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ANIMAL MODEL FOR CARPAL TUNNEL SYNDROME BY CHRONIC CONSTRICTION OF THE MEDIAN NERVE IN RATS

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ABSTRACT

Carpal tunnel syndrome (CTS) is one of the common hand disorders and it occurs with compression of the median nerve. Experimentally, limited methods are available in the development of CTS. The compression of the median nerve not explored for the CTS. The present study showed chronic constriction of median nerve produced the symptoms of CTS in rats. The CTS was developed by chronic constriction of median nerve and symptoms were assessed by thermal hyperalgesic method i.e., plantar test and grip strength by wire hang test method at different time intervals *i.e.*, day 8 and 16th day. Chronic constriction of median nerve produced the CTS like symptoms. Statistically significant ($p< 0.05$) were observed between the sham and CTS groups. Therefore, chronic constriction of median nerve can be a newer method for the induction of CTS in experimental pharmacology. And it would be a major model for the screening of neuro-analgesic agents for the treatment of CTS.

Keywords: Carpal tunnel syndrome, chronic constriction injury, Wire hang test, Median nerve, Pain.

INTRODUCTION

Carpal tunnel syndrome (CTS) is a common form of work-related neuropathic pain [1]. The compression of the median nerve is one of the primary etiological factors for the development of CTS [2]. The sensory changes are indicated on the palm side of the median nerve location [3]. In addition, the pain sensations are the ability to spread into the thumb, index finger, long finger and part of the ring finger [4]. The impulse propagation initiates from median nerve and spreading over to the muscle going to the fingers [5]. It can be observed in ipsilateral (same or injured side) as well as a contralateral side (another side) of the median nerve-muscle locations [6]. In addition to that of pain sensation, spreading of burning sensation towards the spinal cord, severe pulsatile pain sensation at night, interference of sleeping process, weakness of median nerve related muscles and difficulty to do day to day simplest hand operated work like writing, lifting the lower weight objects, holding of materials etc. which leads to alters the quality of life [7].

The swelling of wrist joints are causing the compression of median nerve leads to progress the CTS [8]. The symptoms of CTS are numbness, weakness, and tingling on the ipsilateral side of the thumb [9]. The swelling of wrist joints are occurred in different ways *i.e.*, idiopathic (any disease or condition *i.e.*, diabetes; thyroid dysfunction; fluid retention from pregnancy or menopause; high blood pressure; autoimmune rheumatoid arthritis; and fractures or trauma of the wrist) related, fibrosis of the sub-synovial connective tissue, and obstructed blood flow leads to cause the CTS [10]. Even, work-related changes of median nerve and carpal bones are likely to produce the CTS such events are assembly like computer keyboard typing based on wrong positioning of wrists, prolong exposure to vibrations during drilling work and rock cutting works with hand tools or power tools, and repeated & overextended movements of wrist like playing with piano or typing work with typewriter and so on [11]. The screening of neuro-analgesic agents for CTS in preclinical as well as clinical subjects is limited due to the difficulty of evaluating the early stages of CTS. Therefore, the development of newer medicines and screening of neuro-acting analgesic agents in clinical setup are yet to be explored for the management of CTS. Hence, validated clinical relevant animal models for CTS are an essential requirement to improve the quality of life from CTS.

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MATERIALS AND METHODS

Animals

The disease-free male Wistar albino rats (150 ± 20 g) were used. Animals maintained with standard laboratory diet with free access of tap water. The 12 h light-dark cycle, 37 ^{o}C temperature and 60 % relative humidity were maintained in animal house and the experimental laboratory. The research design was approved by Institutional Animal Ethics Committee (IAEC; No.: 288/2018) and animal care was followed as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA: Reg. No.: 155/PO/Re/S/99/CPCSEA) guidelines, Ministry of Environment and Forest, Government of India.

