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Research Article

Controlled Trial of Efficacy of Dexketoprofen in Sciatic Nerve Crush Injury in Rats

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Summary

Object: Local applications of Dexketoprofen trometamol (dex) have been shown to increase in the sciatic nerve functional tests following the nerve injuries. The aim of the current study was to compare the efficacy of dex application for 1 and 5 days following nerve injury rat model. Possible effects of dex were checked by means of the Sciatic Function Index (SFI), Withdrawal of the Reflex Leg (WRL), number of axons, axon diameter, and nerve diameters.
Material Methods: The animal crushed model was done through Aesculap -Yasargil aneurysm method on Wistar albino (N=21) right sciatic nerves. In order to achieve desired purpose 3 experimental groups were organized: Group 1: control (injured and no drug); Group2: dexketoprofen 1 days (injured and single dose of drug); Group3: dexketoprofen 5 days (injured and single dose of 5 times drug applications). Injections were done through the tissue expander's injection port with a connecting tube directed at the crush-injured site. Functional recovery of the sciatic nerves was evaluated with the improvement in the SFI values. Additionally, recovery of sensory function was assessed through WRL results and histopathological examination was performed 6 week following the injury.
Results: At the end of the experimental periods there was no significant differences were found between the experimental group of animals on the bases of the number of axons, axon diameter, and nerve diameter. Moreover, up 30th day of the experimental period our functional test results have shown that there was no difference between the groups. But following the induction of the injury, the statistical significances were seen on the functional tests. This effect was seen only if the multi-dose application of the dex.
Conclusions: This study suggests that; dexketoprofen trometamol of 5 consecutive days, has revealed positive significant changes in the sciatic nerve injury through analgesic effect of it.

Key words: Nerve injury, clipping, dexketoprofen trometamol, fiber diameter, axon number and nerve diameter

Sıçanlarda Siyatik Sinir Yaralanmasında Deksketoprofenin Etkinliğinin Kontrollü Çalışması

Özet

Amaç: Bu çalışmanın amacı, sıçanlarda siyatik sinir ezilme yaralanmalarında lokal olarak uygulanan deksketoprofen'in etkilerini karşılaştırmaktır. Bunun için sıçan modelinde yaralı siyatik sinirlerin Siyatik Fonksiyon Endeksi (SFI), Refleks Bacak geri çekilmesi (WRL), akson sayısı, parametreleri üzerinde 1 gün, 5 gün ve kontrol gruplarında akson çapı ve sinir çapı birlikte değerlendirilerek nöronal hasar incelendi.

Yöntem ve Gereç: Wistar albino sıçanlar (21 hayvan) kullanıldı. Sağ siyatik sinir bir Aesculap - Yaşargil anevrizma kullanılarak 10 saniye ezildi ve 10 saniye sonra anevrizma klibi açıldı. Deney hayvanları Grup 1, Grup 2 ve Grup 3 olarak üç gruba ayrıldı. Duyusal fonksiyonun iyileşmesi WRL ile değerlendirildi . Histo-patolojik inceleme yapıldı.
Bulgular: Akson sayısı, akson çapı ve sinir çap bazında gruplar arasında anlamlı fark yoktu. Ama yaralanma indüksiyonundan sonra, istatistiksel olarak anlamlı fonksiyonel testler görüldü. Bu etki yalnızca çok dozlu Dex uygulamalarında etkili olarak bulundu.
Sonuç: Bu çalışma; 5 gün üst üste deksketoprofen trometamol uygulanmasının, analjezik etkisi ile siyatik sinir hasarı üzerinde olumlu önemli etkilerinin olduğunu ortaya çıkarmıştır.

Anahtar Kelimeler: Sinir hasarı, klipleme, deksketoprofen trometamol, fiber çapı, akson sayısı, sinir çapı

INTRODUCTION

Sciatic nerve injuries are often caused by injections, lacerations, contusions, compressions, and iatrogenic causes. Injuries to sciatic nerves cause partial or total loss of motor, sensory and autonomic functions due to the axon discontinuity, degeneration, and eventual death which finally result in substantial functional loss and decreased quality of life[21]. For the majority of the cases it is a life saving phenomenon to recover the lost neuronal function[1,9] Nerve crush injuries are also a well-known model in experimental regeneration studies to investigate the impact of various pharmacological treatments. Although both morphological and functional data have been used to evaluate the neural regeneration after induced crush injuries, the correlation between these two types of assessment is usually poor. With this point of view, research carried on peripheral nerve injury needs to combine both functional and morphological assessment together[11]. For the majority of the cases together with morphological examinations the most common function tests that were used are Withdrawal Reflex Latency (WRL) and Sciatic Functional Index (SFI).

