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PUNICA GRANATUM ATTENUATES SCIATIC NERVE LIGATION INDUCED-NEUROPATHIC PAIN

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ABSTRACT

The study has been designed to investigate the effect of aqueous extract of rind of Punica granatum in sciatic nerve ligation induced-neuropathic pain in rats. Surgical procedure was performed with sciatic nerve ligation to develop neuropathic pain in rats. The development of neuropathic pain was assessed by employing behaviour parameters such as hyperalgesia and allodynia. Further, the functionality of sciatic nerve was assessed using the histopathological study of myelinated and unmyelinated fibers in sciatic nerve. Moreover, the oxidative stress was assessed by estimating serum thiobarbituric acid reactive substances (TBARS), catalase, glutathione and tissue TBARS and Superoxide dismutase (SOD). Rats exposed to sciatic nerve ligation produced marked increase in oxidative stress, which was assessed in terms of TBARS and SOD along with decrease in the level of catalase and glutathione. Moreover, it develops neuropathic pain by impairing the normal functions of myelinated and unmyelinated fibers in sciatic nerve. Treatment with aqueous extract of Punica granatum extract (100mg/kg, p.o) markedly prevented sciatic nerve ligation-induced neuropathy and oxidative stress by increasing the pain threshold, by improving the functionality of sciatic nerve, by decreasing serum and tissue TBARS and tissue SOD, by increasing levels of serum glutathione and catalase. It may be concluded that Punica granatum extract reduced the oxidative stress via inhibiting p^{38}MAPK and alleviates neuropathic symptoms and consequently improved the functionality of sciatic nerve and prevents sciatic nerve ligation–induced neuropathic pain.

INTRODUCTION: 'Pain according to International Association for the study of pain (IASP) is an unpleasant sensory and emotional experience associated with tissue damage'. Numerous noxious stimuli are responsible for perception of pain. Neuropathic pain (NP) is one of the chronic pain caused by injury to either neural or non neural tissue [1]. NP is a maladaptive and pathological pain [2], which is triggered by lesions to the somatosensory nervous system [3]. A review of the epidemiology of chronic pain found that still no accurate data is available for the prevalence of NP [4]. It is estimated that 3% of the population suffers from NP [5]. About 25% of patients with chronic pain will have NP [6]. The prevalence of NP has been estimated at between 1% and 2% in UK [7], 0.6% in US [4]. NP occurs in about 1 in every 10 adults over age 30 [8].

Various reports indicate that neuropsychologic and neuroanatomic changes too are responsible in the progression of NP [9, 10]. Nerve damage due to NP results in allodynia (Pain due to stimulus that does not normally provoke pain, it can be provoked by touch stimulation or cooling) and Hyperalgesia (Provoked by heat stimulation) [11]. Various mediators are implicated in the pathogenesis of NP such as neuropeptides [12],

neurokinins [13], inflammatory mediators like tumour necrosis factor (TNF-α) and interleukins (2), growth factors and adhesions molecules such intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) [14]. It has been shown that during neuropathic pain, Neuropeptides such as cholecystokinin (CCK), Vasointestinal polypeptide (VIP), galanin, and Nitric oxide (NO) production increases with increased level of neurokinins like substance P and calcitonin gene related protein (CGRP) in large myelinated fibers [15, 16].

Various evidences indicate that in NP there is activation of microglial and astrocytes that in return activate p^{38} mitogen activated protein kinase (MAPK) including extracellular regulating kinase (ERK) [17]. Further interleukins (IL's) and TNF-α are responsible for both central and peripheral NP. Increased level of TNF- α at the injury site is responsible for inflammation [2]. Inflammation has been documented in the induction of cycloxygenase-2 (COX-2), an inducible enzyme localized primarily in inflammatory cells and tissues, leading to the release of prostanoids, which sensitize peripheral nociceptor terminals and produce localized pain hypersensitivity [18].

Sciatic nerve ligation is one of the best methods for inducing NP as described by Bennet and Xie, 1988 [19, 20]. Sciatic nerve ligation induced NP leads to generation of reactive oxygen species (ROS) and release of various inflammatory mediators such as TNF-α, ILs [21]. The inflammatory process and its mediators have been implicated in the regulation of both the axonal degenerative and regenerative processes after injury [22].

