**+++
TCALE-II	26.11±0.8720	10.45±0.5426***+++	18.50±0.3416**++	24.46±0.7638*+++

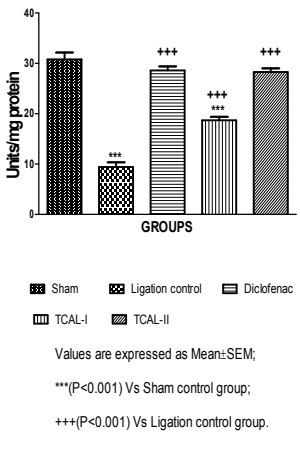
Values are expressed as Mean ± SEM [n=6]
*,**,*** (P<0.05),(P<0.01),(P<0.001) Vs Sham control Group
+,++,+++(P<0.05),(P<0.01),(P<0.001) Vs Ligation control Group

Fig.1 Effect of aqueous extract of TC on SOD levels in sciatica pain induced rats



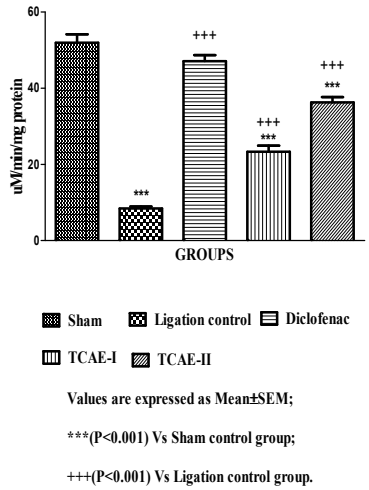
Values are expressed as Mean±SEM;
***(P<0.001) Vs Sham control group;
+++(P<0.001) Vs Ligation control group.

Fig.2 Effect of alcoholic extract of TC on SOD levels in sciatica pain induced rats



Values are expressed as Mean±SEM;
***(P<0.001) Vs Sham control group;
+++(P<0.001) Vs Ligation control group.

Fig.3 Effect of aqueous extract of TC on catalase levels in sciatica pain induced rats

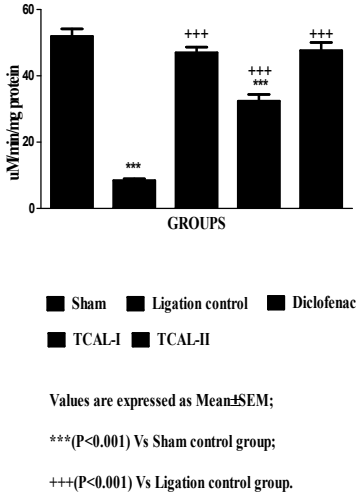


Values are expressed as Mean±SEM;

***(P<0.001) Vs Sham control group;

+++(P<0.001) Vs Ligation control group.

Fig.4 Effect of alcoholic extract of TC on catalase levels in sciatica pain induced rats

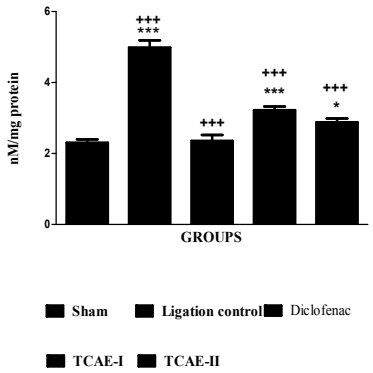


Values are expressed as Mean±SEM;

***(P<0.001) Vs Sham control group;

+++(P<0.001) Vs Ligation control group.

Fig.5 Effect of aqueous extract of TC on lipid peroxidation levels in sciatica pain induced rats

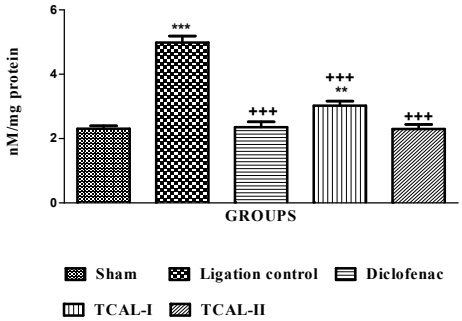


Values are expressed as Mean±SEM;

*,***(P<0.05),(P<0.001) Vs Sham control group;

+++(P<0.001) Vs Ligation control group.

Fig.6 Effect of alcoholic extract of TC on MDA levels in sciatica pain induced rats

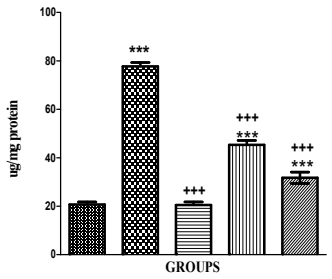


Values are expressed as Mean±SEM;

,*(P<0.05), (P<0.001) Vs Sham control group;

+++(P<0.001) Vs Ligation control group.

Fig. 7 Effect of aqueous extract of TC on calcium levels in sciatica pain induced rats

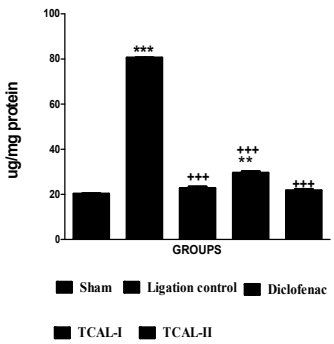


Values are expressed as mean±SEM [n=6];

***(p<0.001) Vs Sham control group

+++(p<0.001) Vs Ligation control group

Fig.8 Effect of alcoholic extract of TC on calcium levels in sciatica pain induced rats



Values are expressed as mean±SEM [n=6];

*,***(p<0.01) ,(P<0.001) Vs Sham control group;

+++(p<0.001) Vs Ligation control group

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