For the measurement of withdrawal reflex latency (WRL), the rat was wrapped in a surgical towel above its waist and then positioned to stand with the affected hind paw on a hotplate at 56 °C. WRL is defined as the time elapsed from the onset of hotplate contact to withdrawal of the hind paw and measured with a stopwatch. The affected limbs were tested three times, with an interval of 2 min between consecutive tests to prevent for sensitization, and the three latencies were averaged to obtain a final result[8].

For the assessment of motor nerve recovery, walking track analysis was carried out as described in previous reports[7,14] with minor modifications. The rats were allowed conditioning trials in an 8.2 × 100 cm walking track with a piece of white paper at the bottom of the track. The hind feet were dipped in red ink, leaving prints on the white paper. The print length (PL), the toe spread (TS), and the intermediary toe spread (IT) were thus obtained. In general, the maximal value was adopted for each measurement, and the data were recorded with the prefix E for the operated side and N for the normal non-operated side. The sciatic function index (SFI), an indicator of the degree of nerve dysfunction, varies from 0 to −100, with 0 corresponding to normal function and −100 to complete dysfunction. It was calculated by the formula[2,10]:

$$SFI = -38.3\left(\frac{EPL - NPL}{NPL}\right) + 109.5\left(\frac{ETS - NTS}{NTS}\right) + 13.3\left(\frac{EIT - NIT}{NIT}\right) - 8.8$$

Dexketoprofen trometamol is the S(+) isomer of ketoprofen, which retains the typical action of its non-steroidal anti-inflammatory drug (NSAID) parent. This drug is an analgesic and anti-inflammatory agent. It is the active optical isomer of ketoprofen, a propionic acid NSAID which has been separated to halve the dose required and halve the metabolic load. The inactive isomer has been discarded in the hope of eliminating or reducing potential unnecessary side effects[22].

With all these results, current study aims to investigate the possible positive effects of dex (5 mg kg^{-1}, i.p.) either for single and a multiple times applications on the crushed nerve injuries.

MATERIAL AND METHODS

Study design and animals

Twenty one male Wistar rats weighing 250-350 g were used for the experiments. Experiments were carried out after obtaining the approval from Kırıkkale University Ethics Committee for Animal Studies (Registration date and number: 22.08.2011 and 11/217). Animals were housed maximum four rats per cage, in an ambient temperature of 22±2 Co, humidity 51% and a 12-hour light-dark cycle during experiment. All animals were allowed free access to food and water, except during surgery and experiments. After having the sciatic nerve injury (see below), the animals were divided randomly into three groups: Group 1 (Con): Control group animals that was no drug application (N=7); Group 2 (Dex1): Single dose of Dexketoprofen application (5 mg kg^{-1}, i.p.) (N=7); Group 3 (Dex5): Five days of consecutive Dexketoprofen application (5 mg kg^{-1} day^{-1}, i.p.) (N=7). All experiments were carried out for six weeks.

Surgical procedure

Under deep Anaesthesia (40 mg/kg Ketamine HCl (Ketalar; Pfizer Inc, USA) and 5 mg/kg Xylazine HCl (Rompun %2; Bayer HealthCare AG, Germany), i.m.) 21 rats received a unilateral sciatic nerve crush injury. Each animal on its right side, the area of 4x2 cm on the right thigh was shaved and the right sciatic nerve was carefully exposed.

Sciatic Nerve Crush

In order to achieve the sciatic nerve crush, aneurysm clip (applying 63 gram force on the sciatic nerve) (Aesculap, Yasargil) was used for 10 seconds. To facilitate the axonal lesion on the sciatic nerves for each animal this procedure was repeated three times. Then the clips were removed and the muscles and skin sutured with 3.0 silk sutures under aseptic conditions. For the recovery period animals were housed under the conditions described above.

Functional assessment of nerve regeneration

Following the peripheral nerve injury, experimental animals were subjected to walking tests (8.2x12x42 width, height and length respectively) on the first, 7^{th}, 14^{th} and 30^{th} days. Then the sciatic nerve function indexes (SFI) were calculated. These values were then used for the calculation of the SFI which was developed by De Medinacelli and modified by Bain et al[3]. Results of calculated values were varies between 0 and 100, showing that the 0 indicates normal while 100 indicates a complete loss of function. Finally the results of the experimental group of animals were analyzed statistically.

