Analgesic effects of Terminalia Chebula is mediated by suppression of brain protein expression of NGF and NF-κB and oxidative markers following neuropathic pain in rats

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Research Article

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Abstract

Background: Due to the complications related to the use of the current pharmacological approach for relief of neuropathic pain, searching for effective compound with less complication is need of present era. Since, the pathophysiology mechanism of neuropathic pain is related to excessive inflammation in nervous system. Hence, the present study focuses on whether the potential analgesic effects of Terminalia Chebula (TC) extract is mediated by the changes of brain protein expression of nerve growth factor (NGF) and nuclear factor-kappa B (NF-κB) in a rat model of sciatic nerve chronic constriction injury (CCI).

Method and Results: Neuropathic pain induced by left sciatic nerve CCI. Male wistar rats assigned into sham, CCI, and CCI+TC (40mg/kg) groups. Animals received normal saline (1 ml) or aqueous-alcoholic extract of TC (40 mg/kg) for 30 days via gavage needles once a day. Cold allodynia and anxiety-like behaviors examined on one day before CCI surgery (day -1) and also on days 2, 7, 14 and 30 following CCI. We also assessed TC extract effects on oxidative stress markers on day 30 following CCI. Furthermore, western blot study performed on day 30 following CCI for evaluation of TC extract effects on protein expression of NGF and NF-κB of the brain. Orally gavage of TC extract significantly decreased cold allodynia on days 2 and 14 following CCI. Additionally, CCI model of chronic pain significantly increased protein expression of NGF and NF-κB of the brain on day 30 following CCI. Furthermore, TC extract significantly decreased protein expression of NGF and NF-κB of the brain. The TC extract significantly increased brain glutathione (GSH) content and decreased malondialdehyde (MDA) content.

Conclusions: It is suggested that analgesic effects of TC extract is mediated by suppression of brain NGF, NF-κB, and also its antioxidant activity in the brain following neuropathic pain in rats.

Introduction

Neuropathic pain is usually considered as a complex condition, resulting from physical injury or disease that affects the peripheral or central nervous system [1]. Approximately 3–18% of the population worldwide suffers from neuropathic pain [2]. The pathophysiology mechanism of neuropathic pain is related to excessive inflammation in both the peripheral and central nervous system which may induce the progression of pain perception [3]. Initiation and maintenance of neuropathic pain is related to excess release of inflammatory cytokines in the peripheral and central nervous system, leading to peripheral and central sensitization [3]. After peripheral nerve injury, neuro-glial cells and inflammatory cells such as macrophages are recruited to the damage site and increase the release of NGF [4]. The binding of NGF to its receptors tropomyosin receptor kinase A receptor (TrkA) and p75 neurotrophin receptor (p75NTR) on peripheral afferent neurons activates various signaling pathways, including activation NF-κB, leading to sensitization of nociceptors and subsequently hyperalgesia [4]. It has been postulated that the NGF/NF-κB signaling pathway is one of critical signaling pathways, which play an important role in the chronicity of neuropathic pain [5]. Indeed, activation of these signaling pathway mediated the generation of several inflammatory factors such as TNF-α and IL-1β, subsequently contributing to excess inflammation and

hypersensitivity [6]. Several studies have clearly highlighted the important role of NGF/NF-κB signaling pathway in the pain modulation. It has been demonstrated that NGF is upregulated in patients with chronic pain syndromes (Bimonte S, 2021). Dos Reis and colleagues in 2016, in a rat model of trigeminal neuropathic pain, demonstrated that Pretreatment with an antibody anti-NGF and antagonists of TrkA attenuated heat hyperalgesia [7]. Additionally, da Silva and colleagues in 2019 identified that local injection of anti-NGF markedly alleviated pain in neuropathic rats, which lasting for 5 h [8]. Recently, a meta-analysis study was shown that anti-NGF antibodies can suppress pain perception in patients with osteoarthritis pain and chronic low-back pain [9]. Furthermore, Xu and colleagues in 2018 have identified that NF-κB signaling pathway is activated in neuropathic pain following chronic constriction injury of sciatic nerve in rats. They have also reported that excess activation of NF-κB increases NGF expression following neuropathic pain. Their study has provided that inhibition of NF-κB signaling pathway decreases pain perception in neuropathic rats [10]. Hence, it is highly likely that finding natural compounds that target NGF/NF-κB signaling pathway can markedly alleviate chronic pain. Additionally, natural compounds would overcome the various side effects of monoclonal antibody chemicals, particularly in elderly adults [11]. Terminalia Chebula (TC) (Family: Combretaceae) is commonly known as black- or chebulic myrobalan [12]. Traditionally, the fruit of TC has numerous pharmacological actions including; cytoprotective, antioxidant activity, anti-inflammatory, analgesic, as well as anti-microbial [13]. Furthermore, the oral administration of the TC did not produce toxicity in rats [14]. Therefore, in the present study, we first investigated the analgesic and anxiolytic effects of hydro-alcoholic extract of TC in neuropathic rats. Next, we evaluated whether its analgesic effects mediated by alteration of the NGF and NF-κB expression of brain following neuropathic pain in rats.

Materials And Methods

Animal preparation

Adult Wistar male rats, (weight 180–200 g, n=6/group) were bought from breeding colony of Baqiatallah University of Medical Sciences, Tehran, Iran. Animals were kept in well ventilated sterile Plexiglas cages in the animal houses of Baqiatallah University of Medical Sciences. Each cage contained equal number of rats (two rats in each cage). Animals were maintained under 12 h light/dark cycle in a room at 22–24 °C with free access to food and water. All experiments conducted in agreement with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and was approved by the local ethical committee of Baqiatallah University of Medical Sciences, Tehran, Iran (Ethical code: IR.BMSU.REC.1400.033).