After nerve damage, transcription and axonal trafficking of sodium channels to the site of injury is increased, with concomitant attenuation of potassium channels. The altered expression of ion channels results in neurons becoming hyperexcitable and generating ectopic activity, which is thought to lead to the genesis of spontaneous and paroxysmal pain [23]. At the cell body of primary afferent neurons within the dorsal root ganglia (DRG), sympathetic neuronal sprouting occurs and may account for sympathetically maintained pain [5]. Peripheral nerve injury causes a multitude of changes in gene transcription and activation of various kinases and proteins such as p^{38}-MAPK pathway, including enhanced N-methyl-D-aspartate (NMDA) receptor activity [24]. NMDA receptors cause hyperexcitability of spinal cord nociceptive neurons induced by C-fiber stimulation which further results in generation of pain [25]. However, nerve injury also elicits hypertrophy and activation of glial cells, including microglia within the grey matter of the spinal cord [26].

Activated microglial expresses ionotropic adenosine triphosphate (ATP) receptors such as P_2X_4 purinergic receptors [27]. Following activation, microglia release various pronociceptive cytokines such as IL-1, TNF-α and neurotrophins including brain-derived neuro-trophic factor (BDNF), which been implicated in nociceptive transmission and contributes to the sensitization and maintenance of NP [5]. Sciatic nerve ligation has been reported to activate COX-2 pathway that is implicated in NP [21].

Punica granatum rind is composed of phenolic punicalagins, gallic acid, catechin [28] and various flavanoids like quercetin, rutin [29] which has been documented to possess free radical scavenging property [30]. Clinically, *Punica granatum* has been implicated in the management of various cardio-vascular and central nervous system disorders like hypertension, Atherosclerosis, Alzheimer's disease [31].

Further, it has been found to possess a good anti-inflammatory activity by inhibiting p^{38}-MAPK pathway [32, 33], which is associated with the increased gene expression of TNF-α, IL-1β, inducible nitric oxide synthase (iNOS) and COX-2 agents that are critical mediators in production of NP [23]. Thus, the study has been designed to explore the effect of aqueous extract of rind of *Punica granatum* on experimentally induced NP by sciatic nerve ligation.

MATERIALS AND METHODS:

Animals: The experimental protocol used in the present study was approved by institutional animal ethics committee (IAEC). Age matched young Wistar rats weighing about 120-140 g were employed in the present study. They were fed on standard chow diet and water *ad libitum* and were acclimatized in the institutional animal house and were exposed to normal light and dark cycle of the day.

Assessment of Behaviour Parameters:

Assessment of Hyperalgesia and Allodynia: The hyperalgesia was assessed in both epsilateral and contralateral hind paws by immersing paws in the water, maintained at a temperature of 52.5°± 0.5°C.

Withdrawal thresholds for allodynia were measured in both epsilateral and contralateral hind paws by immersing in the water, maintained at a temperature of 4°± 0.5°C.

Assessment of Oxidative Stress: At 14th day, the blood samples were collected by retro-orbital sinus puncture. Serum samples were prepared for the evaluation of oxidative stress markers like thiobarbituric acid reactive substances (TBARS), reduced glutathione and catalase changes in rats. For the evaluation of superoxide dismutase (SOD) and TBARS in tissue, animals were sacrificed after 14th day by cervical dislocation.

Assessment of Oxidative Stress in Serum:

Estimation of thiobarbituric acid Reactive Substances (TBARS): 1ml of 20% trichloroacetic acid (TCA) was added to 100µL of serum and 1mL of thiobarbituric acid (TBA) reagent. The mixture was then incubated at 100 °C for 30 min. After cooling the samples they were centrifuged for 20 minutes. Serum concentration of TBARS was measured spectrophotometrically at 532 nm. A standard graph using 1, 1, 3, 3 tetra-methoxypropane (1-50µM) was plotted to calculate the concentration of TBARS [34, 35].

Glutathione estimation: 0.5 ml of serum was added to 0.5 ml Ellman's reagent (0.1% sodium nitrate and 19.8 mg 5', 5- dithio (bis)-2- nitrobenzoic acid (DTNB). Then 3 ml of phosphate buffer (pH 8.0) was added to the mixture. After 10 minutes the optical density of the yellow-coloured complex formed by the reaction of GSH and DTNB was measured at 412 nm. A standard curve was obtained with standard GSH and absorbance was measured at 412nm [36].

Catalase Estimation: The catalase activity was estimated using method of Aebi *et al*., 1984. 0.1 ml of serum was added to 1.9 ml of 50 mM phosphate buffer (pH 7.0). 1.0 ml of 30 mM hydrogen peroxide (H_2O_2) was added and a change in absorbance was followed for 30 sec at 240 nm at 15-sec intervals. The catalase activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.071 mmol cm^{-1}) and the activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

$$\text{CAT activity} = \frac{\delta \text{ O.D.}}{\text{E X Vol. of sample (ml)} \times \text{mg of protein}}$$

Where δ O.D. = Change in absorbance / minute; E= Extinction coefficient (0.071 mmol cm^{-1}) [37]. Protein content was estimates using Lowry's method) [38].