Sensory functions of the sciatic nerves detected through withdrawal of the reflex

leg (WRL). The test of WRL was done through nociceptive hot plate technique which was done by Master et al[15]. Briefly, the hot plate was maintained at 56 ± 0.1°C and the paw withdrawal latency (WRL) was measured. The test was repeated three times at an interval of 15 min, and each test was carried out by a different blind observer. The final response was reported as an average of the three tests. In order to prevent the skin damages hot plate measurements were ended at the 12th seconds of the procedure and the animals having longer reflex times were reported as 12 seconds[24].

Histopathological Evaluation

All of the rats, at the end of the sixth week, were sacrificed for the histopathological examination. Sciatic nerves of right legs were removed in one piece including the damaged area in them. Each sciatic nerve was then placed in 10% formalin for fixation. Both for the histopathological condition of the damaged area and the effect of dexketoprofen treatment were checked by means of fibroblast proliferation, inflammatory cell density and axon counts.

Serial sections of 5 μm stained with haematoxylin–eosin were examined and photographed by a microscope (Leica® Microsystems, Wetzlar GmbH). Each section was evaluated by experienced histopathologist blinded to the groups, and study drug (Figure 4).

Statistical Analysis

Statistical analysis was performed with SPSS for Windows 11.0 (SPSS Inc., Chicago, II., USA). Unless otherwise stated, data were expressed as means ± standard deviation. All results were evaluated with the Kolmogorov-Smirnov for testing the normal distribution. Due to homogeny distributions of the measured axon diameter, axon number and nerve diameter values, they were evaluated by One Way Anova. For the comparison of differences among the groups Tukey post hoc analysis were used. Furthermore in order to eliminate the type 2 errors Bonferroni corrections were done and $p < 0.008$ were assigned to be different.

RESULTS

Histopathological Analysis

At the end of the experimental periods, animals were sacrificed under light anesthesia (30 mg/kg sodium pentobarbital) and dissected sciatic nerve of animals was used for the histopathological examinations. Mean values of the nerve diameter and the number of the fibers of experimental group of animals were summarized in Figure 1. Statistical analysis of both nerve diameter and the fiber numbers results in insignificance among the experimental group of animals. Neither the single (Dex 1) nor the cumulative dose (Dex 5) of the dexketoprofen application did not produce any significant effect on the nerve diameter and axon numbers (Figure 2).

The mean axon diameter values were summarized in Table 1. The statistical comparison of these measured values (Table 1B) resulted in an insignificance among the experimental group of animals (one way ANOVA post hoc Tukey examined data was corrected by Bonforreni in order to eliminate the type 2 errors).

The assessment of the grading values of inflammation (I) was done by the grading system starting from 0 to 3 indicating absent, slight, moderate and strong, respectively. Comparison of the mean graded values showed that either there were no or a few inflammatory cells was present. (I_{Con}=0.63±0.52, I_{Dex1}=0.50±0.76 and I_{Dex5}0.63±0.52) (Kruskall Wallis, p=0.203).

Functional assessment of nerve regeneration

Mean withdrawal reflex latency (WRL) measurements on the first and seventh day were found to be more than 12 seconds for

all the experimental groups (data not shown here). The measurements on the following days (14th and 21th) did not produce any statistical significance among the animals (p=0.351). p values of WRL tests for the 30th and 45th measurements were summarized in Figure 2. Post hoc analyses of the WRL results have shown that cumulative application of Dex (Dex5) produced a positive effect both on the 30th and 45th days compared the injured group of animals (Con). Meanwhile, although there was no significance between the Dex1 and Dex5 group animals on 30th day, 45th day results have resembled significance between these group animals.

No significance was found on sciatic functional index (SFI) measurements on the 1st and 7th day of the experimental periods (p1=0.657 and p7=0.632). Similar to WRL test measurements SFI results did not produce any significance for the following two weeks of the experimental period (p=0.472). p values of SFI tests for the 30th and 45th measurements were summarized in Figure 3. Single dose of Dex application produced a positive effect only on the 30th day of experimental period. Meanwhile, post hoc analyses of the SFI results have shown that cumulative application of Dex (Dex5) produced a positive effect both on the 30th and 45th days compared the injured group of animals (Con).