Experimental design and groups

In the current study, animals were divided into 3 groups (n=6 per group). These groups were as follows: [Group 1: sham group]; [Group 2: CCI group]; [Group 3: CCI+TC group]. Animals (in the CCI+TC group) received TC aqueous extract (40 mg/kg) [15] for 30 days via gavage needles once a day. Sham animals received saline 0.9% by gavage needle for the same period of time. Cold allodynia was assessed

1 day prior to neuropathic surgery (CCI model), and on days 2, 4, 7, 14 and 30 post-surgery, using acetone test (Figure 1). Anxiety-like behaviors were also assessed 1 day prior to neuropathic surgery (day -1), and on days 2 and 7 post-surgery, using EPM (Figure 1).

Induction of neuropathic pain (CCI surgery)

Neuropathic pain (CCI model) was induce, as it was previously introduced by Bennett and Xie [16]. Briefly, after anesthetizing the animals with chloral hydrate (350 mg/kg, i.p), the left body of sciatic nerve (1 cm) was exposed and then four loss ligatures (4/0 catgut) was tied around the nerve, about 1 mm apart, until a brief twitch in the hind limb was observed. In sham animals, only the left sciatic nerve was exposed, but not ligated.

Cold Allodynia (Acetone Test)

To assess cold allodynia in neuropathic rats, foot withdrawal (as a positive response) in response to acetone drop (acetone test) was evaluated [17]. Briefly, the rat was placed under a transparent Plexiglas chamber with a metal mesh floor. After 10 min accommodation period, a drop of acetone was applied with a syringe to the plantar surface of the left hind paw (ipsilateral to spinal nerve injury). Withdrawal of the paw or licking/shaking of the toes are considered as a positive response. The acetone was applied 5 times (every 5 min) to the left hind paw (neuropathic paw). The frequency of paw withdrawal was expressed as a percent as follows: (Number of positive response × 100)/(5 trials).

Anxiety-like behaviors (Elevated Plus Maze)

Assessment of anxiety-like behaviors performed using EPM test. The EPM is a cross-shaped platform consisted of two open and two closed arms (opposing each other). All arms communicate via a central zone, allowing rats to move freely into each arm. Rats were placed on the central zone, facing an open arm for 5 min. Then, their movements on the maze monitored for 5 min period with a camera. The percent of open arms spent time and also the percent of open arms entries were assessed as an index of anxiety-like behaviors. Less time spent into the open arms and less number of entrances into the open arms were in favor of anxiety [18].

Scarification of animals and brain tissue dissection

At the end of experiment (day 30), the animals were anesthetized with an intraperitoneal injection of 100/10 mg/kg of ketamine/xylazine mixture. After that, laparotomy was performed to isolate the complete brain tissue and kept at −80 °C for the evaluation of GSH, MDA, SOD and CAT as well as the Western blot assays.

Determination of protein levels in the brain

The method of Bradford was used to measure the protein levels of brain samples [19]. Bovine serum albumin (BSA; Sigma, Germany) was used as a standard.

Biochemical analysis

The activity of the brain catalase (CAT) enzyme was assessed according to method of Aebi in 1984 [20]. The activity of brain superoxide dismutase (SOD) was assessed based on the nitroblue tetrazolium (NBT) reduction by SOD [21]. Lipid peroxidation in the brain was assessed by measuring the malondialdehyde (MDA) content. The protocol follows that described by Yazdanparast et al. [22]. Brain glutathione (GSH) content was assessed by the method of Moron et al. [23].

Western blotting analysis for brain NGF and NF-kB protein expression

The brain tissues were subjected to homogenization in a RIPA buffer solution to which protease and phosphatase inhibitors were added. To remove the insoluble substances, the homogenates were subjected to centrifugation at 12,000× g for 25 min. Then, 5 µL of the supernatant was utilized for assessment of the concentration of the protein using a Bio-Rad Quick StartTM Bradford protein assay kit. Proteins from the tissue homogenates were subjected to a denaturation step using 4X Bio-Rad Laemmli sample buffer and then loaded on sodium dodecyl sulfate polyacrylamide gel. Then, the proteins were electrophoresed and transferred from the gel to nitrocellulose membranes. In order to block the membrane's free sites, it was incubated in 5% Bio-Rad non-fat dried milk for an hour and finally washed before overnight incubation with the selected primary antibodies: NGF (catalog number: orb228196) and NF-kB (catalog number: GTX102090) at 4 °C with gentle agitation. The following step was the washing of the blots and incubation with secondary antibodies (rabbit, PZ5610) and GAPDH (ab181602). The reacted antigens were visualized by enhanced chemiluminescence by a commercial detection kit. The densitometric analysis for the color intensity was measured by ImageJ software [24].

Statistical analysis

Statistical analyses were conducted with the SPSS software (version 24.0). Data are expressed as mean ± SEM. The two-way ANOVA used to understand if there is an interaction effects between the two independent variables (groups and time) on the dependent variable (cold allodynia and anxiety like behaviors). Then, we use one-way ANOVA to compare mean difference of between groups, followed by the Tukey HSD post hoc test, and $p<0.05$ was considered significantly.