Assessment of Oxidative Stress in Tissue:

Estimation of Superoxide dismutase (SOD): A weighed amount of nerve tissue was taken in 5 mL phosphate buffered saline containing 100 mM of nitroblutetrazolium (NBT) at 37°C for 1.5 h. The NBT reduction was stopped by adding 5 mL of 0.5 M hydrochloric acid (HCl). The tissue was minced and homogenized in a mixture of 0.1 M sodium hydroxide (NaOH) and 0.1% sodium dodecyl sulphate (SDS) in water containing 40 mg/L diethylene triamine penta acetic acid (DTPA).

The mixture was centrifuged for 20 min and the resultant pellet was suspended in 1.5 mL of pyridine and kept at 80°C for 1.5 h to extract formazan, an adduct formed after reaction of NBT with superoxide anions. The mixture was again centrifuged at 10,000 g for 10 min and absorbance of formazan was determined spectrophotometrically at 540 nm. The amount of reduced NBT was calculated using the formula:

$$\text{Amount of reduced NBT} = \frac{A \times V}{T \times WT \times e \times l}$$

Where A is absorbance, V is volume of pyridine, T is time for which the tissue was incubated with NBT (1.5 h), Wt is blotted wet weight of tissue, e is extinction coefficient (0.72 L/mM per mm), and l is length of light path. Results were expressed as reduced NBT picomole per minute per milligram of wet tissue [39].

Estimation of Thiobarbituric Acid Reactive Substances (TBARS): Tissue estimation of TBARS was performed according to the method of Ohkawa *et al*., 1979. 0.2 ml of supernatant obtained from homogenate(prepared in phosphate buffer) was pipetted out in a test tube, followed by addition of 0.2ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of thiobarbituric acid and the volume was made up to 4ml with distilled water. The test tubes were incubated for 1 h at 95^{o}C, then cooled and added 1 ml of distilled water followed by addition of 5 ml of n-butanol-pyridine mixture (15:1v/v). The tubes were centrifuged at 4000 g for 10 min. the absorbance of the developed pink colour was measured spectrophotometrically at 532 nm. A standard calibration curve was prepared using 1-10 nM 1, 1, 3, 3-tetra methoxy propane. The TBARS value was expressed as nanomoles per mg of protein [40].

Histopathological Study:

Assessment of Axonal Degeneration: Samples of sciatic nerve was stored in the fixative solution (10% formalin) and cut into 4 µm thickness size. Staining was done by using haematoxylin and eosin as described by Yukari method. Nerve sections were analyzed qualitatively under light microscope (450 ×) for axonal degeneration [41].

Neuropathic pain (NP) Animal Model:

Partial Sciatic nerve ligation (pSNL) induced Neuropathic pain in rat:

Surgical Procedure: The rats were anesthetized with Ketamine (50 mg/kg, i.p.). The right hind legs were shaved, and the skin was sterilized with iodine. All surgical instruments were sterilized before surgery. The sciatic nerve of right hind paw was exposed at the level of middle of thigh by dissection through biceps femoris.

The nerve was then freed from adhering tissues and nerve was tightly ligated with silk suture. After checking homeostasis, the muscle and the adjacent fascia were closed with sutures, and the skin was closed with clips. The animals were placed under heating lamps until they recovered from anaesthesia [19].

Experimental Protocol: In the present study four groups were employed using six animals in each group.

Group I: Normal control

Rats were not subjected to any treatment and received distilled water/saline for 14 days. All the animals were sacrificed at end of the 14th day and the behavioural and biochemical analysis was done for estimation of oxidative stress and histopathological studies.

Group II: Sham control

Rats were subjected to surgical procedure. Only sciatic nerve was exposed. The animals were anesthetized with Ketamine (50 mg/kg, i.p.). The animals were taken care off until they recovered from anaesthesia.

Group III: Sciatic nerve ligated rats

Rats were subjected to surgical procedure. Sciatic nerve was ligated. The animals were anesthetized with Ketamine (50 mg/kg, i.p.). Neosporin powder was applied to the leg prior to surgery to prevent infection.

Group IV: *Punica granatum* treated sciatic nerve ligated rats

Rats undergone sciatic nerve ligation were treated with *Punica granatum* (100 mg/kg/day, p.o) and the treatment was started 3 days before doing surgical procedure and was continued for 14 days from the day of surgery.

Statistical Analysis: All the results are expressed as mean ± standard deviation (SD) followed by one way ANOVA along with Turkey's multiple comparison test. The $p<0.05$ was considered to be statistically significant.