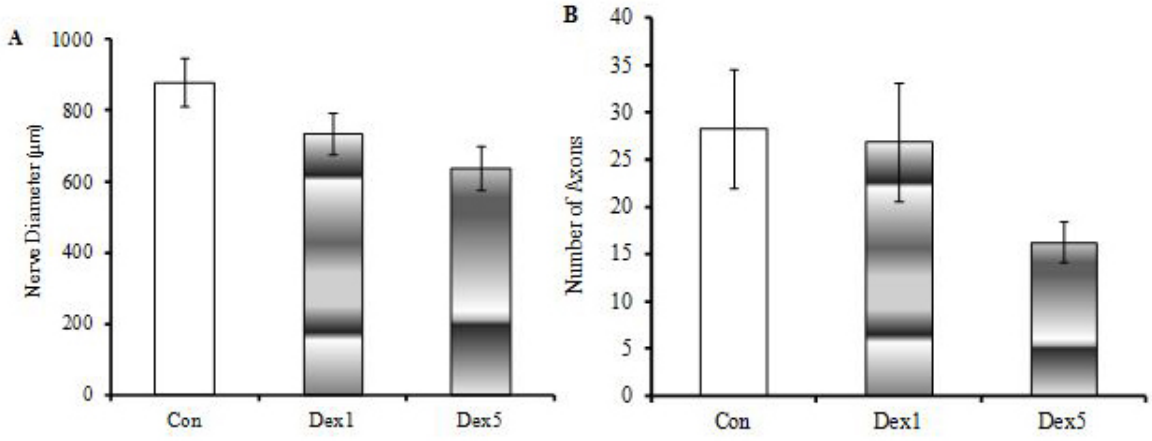

Figure 1: *Nerve diameters and the axon numbers of the experimental group of animals. A shows mean sciatic nerve diameters while B shows the mean axon numbers. In the figure; Control (Con,), single dose of dexketoprofen treated group (Dex1) and five day dexketoprofen treated group (Dex5). Values are given as mean ± SEM.*

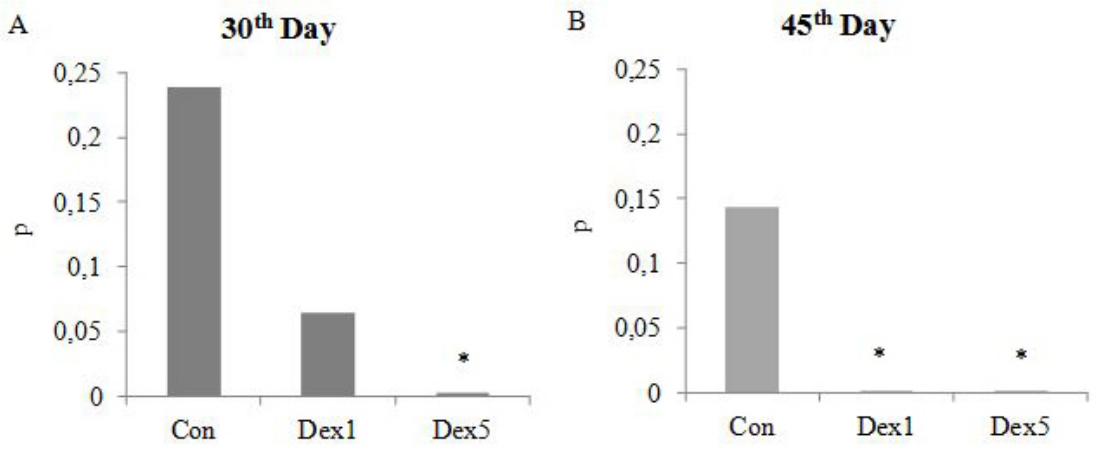

Figure 2: *Post-hoc analysis results of WRL tests on the 30th (A) and 45th (B) days. Control (Con), single dose of dexketoprofen treated group (Dex1) and five day dexketoprofen treated group (Dex5). Values are given as mean ± SD. *p<0.008 values were considered to be significant following the Bonforreni correction to eliminate the type 2 errors.*

Table 1 Axon diameters of the experimental group of animals.