Results

The effects of hydro-alcoholic TC extract on cold allodynia

The results of the two-way ANOVA revealed that there was significant main effect of groups on cold allodynia ($F_{(2,54)}$ =14.91, p=0.001, ηp2 = 0.35). However, there was not significant main effect of the time on cold allodynia ($F_{(4,54)}$ =2.24, p=0.07, ηp2 = 0.14). Furthermore, two-way ANOVA confirmed a significant interaction ($F_{(8,54)}$ =4.04, p=0.001, ηp2 = 0.37) between the groups and the time on the cold allodynia. As shown in the figure 2, following CCI surgery, the PWR significantly increased in CCI group [Figure 2, (**p<0.004 on Day 2; 80.00±5.77), and (*p<0.02 on Day 7; 83.33±6.14), and (***p<0.001 on Day 14;

92.00±4.89)] as compared with sham group [Figure 2, (Day 2; 40.00±8.16), and (Day 7; 26.66±6.66), and (Day 14; 35.00±5.00)] from 2 days up to 14 days' post-surgery of neuropathy. Additionally, intra-orally administration of TC significantly decreased PWR as compared with CCI rats [Figure 2, (##p<0.004 on Day 2; 40.00±8.16) and (###p<0.001 on Day 14; 40.00±11.54)] (it indicating analgesic activity of TC).

The effects of hydro-alcoholic TC extract on anxiety-like behaviors

The results of the two-way ANOVA revealed that there was significant main effect of groups ($F_{(2,50)}$ =11.52, p=0.001, ηp2 = 0.31) and time ($F_{(2,50)}$ =10.77, p=0.001, ηp2 = 0.30) on the percentage of entries into open arms. Aditionally, there was significant interaction effects of the groups and the time ($F_{(4,50)}$ =3.11, p=0.023, ηp2 = 0.19) on the percentage of entries into open arms. In the EPM test, CCI surgery significantly decreased percentage of entries into open arms (0.00±0.00, p=0.003) on day 7 after neuropathy as compared with sham group (43.02±6.0, Figure 3A). Furthermore, CCI surgery significantly decreased percentage of spent time into open arms on days 2 (1.00±0.52, p=0.001) and 7 (1.03±0.68, p=0.009) after neuropathy, as compared with sham group (29.86±9.52 on day 2 and 26.11±8.02) (Figure 3B). A decline in percentage of entries or spent time into open arms displayed increased anxiety. Orally gavage of TC significantly increased only percentage of entries into open arms (50.00±0.00, p=0.004) on day 7 after neuropathy as compared with CCI group (Figure 3A). However, application of TC did not induce significant alteration in percentage of spent time into open arms on days 2 and 7 as compared with the CCI groups (Figure 3B).

The effects of hydro-alcoholic TC extract on oxidative stress-related markers

Our data analysis identified that CCI surgery significantly decreased the GSH level (8.32±0.80, P<0.05) and SOD activity (12.94±1.03, P=0.001) in the brain in CCI group on day 30 after CCI surgery as compared with sham group (11.28 ± 0.67 for GSH level and 29.17±1.53) (Figure 4A and 4C). However, CCI surgery could not significantly have changed the CAT activity and MDA level in the brain in CCI group on day 30 after neuropathy as compared with sham group (Figure 4B and 4D). Additionally, orally gavage of TC significantly increased GSH level in the brain (16.66 ± 0.90, p=0.001) as compared with sham and CCI group (Figure 4A). Furthermore, application of TC significantly decreased MDA level of brain in CCI+TC group (4.10±0.32) as compared with the CCI group (5.96±0.27) (Figure 4D).

The effects of hydro-alcoholic TC extract on protein expression of NGF and NF-kB of the brain

As shown in the Figure 5, CCI surgery significantly increased the protein expression of NGF (0.99 ± 0.02, p=0.001) and NF-kB (1.07 ± 0.05, p=0.001) in the brain as compared with sham group (0.12 ± 0.01 for NGF and 0.13 ± 0.03 for NF-kB). Interestingly, orally gavage of TC significantly decreased the protein expression of NGF (0.54 ± 0.01, p=0.001) and NF-kB (0.49 ± 0.06, p=0.001) in the brain as compared with CCI group (Figure 5).

Discussion

The major findings of the present study were as follows: (1) the sciatic nerve injury induce cold allodynia and anxiety-like behaviors, (2) the TC extract has analgesic and anxiolytic effects, (3) the analgesic effects of TC extract is mediated by downregulation of brain NGF and NF-κB protein expression following sciatic nerve injury in rats, (4) the analgesic effects of TC extract is also mediated by improvement of brain oxidative status via suppression of GSH and MDA content following sciatic nerve injury in rats. To our knowledge, this study is the first report of analgesic mechanism of TC extract in neuropathic pain. The current pharmacological approach has either limited efficacy or unacceptable complication. Several studies have indicated that antioxidant interventions are a particularly promising therapeutic option for chronic pain as far down as the cellular level [25]. One particular herbal remedy is fruit of TC because of its various types of constituents; including flavonoids, alkaloids, and glycosides. In traditional medicine, it has several pharmacological activities such as anti-cancer, anti-arthritis, anti-oxidant, anti-inflammatory, and cardiovascular protective [26]. In the present study, the extract of TC has both analgesic and anxiolytic effects up to 30 days after induction of neuropathic pain. Our study is in line with previous reports regarding analgesic effects of TC extract. For example, Seo and colleagues in 2012 have reported anti-arthritic and analgesic effect of NDI10218, a standardized ethanol extract of TC, on collagen-induced arthritis and visceral pain model in rat [27]. Hear, we assessed the possible analgesic mechanism of TC extract in chronic neuropathic pain. In the present study, we assessed whether the analgesic and anxiolytic effects of TC extract is mediated by alteration of brain NGF and NF-κB protein expression or improvement of oxidative stress following sciatic nerve injury in rats. Ample evidence points that oxidative stress, along with inflammation, constitute the physiopathological mechanisms for the initiation and maintenance of the chronic neuropathic pain. As shown in the Fig. 6, after nerve injury, it is widely accepted that excess generation of oxidative stress markers (such as reactive oxygen species, ROS) as well as pro-algesic and pro-inflammatory cytokines (such as NGF, NF-κB, and tumor necrosis factor-α, TNF-α), potentiate the release of each other by specific signaling pathways in a vicious cycle, result in nociceptor sensitization and neural apoptosis [28]. Although, NGF activates neurotrophic signaling via activation of TrkA, it can also activates apoptotic signaling via both TrkA and p75NTR. Aditionally, the NGF binding to p75NTR over-activates the expression of the transcription factor NF-κB [29]. Therefore, it is highly likely that the nerve injury-induced vicious cycle of oxidative stress and inflammation, can be pharmacologically modulated through suppression of NGF/ NF-κB signaling pathway. We observed that orally gavage of the TC extract decreased brain NGF and NF-κB protein expression following sciatic nerve injury in rats. Growing evidence points the link between NGF/ NF-κB pathway activity and allodynia. For example, Lippoldt and colleagues in 2016 have reported among pro-algesics markers to maintain chronic pain, only NGF and the glial cell line-derived neurotrophic factor family ligand (GFL) leading to cold allodynia after nerve injury [30]. Additionally, Sobeh and colleagues (2020) have been reported that sciatic nerve injury induced macrophages and Schwann cells activation, and also excess generation and release of pro-algesic cytokines, particularly NGF and NF-κB, which cause chronic pain and allodynia [31]. Furthermore, NGF have been found to induce cold allodynia in mice when administered by intraplantar injections [30].We further observed that TC extract improved brain oxidative status via decrease of GSH and MDA content following sciatic nerve injury in rats.