Drugs and Chemicals: Trichloroacetic acids (TCA), Thiobarbituric acid (TBA), Diethylene triamine penta acetic acid (DTPA), Thiopental sodium were procured from Samarth Life Sciences (Mumbai, India). While 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), Reduced glutathione, Nitroblutetrazolium (NBT) were purchased from Sanjay biologicals, Amritsar, India. 1, 1, 3, 3 tetramethoxypropane was purchased from V.K Chemicals, India. All other reagents used in the present study were of analytical grade.

Procurement of Extract: Aqueous dry extract of *Punica granatum* (rind of fruit) was procured as a gift sample from Amsar Pvt. Ltd., Indore, India

RESULTS: The treatment with extract of *Punica granatum* (100 mg/kg/day, p.o) to normal rats did not produce any significant *per se* effects on behaviour parameters like hyperalgesia and allodynia and oxidative stress parameters such as TBARS, catalase, SOD and glutathione levels.

Effect of *Punica granatum* on Behaviour Parameters: Rats subjected to neuropathy by sciatic nerve ligation shows a marked decrease in the pain threshold on 7th and 14th day assessed by hyperalgesia and allodynia in comparison to normal rats. However, a significant increase in pain threshold in hyperalgesia and allodynia was observed in rats when subjected to treatment with extract of *punica granatum* in comparison to neuropathy control (**Fig. 1-4**).

Effect of *Punica granatum* on serum TBARS: The lipid peroxidation measured on 14th day in terms of TBARS was noted to be increased significantly in sciatic nerve ligated rats when compared with normal rats. However, treatment with extract of *Punica granatum* significantly attenuated level of TBARS in comparison to sciatic nerve ligation- induced diseased group (**Fig. 5**).

Effect of *Punica granatum* on serum Glutathione Activity: The result depicts a significant decrease in glutathione level in sciatic nerve ligated rats when compared with normal rats. However, treatment with extract of *Punica granatum* significantly increased the level of reduced glutathione on 14th day as compared to the sciatic nerve ligation induced neuropathic rats (**Fig. 6**).

Effect of *Punica granatum* on serum Catalase Activity: A significant decrease in catalase level was observed in sciatic nerve ligated rats when compared with normal rats. However, treatment with extract of *Punica granatum* significantly elevates the level of catalase as compared to the sciatic nerve ligation induced neuropathic rats (**Fig. 7**).

Effect of *Punica granatum* on Superoxide Anion Generation (SAG) and tissue TBARS: The superoxide anion generation was estimated on the basis of reduction of NBT. It has been observed that sciatic nerve ligation in rats increases the generation of superoxide anion significantly when compared with normal rats. However, rats treated with extract of *Punica granatum* significantly attenuated the superoxide anion generation in contrast to sciatic nerve ligated rats (**Fig. 8**). Further, there is a significant increase in the level of tissue TBARS in sciatic nerve ligated rats when compared with normal rats. However, treatment with *Punica granatum* extract significantly attenuated sciatic nerve ligation-induced increase in lipid peroxidation in diseased group (**Fig. 9**).

Effect of *Punica granatum* on histopathological assessment of neuropathy: The results depict that in normal animals the normal functionality of sciatic nerve was maintained in normal rats. Sciatic nerve ligation results in the reduction and swelling of no. of myelinated and unmyelinated fibers whereas rats treated with *Punica granatum* extract shows milder swelling with increased no. of myelinated and unmyelinated fibers (**Fig. 10**).

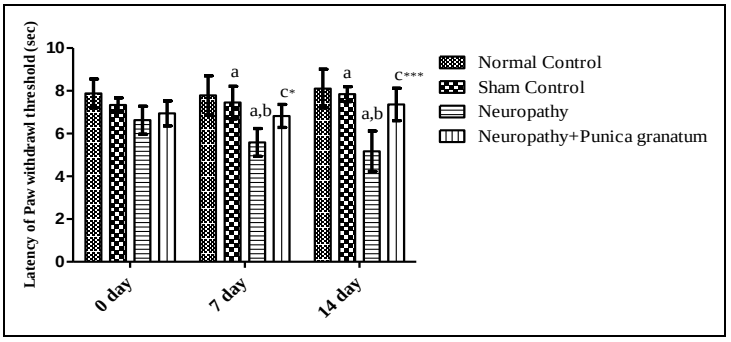


FIG. 1: EFFECT OF *PUNICA GRANATUM* EXTRACT ON HYPERALGESIA ASSESSED BY THE THERMAL SENSATION EVOKED IPSILATERAL HIND PAW WITHDRAWAL THRESHOLD