Induction of CTS by chronic constriction of the median nerve (CCM)

CTS was induced by chronic constriction of the median nerve as described method of Clark *et al.* [12] with a

slight modification of Yi *et al.* [13]. Briefly, rats were anesthetized with ketamine hydrochloride (50 mg/kg, *i.p.*) [14]. The front right leg hair was removed and the skin was sterilized with 0.5 % w/v povidone solution [15]. The right median nerve was exposed at elbow level. The four loose ligatures (silk thread no.: 4) were placed around the median nerve. The loose ligature was applied until the short flick response appearance in ipsilateral hind paw. The opening of muscle and skin layers were sutured and the topical antibiotic powder was applied at once [16-17]

Experimental protocol

Three groups were employed in the present study. Each groups comprising six Wistar albino rats (n=6). *Group I (Normal control):* Rats were not subjected to any surgical procedure and kept for 16 consecutive day's normal research laboratory conditions. *Group II (Sham control):* Rats were subjected to expose the right median nerve without any nerve ligation. *Group III (CCM):* Rats were subjected to expose and ligation the right median nerve under anesthetic condition. The procedure was described in the induction of neuropathic pain section. The plantar and wire hang test were performed on different time intervals i.e., 0, 8 and 16th day.

Pain sensitivity assessment by plantar test

The radiant heat sensation was assessed in ipsilateral (CCM operated) paw as described by Hargreaves *et al.* [18] with a slight modification of Muthuraman *et al.* [19]. Clinically, it mimics the CTS like thermal sensation effects. Briefly, the CCM operated paw was placed near (1 cm distance) the radiant heat lamp source without touching the lamp source. The radiant heat sensitivity of ipsilateral paw was noted as paw withdrawal threshold. The cut off time was maintained at 20 seconds.

Assessment neuromuscular function by wire hang test

The neuromuscular function was assessed as described method Jansone *et al.* [20]. The wire hang test was used for the assessment of muscle grip strength and ability forelimb neuromuscular function. Briefly, the apparatus consisted with 60 cm length and5 mm in diameter stainless steel wire and it was fixed horizontally between two vertical supports and 60 cm above a soft sponged surface. On the first day, rats were trained to grasp the central position of the wire by forepaws. The hanging time from the wire was noted. The observation was made for 2 minutes duration. Triplicate assessment was made in each rat and the longest duration was taken for further statistical evaluation. The gap (resting) duration between each assessment was maintained for three minutes.

Statistical analysis

All the results were expressed as mean ± standard deviation (SD). Data obtained from behavioral tests were statistically analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni's*post-hoc* analysis were applied by using Graph pad prism Version-5.0 software. A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The performance of CCM procedure significantly ($p < 0.05$) raised the thermal pain sensation by decreasing of ipsilateral paw withdrawal threshold when compared to the sham control group (Figure 1).

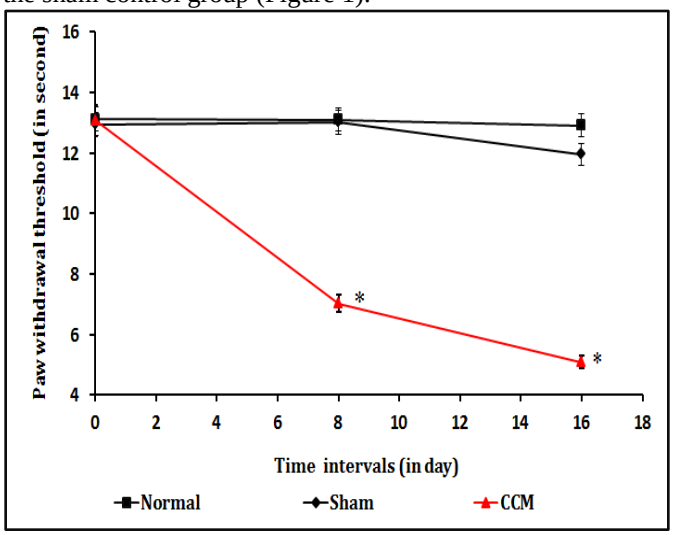

Figure 1: Role of CCM in the plantar test. Data were expressed as mean ± SD, n=6 rat per group. $^{*}p < 0.05$ Vs sham control group. Abbreviation: CCM, chronic constriction of the median nerve.