Conclusion

Sciatic nerve injury can up-regulate brain protein expression of NFG and NF-κB along with oxidative stress in rat. It is likely that analgesic and anxiolytic effects of TC extract are mediated by suppression of brain protein expression of NFG and NF-κB and oxidative markers following neuropathic pain in rats.

Declarations

Acknowledgements

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Author Contributions

Z.J. and M.H. carried out the study. M.J. interpreted the data, and drafted the manuscript. G.H.M. and Z.J. managed the data, carried out the statistical analyses, interpreted the data. M.J.B. revised the manuscript. Z.B. helped design the study and revised the manuscript. All authors have read and approved the final version of the manuscript and agree with the order in which the authors are listed.

Data availability

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no fnancial or non-fnancial confict of interest.

Research involving human and animal rights

The present study was an animal study. All experiments conducted in agreement with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and was approved by the local ethical committee of Baqiatallah University of Medical Sciences, Tehran, Iran (Ethical code: IR.BMSU.REC.1400.033).

References

1. Rezq S, Alsemeh AE, D'Elia L, El-Shazly AM, Monti DM, Sobeh M (2020) Thymus algeriensis and Thymus fontanesii exert neuroprotective effect against chronic constriction injury-induced neuropathic pain in rats. Sci Rep 10(1):1-5.
2. Cohen SP, Mao J (2014) Neuropathic pain: mechanisms and their clinical implications. BMJ 348:7656-7656.

3. Viswanath O, Urits I, Burns J, Charipova K, Gress K, McNally A (2020) Central neuropathic mechanisms in pain signaling pathways: current evidence and recommendations. Adv Ther 37(5):1946-1959.
4. Ahmed AS, Berg S, Alkass K, Druid H, Hart DA, Svensson CI (2019) NF-κB-associated pain-related neuropeptide expression in patients with degenerative disc disease. Int J Mol 20(3):658.
5. Dai WL, Yan B, Bao YN, Fan JF, Liu JH (2020) Suppression of peripheral NGF attenuates neuropathic pain induced by chronic constriction injury through the TAK1-MAPK/NF-κB signaling pathways. Cell Commun Signal 18(1):1-3.
6. Üçeyler N, Rogausch JP, Toyka KV, Sommer C (2007) Differential expression of cytokines in painful and painless neuropathies. Neurology 69:42-49.
7. Dos Reis RC, Kopruszinski CM, Nones CF, Chichorro JG (2016) Nerve growth factor induces facial heat hyperalgesia and plays a role in trigeminal neuropathic pain in rats. Behav Pharmacol 27(6):528-35.
8. da Silva JT, Evangelista BG, Venega RAG, Seminowicz DA, Chacur M (2019) Anti-NGF treatment can reduce chronic neuropathic pain by changing peripheral mediators and brain activity in rats. Behav Pharmacol 30(1):79-88.
9. Yang S, Huang Y, Ye Z, Li L, Zhang Y (2020) The efficacy of nerve growth factor antibody for the treatment of osteoarthritis pain and chronic low-back pain: a meta-analysis. Front Pharmacol 11:817.
10. Xu L, Liu Y, Sun Y, Li H, Mi W, Jiang Y (2018) Analgesic effects of TLR4/NF-κB signaling pathway inhibition on chronic neuropathic pain in rats following chronic constriction injury of the sciatic nerve. Biomed Pharmacother 107:526-533.
11. Zhao L, Tao X, Song T (2021) Astaxanthin alleviates neuropathic pain by inhibiting the MAPKs and NF-κB pathways. Eur J Pharmacol 912:174575.
12. Ahmed S, Naved A, Khan RA, Siddiqui S (2015) Analgesic activities of methanol extract of Terminalia chebula fruit. J Pharm Pharmacol 6(12):547.
13. Sireeratawong S, Jaijoy K, Khonsung P, Soonthornchareonnon N (2014) Analgesic and anti-inflammatory activities of the water extract from Terminalia chebula Rezt. Afr J Tradit Complement Altern Med 11(6):77-82.
14. Panunto W, Jaijoy K, Soonthornchareonnon N, Lertprasertsuke N, Sireeratawong S (2010) Acute and chronic toxicity studies of the water extract from dried fruits of terminalia chebula rezt in rats. Int J Appl Res 3: 36-43.
15. Gupta A, Chandra pal V (2018) Pharmacological evaluation of memory enhancing activity of aqueous extracts of Terminalia chebula (Haritaki) in rats. World J Pharm Pharm Sci 7(10):1608-1620.
16. Bennett GJ, Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33:87-107.