All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.001 *vs* neuropathy on 14th day

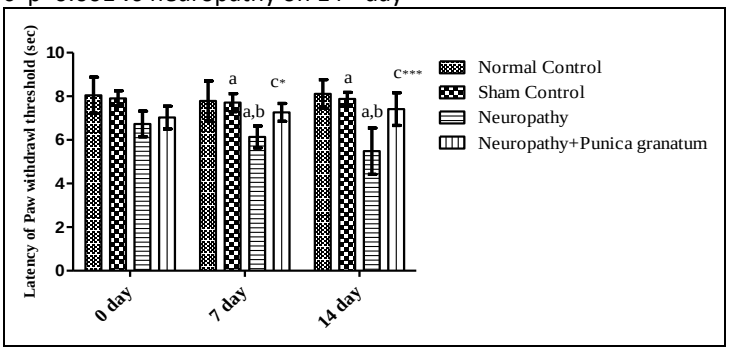


FIG. 2: EFFECT OF *PUNICA GRANATUM* EXTRACT ON HYPERALGESIA ASSESSED BY THERMAL SENSATION EVOKED CONTRALATERAL HIND PAW WITHDRAWAL THRESHOLD

a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.001 *vs* neuropathy on 14th day

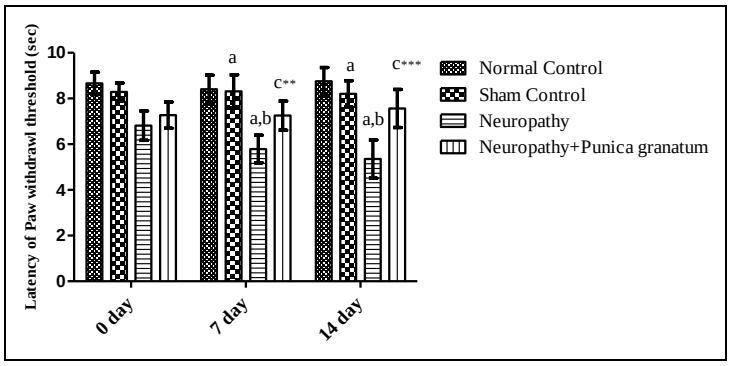


FIG. 3: EFFECT OF *PUNICA GRANATUM* EXTRACT ON ALLODYNIA ASSESSED BY THE NON-NOXIOUS STIMULI EVOKED IPSILATERAL HIND PAW WITHDRAWL THRESHOLD

All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.001 *vs* neuropathy on 14th day

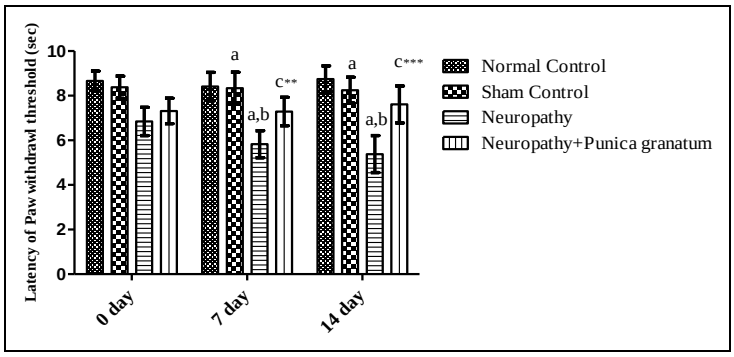


FIG. 4: EFFECT OF *PUNICA GRANATUM* EXTRACT ON ALLODYNIA ASSESSED BY THE NON-NOXIOUS STIMULI EVOKED CONTRALATERAL HIND PAW WITHDRAWL THRESHOLD

All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.001 *vs* neuropathy on 14th day

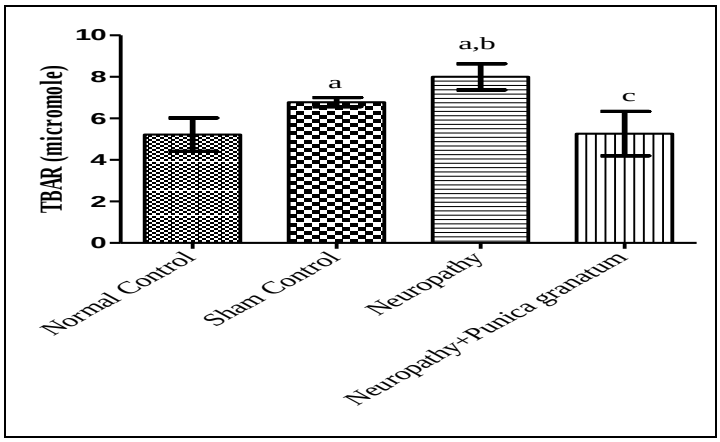


FIG. 5: EFFECT OF *PUNICA GRANATUM* EXTRACT ON LIPID PEROXIDATION BY ESTIMATING THIOBARBITURIC ACID CONCENTRATION (TBARS) IN SERUM EXPRESSED AS µM

All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.05 *vs* neuropathy.