Similarly, CCM also develops the impairment of neuromuscular dysfunction decreasing of hanging time from the horizontal wire setup when compared to the sham control group (Figure 2).

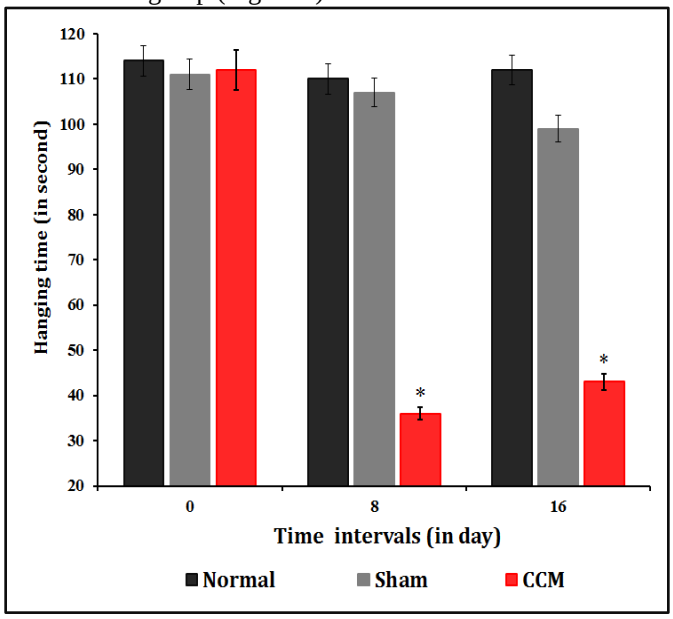

Figure 2: Role of CCM in the wire hang test. Data were expressed as mean ± SD, n=6 rat per group. $^{*}p < 0.05$ Vs sham control group. Abbreviation: CCM, chronic constriction of the median nerve.

This result indicates that CCM has been inducing the CTS with mimicking of clinically relevant symptoms of CTS. The good preclinical CTS model has compared to that of

anatomy, physiological function, and similar etiological principles of human CTS. In addition, CTS is also characterized by rising of carpal tunnel pressure. Practically, injection of toxic chemicals like carrageenan and complete Freund's adjuvant are produced the joint inflammation and it is frequently employed for the induction of inflammation and rheumatoid arthritis. The ultimate goal of the CTS model development is to makes the compression effect on the median nerve. Various methods are employed in the induction of median nerve injury like nerve trauma with accelerated workforce [21], crushing of median nerve [22] and partial transection [23]. However, these models are claimed as screening models for neuroinflammation, neurodegeneration and neuropathic pain. The chronic constriction of the median nerve is not explored in rats to reveals the models for CTS. The present work is shown the CTS like symptoms with compression of the median nerve. The CTS symptoms were expressed by rising of thermal pain sensation and alteration of neuromuscular strength & functions. This is the first report to claim the models for the CTS by chronic constriction of median nerve due to its clinical relevant symptoms.

However, the improvement of this model is still required with the assessment of multiple parameter evaluations. The behavioral evaluation like sleep pattern; pain speeding nature, intensity and referred pain pattern; electrophysiological evaluation like nerve conduction velocity, electromyography observation, tracing of impulse transmission, patch clamp based ion exchange pattern; and biochemical estimation like ions content, changes of enzymatic reaction, inflammatory mediators, neurotransmitters, neuropeptides, DNA fragmentations and expressed proteins. In addition, histopathological and immunohistochemistry based observations also help to explore the best model for CTS and relevant of human pathology associated CTS.

CONCLUSION

Therefore, the present research communication can help to screen newer neuro-analgesic agents for CTS and also supports the validation, improvements, and development of clinical relevant CTS.

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