17. Mirasheh MH, Zohrehvand MR, Kazemi R, Bahari Z, Bahrami F, Jangravi Z (2020) The analgesic and anxiolytic activity of resveratrol mediated by different sub-types of α-adrenoceptors of anterior cingulate cortex following neuropathic pain in male rats. J Adv Med Biomed 28(129):183-190.
18. Akçali D, Belen AD, Babacan A, Bolay H (2017) Nitroglycerin challenge induces lateralized headache in nasociliary nerve-ligated rats: implications for chronic migraine. Turk J Med Sci 47:681-688.
19. Mei-Jing KO, Zhe XU, Yan-Yun LI, Yue-Yun LI, Yan LI, Jia-Xu CH (2018) Differentially expressed proteins in rat hippocampus after chronic immobilization stress and intervention using xiao yao san decoction. DCM 1(3):219-227.
20. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121-126.
21. Namadr F, Bahrami F, Bahari Z, Ghanbari B, Elahi SA, Mohammadi MT (2019) Evaluation of the effects of fullerene C60 nanoparticles on oxidative stress parameters in normal rats liver and brain. J Adv Med Biomed Res 27(124):8-15.
22. Yazdanparast R, Bahramikia S, Ardestani A (2008) Nasturtium officinale reduces oxidative stress and enhances antioxidant capacity in hypercholesterolaemic rats. Chem Biol Interact 172(3):176-184.
23. Moron MS, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. BBA 582(1):67-78.
24. Alomar SY, Gheit RE, Enan ET, El-Bayoumi KS, Shoaeir MZ, Elkazaz AY (2021) Novel mechanism for memantine in attenuating diabetic neuropathic pain in mice via downregulating the spinal HMGB1/TRL4/NF-kB inflammatory axis. Pharmaceuticals 14(4):307.
25. Kaushik AS, Strath LJ, Sorge RE (2020) Dietary Interventions for Treatment of Chronic Pain: Oxidative Stress and Inflammation. Pain Ther 1-12.
26. Afshari AR, Sadeghnia HR, Mollazadeh H (2016) A review on potential mechanisms of tTerminalia chebula in alzheimer's disease. Adv Pharmacol Sci 2016.
27. Seo JB, Jeong JY, Park JY, Jun EM, Lee SI, Choe SS (2012) Anti-arthritic and analgesic effect of NDI10218, a standardized extract of terminalia chebula, on arthritis and pain model. Biomol Ther 20(1):104.
28. Dai RP, Zhou XF (2014) Neurotrophins and Pain. In: Kostrzewa R. (eds) Handbook of Neurotoxicity. Springer, New York, NY.
29. Barker PA, Mantyh P, Arendt-Nielsen L, Viktrup L, Tive L (2020) Nerve growth factor signaling and its contribution to pain. J Pain Res 13:1223.
30. Lippoldt EK, Ongun S, Kusaka GK, McKemy DD (2016) Inflammatory and neuropathic cold allodynia are selectively mediated by the neurotrophic factor receptor GFRα3. PNAS 113(16):4506-4511.
31. Sobeh M, Mahmoud MF, Rezq S, Abdelfattah MA, Mostafa I, Alsemeh AE (2020) Haematoxylon campechianum extract ameliorates neuropathic pain via inhibition of NF-κB/TNF-α/NOX/iNOS signalling pathway in a rat model of chronic constriction injury. Biomolecules 10(3):386.

Figures

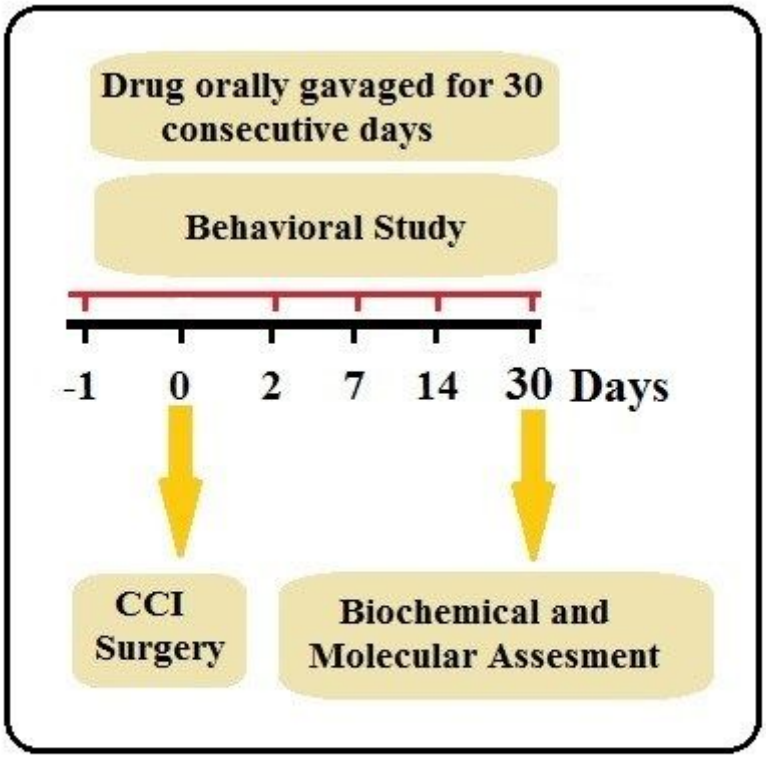

Figure 1

Figure 1

Timeline schematic of experimental paradigm. Baseline recordings of behavioral experiments were performed one day before CCI surgery (day -1). The neuropathic pain was induced by CCI surgery on day 0. Animals received aqueous-alcoholic extract of TC (40 mg/kg) for 30 days via gavage needles once a day. Behavioral experiments (cold allodynia and anxiety-like behaviors) were examined on days 2, 7, 14 and 30 following CCI surgery. Biochemical (Oxidative stress markers) and molecular (western blot) assessment were performed on day 30 following CCI. CCI; chronic constriction injury.