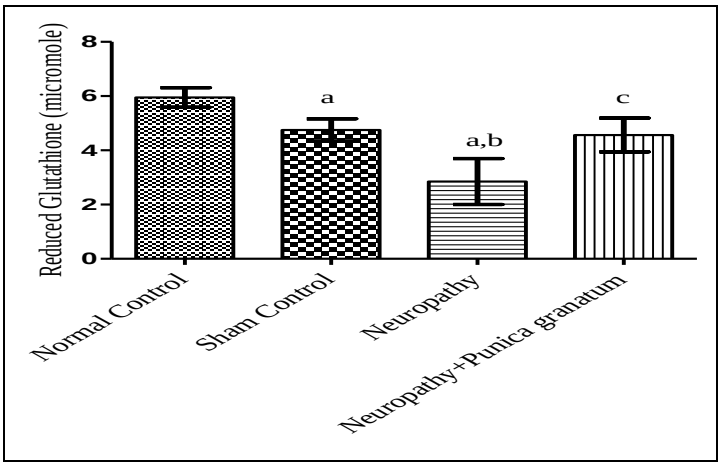


FIG.6. EFFECT OF *PUNICA GRANATUM* EXTRACT ON REDUCED GLUTATHIONE (GSH) (MICROMOLE/MG OF PROTEIN)

All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.05 *vs* neuropathy

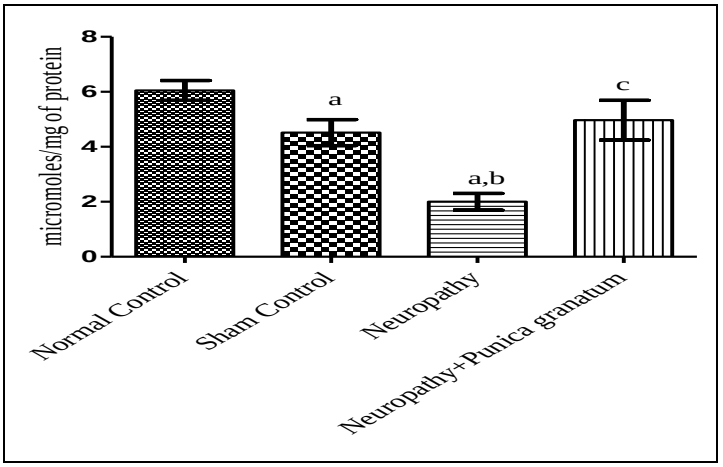


FIG.7. EFFECT OF *PUNICA GRANATUM* EXTRACT ON CATALASE (MICROMOLE/MG OF PROTEIN)

All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.05 *vs* neuropathy.

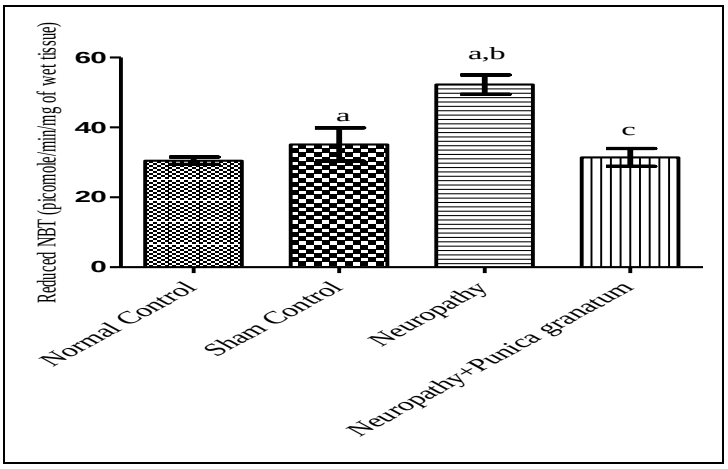


FIG. 8: EFFECT OF *PUNICA GRANATUM* EXTRACT ON SUPEROXIDE ANION GENERATION AS ASSESSED BY ESTIMATING REDUCED NITROBLUTETRAZOLIUM (NBT) (PICOMOLE/MIN./MG OF WET TISSUE).

All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.05 *vs* neuropathy.