A **Groups**	**Axon Diameter (µm)**
Con (N=7)	3.52±1.18
Dex1 (N=7)	3.83±0.89
Dex5 (N=7)	5.72±1.18

B	**Groups**	**P***
Con	Dex1	0.944
	Dex5	0.009
Dex1	Con	0.944
	Dex5	0.019
Dex5	Con	0.009
	Dex1	0.019

In the table, mean axon diameters of the experimental group of animals were given. Control (Con), single dose of dexketoprofen treated group (Dex1) and five day dexketoprofen treated group (Dex5). Values are given as mean ± SD. *p<0.008 values were considered to be significant following the Bonforreni correction to eliminate the type 2 errors.

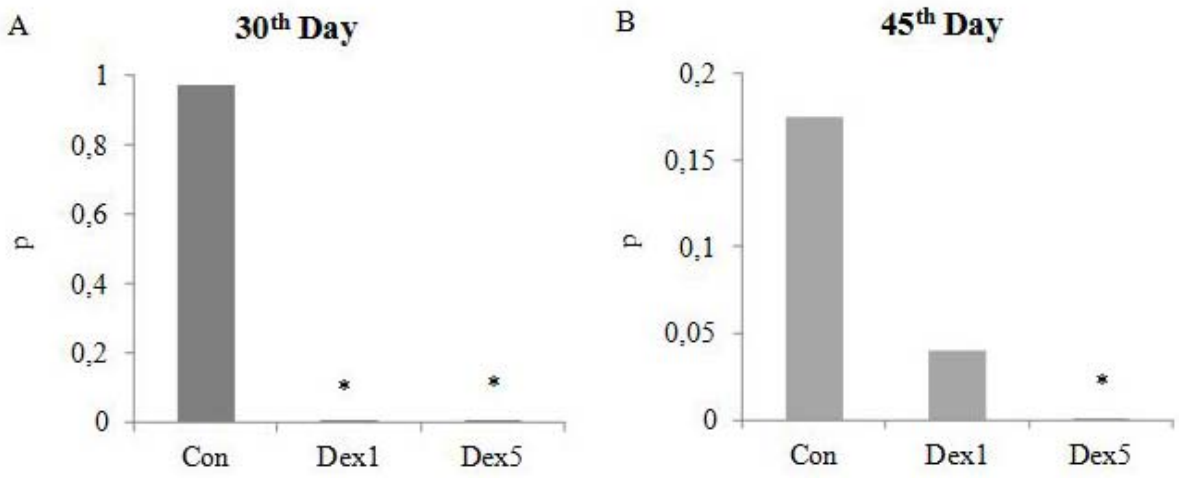

Figure 3: *Post-hoc analysis results of SFI tests on the 30th (A) and 45th (B) days. Control (Con), single dose of dexketoprofen treated group (Dex1) and five day dexketoprofen treated group (Dex5). Values are given as mean ± SD. *p<0.008 values were considered to be significant following the Bonforreni correction to eliminate the type 2 errors.*