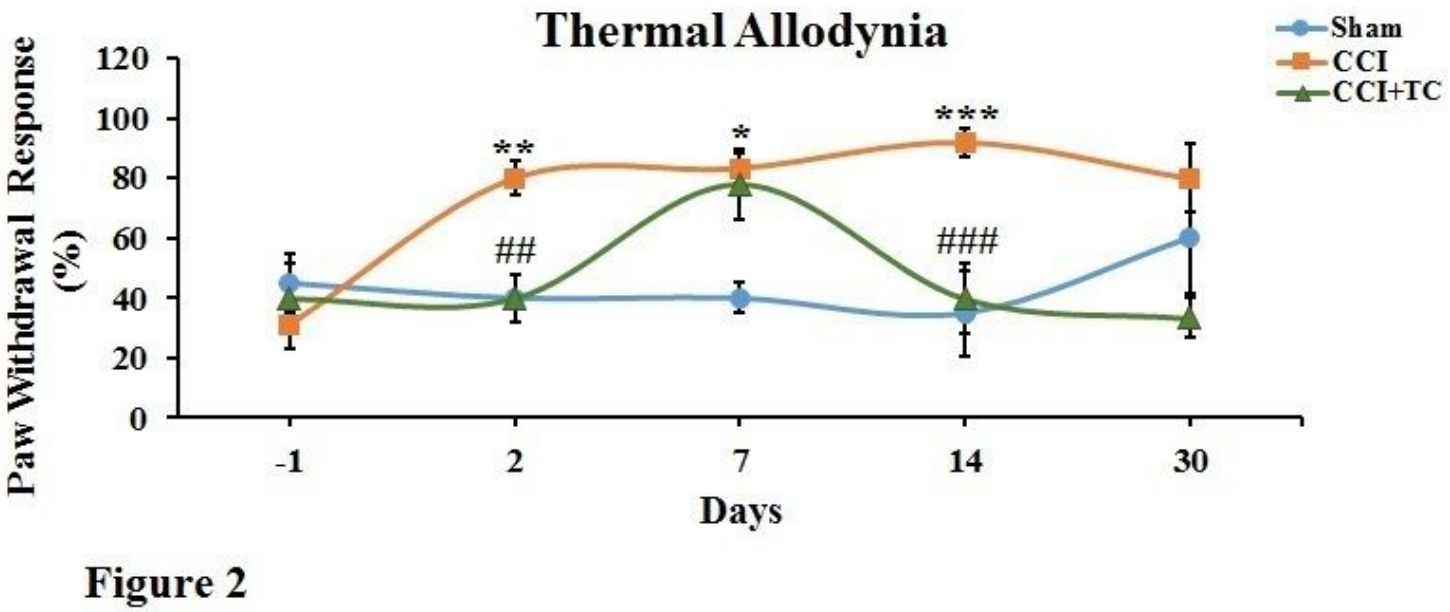

Figure 2

Figure 2

The effects of hydro-alcoholic TC extract on cold allodynia. Statistics were done with two-way ANOVA (for analysis of the interaction of group and time within groups) and one-way ANOVA (for between groups analysis). Values represent the mean±SEM (n=6). * $p<0.05$ as compared with sham group, ** $p<0.01$ as compared with sham group, *** $p=0.001$ as compared with sham group, ## $p<0.01$ as compared with CCI group, ### $p=0.001$ as compared with CCI group. CCI; chronic constriction injury, CCI+TC; chronic constriction injury+ Terminalia Chebula.