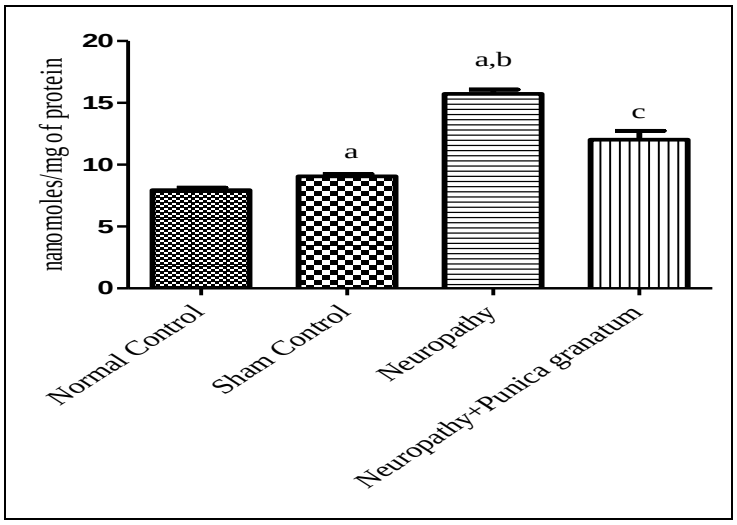
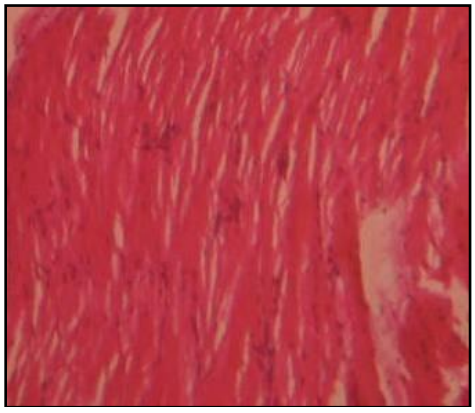
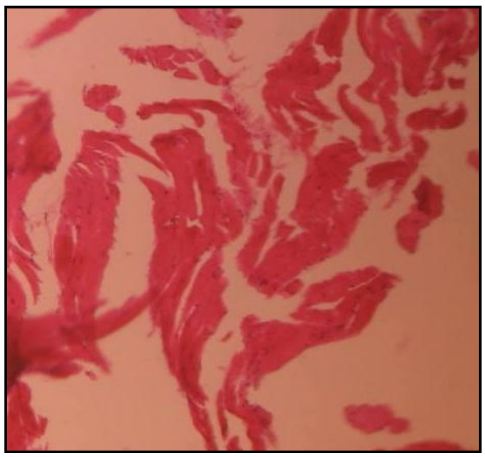


FIG. 9: EFFECT OF *PUNICA GRANATUM* EXTRACT ON TBARS (NANOMOLES/MG OF PROTEIN)

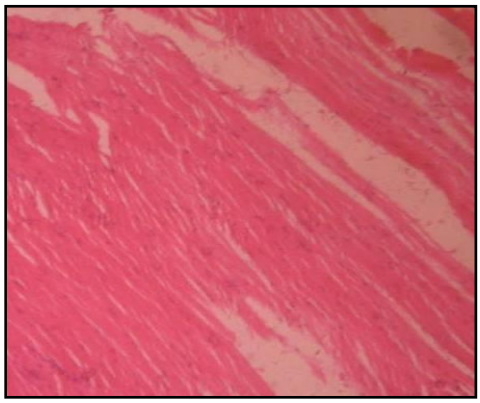
All values as expressed as mean± S.D. a=p<0.05 *vs* normal control; a,b=p<0.05 *vs* normal control and sham control respectively; c=p<0.05 *vs* neuropathy.



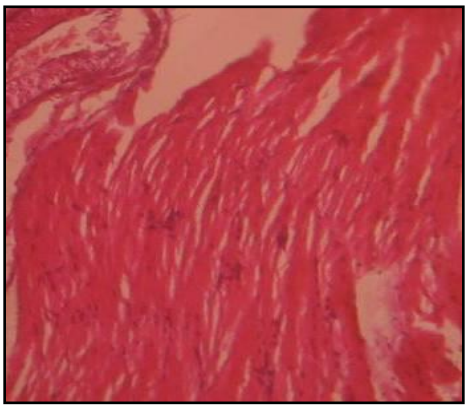
NORMAL CONTROL



SCIATIC NERVE LIGATION INDUCED NEUROPATHY



SHAM CONTROL



***PUNICA GRANATUM* TREATED SCIATIC NERVE LIGATED RATS**

FIG. 10: SHOWS HISTOPATHOLOGY OF SCIATIC NERVE

DISCUSSION: The results from the present study indicate that *Punica granatum* attenuates sciatic nerve ligation induced NP. Sciatic nerve ligation has been reported to play a pivotal role in the pathogenesis of NP [19, 20]. NP is triggered by lesions to the somatosensory nervous system that alter its structure and function that results in spontaneous pain and responses to noxious and innocuous stimuli are pathologically amplified [3].