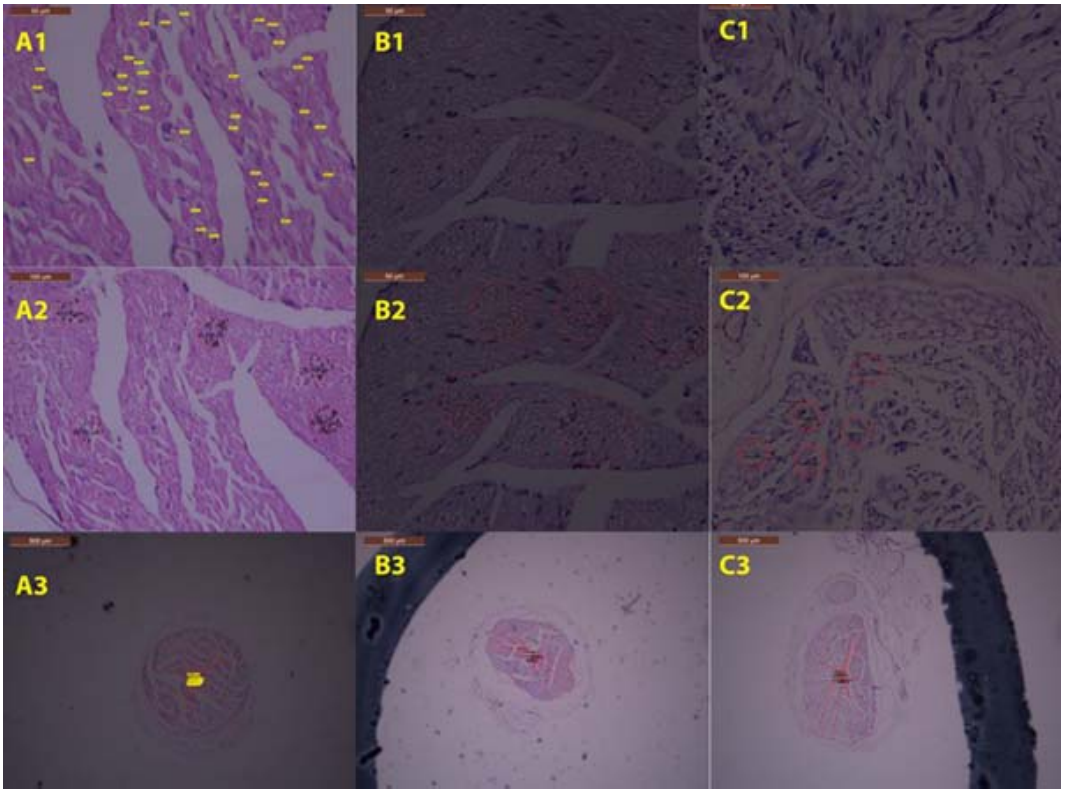

Figure 4: *Histopathological appearances of the experimental group of animals. In the figure letters A, B and C represents injury, single dose of dexketoprofen treated group (Dex1) and five day dexketoprofen treated group (Dex5) respectively. Numbers designated with the letters 1, 2 and 3 represents axon diameter, number of axon and nerve diameter respectively.*

DISCUSSION

Animal models for the study of peripheral nerve regeneration especially models on rat and mouse frequently used due to the extensive availability of these animals as well as the distribution of their nerve trunks which is similar to humans[13,19].

Furthermore, the speed of spontaneous regeneration made them applicable for the short-term studies especially for the screening of the possible drug administration effects. Majority of the studies dealing with the sciatic nerve regeneration after axonotmesis include a post-surgery follow-up period of 4–8 weeks based on the hypothesis that, by the end of this time, functional recovery is complete. Justification of this idea is supported mainly by the data obtained from the WRL and SFI test result analysis[11,13,19].

In this study, we test the therapeutic potential of dexketoprofen treatment of either single or multiple doses (five days) over the 45 days on rat sciatic nerve injuries. Our histopathological evaluations have shown that both of these doses of administrations have no positive effects on nerve diameter, axon diameter and axon numbers. Indicating that in the early phases of nerve injuries Dex cannot be considered as potential drug at least for the morphological recovery.

The fact that there was no change on the morphological examinations the functional checks of the injured nerves produce positive results starting from the 30 day of the experimental period. This short term positive effect of Dex applications starting from 30th day was still seen on the last day of experimental period (45th). The positive effect seen on the functional tests (SFI and WRL) can only be seen in the multi-dose applications.

Dex, as a drug, shows its potency through either anti-inflammation and/or analgesia on the applied system[17]. In agreement with the previous findings[3,4,6,12] we cannot detect sufficient anti-inflammatory effect. Indeed we thought that in order for an effective anti-inflammation the testing period should be increased.

Peripheral nerve researchers frequently use the rat sciatic nerve crush as a model for axonotmesis[16,19].

Axonotmesis or second degree Sunderland injury designates a breakdown of the axon and distal Wallerian degeneration but with preservation of the continuity of the endoneurial sheath. After this type of injury, spontaneous regeneration through the distal nerve stump with good functional return can be expected[20]. The most commonly used method of functional assessment after in nerve repair the rat is walking track analysis. The most commonly used method of functional assessment after in nerve repair the rat is walking track analysis. Also we found good functional recovery for SFI in Dex 5 group after 6 weeks follow-up time after sciatic nerve our study. But single dose of Dex application produced a positive effect only on the 30th day and there was no difference between Con group and Dex1 groups on the 45th day. However we found similar results for WRL tests. Varej˜ao et al. reported the withdrawal response to thermal noxious stimulation after 4 week[5,11,18,23]. And we found difference for WRL test between in all groups from 4th week. This results is shown with dose-dependent effect of dexketoprofen that caused by improve on functional tests (SFI, WRL) after sciatic nerve injury.

CONCLUSION

The present study demonstrated that multiple dose application of Dexketoprofen trometamol could recovery after injury of sciatic nerve. It may be poor anti-inflammatory effect in traumatic nerve injury but it can be effective in neuropathic pain due to trauma. This study needs further investigations especially focused on the analgesic side of effect.

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