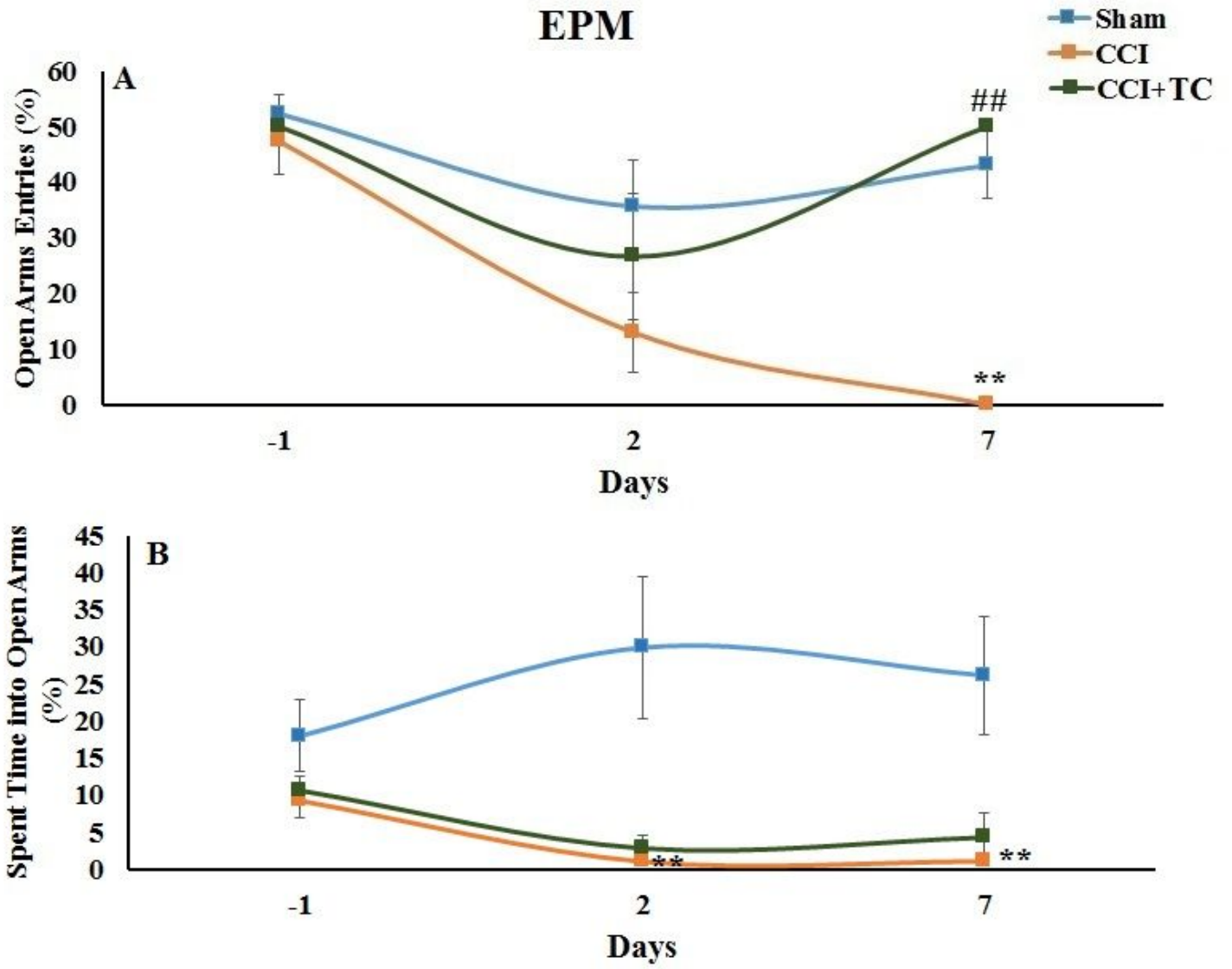

Figure 3

Figure 3

The effects of hydro-alcoholic TC extract on anxiety-like behaviors. Percentage of (A) open arms entries, and (B) spent time into open arms were evaluated as an anxiety index. The two-way ANOVA used to understand if there is an interaction effects between the two independent variables (groups and time) on the dependent variable (cold allodynia and anxiety like behaviors). Then, we use one-way ANOVA to compare mean difference of between groups, followed by the Tukey HSD post hoc test. **$p<0.01$ denote a significant difference with sham group. $^{\#\#}p<0.01$ denote a significant difference with CCI group. EPM: elevated plus maze; CCI; chronic constriction injury, CCI+TC; chronic constriction injury+ Terminalia Chebula.