Further, no satisfactory therapeutic intervention is still available to treat sciatic nerve ligation-induced NP. Thus, the present study has been designed to explore the possible therapeutic strategy to prevent sciatic nerve ligation-induced NP. In the present study, sciatic nerve ligation produced neuropathic pain in 14 days which was assessed in terms of behaviour parameters, oxidative stress markers and histopathology of sciatic nerve. The decrease in pain threshold behaviour, antioxidant enzymes such as catalase, glutathione and decrease in number of myelinated and unmyelinated nerve fibers in sciatic nerve have been marked as index of experimental NP.

NP induced by Sciatic nerve ligation is characterized by hyperalgesia (An increased response to a stimulus that is normally painful) and allodynia (Pain due to a stimulus that does not normally provoke pain) [42]. In NP, marked decrease in pain threshold was observed in allodynia and hyperalgesia that depicts the progression of NP [43]. Sciatic nerve ligation has been reported to cause hyperalgesia and allodynia [44]. This contention is supported by the fact that in rats subjected to sciatic nerve ligation there is significant decrease in pain threshold leading to hyperalgesia and allodynia.

Treatment with *Punica granatum* has been reported to increase the pain threshold. This contention is supported by the fact that treatment with *Punica granatum* alleviates hyperalgesia and allodynic symptoms as significant increase in paw withdrawal threshold was recorded in *Punica granatum* treated rats as compare to neuropathic rats. Sciatic nerve ligation has been reported to produce neuropathic pain by interfering with various signalling pathways [2]. NP involves various signalling pathways such as NFκB, PKA, COX-2, iNOS, MAPK [45].

Further, sciatic nerve ligation has been reported to cause NP by interfering with p^{38}MAPK pathway [46]. Further, there is increased oxidative stress that causes increased generation of free radicals [20]. Moreover, sciatic nerve ligation has been reported to cause imbalance in ROS and antioxidant enzymes [47]. Thus, the noted marked induction of NP in sciatic nerve ligated rats may be due to the development of high degree of oxidative stress. Further, this is strongly supported by the fact that in the present study, sciatic nerve ligated rats showed high degree of oxidative stress which is expressed by high levels of lipid peroxidation and marked reduction in serum glutathione and catalase.

Further, various evidence shows that oxidative stress occur due to activation of p^{38} MAPK [48]. Moreover, it has been reported that after nerve injury, p^{38}MAPK pathway increases the production of various inflammatory mediators such as TNF-α, ILs, COX-2 which are important mediators for the progression of NP [49].

The pharmacological treatment with *Punica granatum* has been noted to prevent sciatic nerve ligation-induced NP by significantly attenuating sciatic nerve ligation-induced neuropathic symptoms and oxidative stress. Further, treatment with *Punica granatum* has been reported to reduce the generation of ROS through its free radical scavenging activity [50]. This contention is supported by the fact that treatment with *Punica granatum* has significantly decreases TBARS and superoxide ion generation along with increase in serum glutathione and catalase. Thus it may conclude that aqueous extract of *Punica granatum* may decrease reactive oxygen species via inhibiting p^{38}MAPK.

Further, *Punica granatum* has been shown to restore the functionality of myelinated and unmyelinated fibres in sciatic nerve. Sciatic nerve ligation has been reported to impair the functionality of sciatic nerve by causing swelling and decrease in no. of myelinated and unmyelinated fibers. This contention is supported by the results obtained in the present study that treatment with *Punica granatum* improves the integrity of sciatic nerve by increasing the no. of fibers to the normal. Further, treatment with *Punica granatum* shows increase in no. of myelinated and unmyelinated fibers with mild swelling as shown in the results obtained from histopathological study of sciatic nerve.

On the basis of the above discussion, it may be concluded that sciatic nerve ligation caused NP is assessed in terms of behaviour parameters, oxidative stress and histopathology of sciatic nerve. Thus, the treatment with *Punica granatum* significantly attenuated the oxidative stress and increases the pain threshold in behaviour tests and consequently retains the normal function of sciatic nerve.

CONCLUSION: The present research opens vista for the management of neuropathic pain with aqueous extract of rind of *Punica granatum*.

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