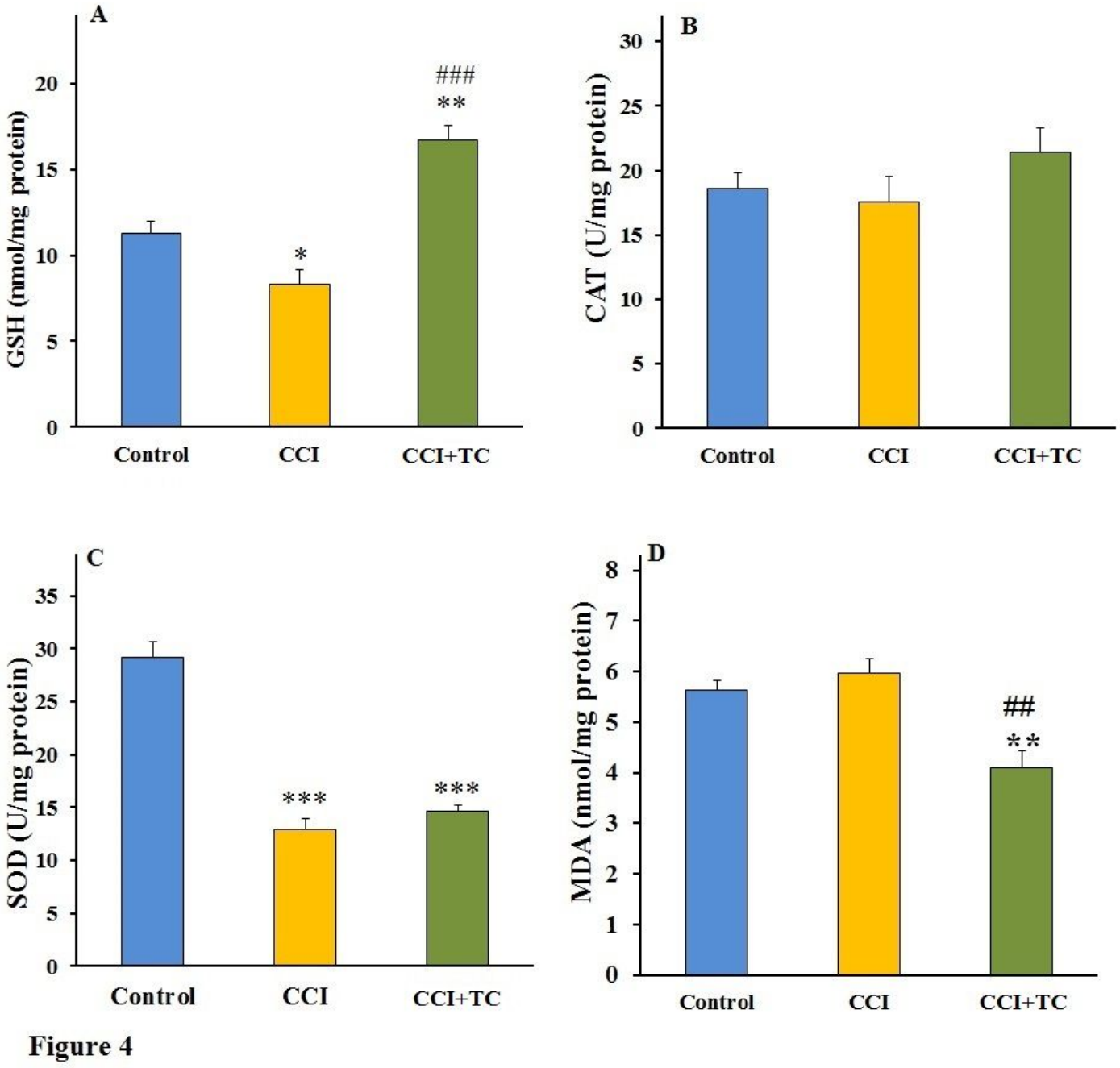

Figure 4

Figure 4

The effects of hydro-alcoholic TC extract on the oxidative stress related markers in the brain. (A) GSH content, (B) activity of CAT, (C) activity of SOD, and (D) MDA content in the brain on day 30 following CCI surgery after orally gavage of saline 9%, or TC extract. All data represent means ± S.E.M. (n = 5-6). Differences in measured parameters among 4 groups analyzed by using one-way ANOVA, followed by the Tukey post hoc test. The symbols * and # denote significant differences from sham and CCI groups, respectively; *P<0.05, **P<0.01, ***P=0.001, ##P<0.01, and ###P<0.001. GSH: glutathione; CAT: catalase;

SOD: superoxide dismutase; MDA: malondialdehyde; CCI; chronic constriction injury, CCI+TC; chronic constriction injury+ Terminalia Chebula.

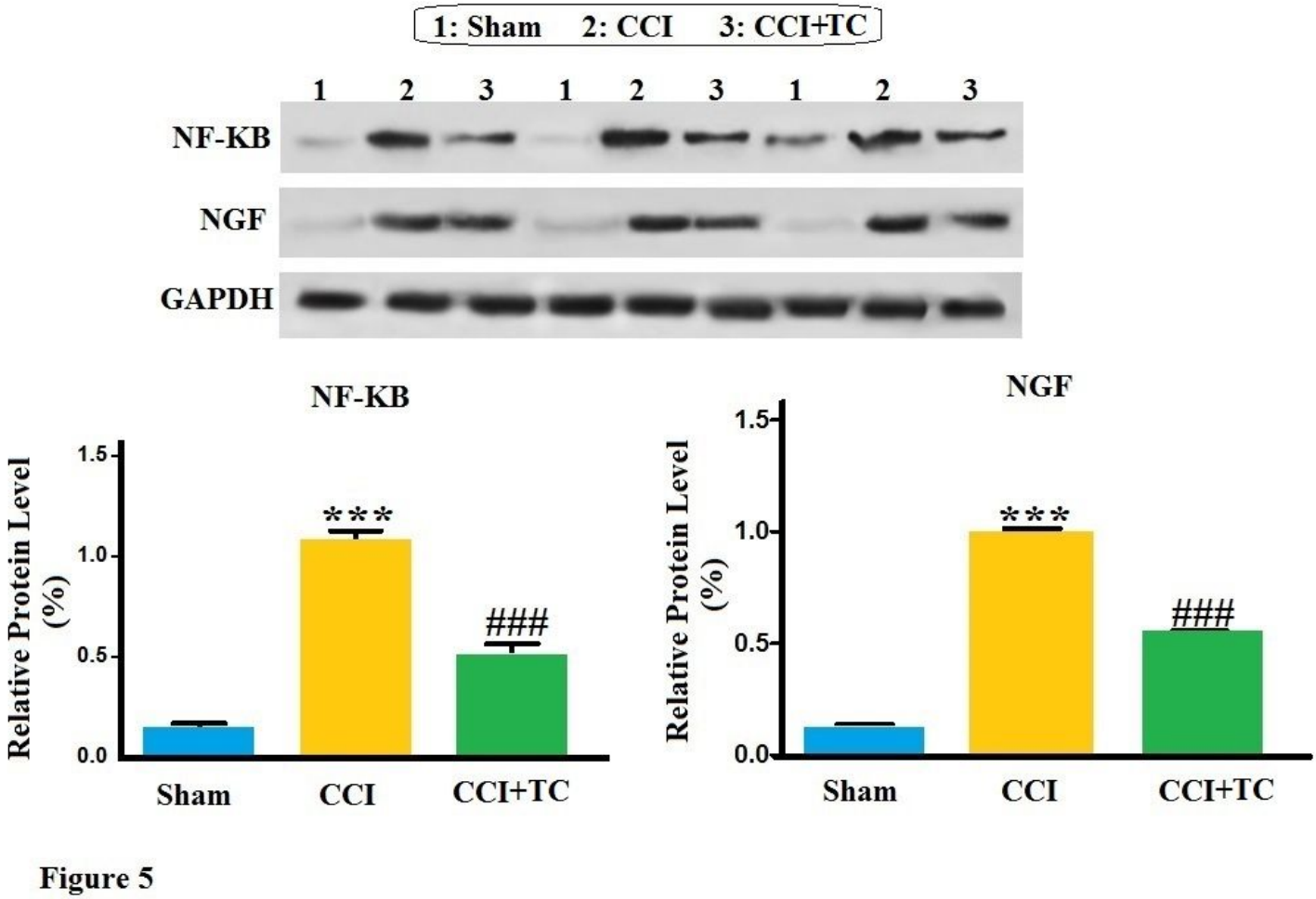

Figure 5

Figure 5

The effects of hydro-alcoholic TC extract on protein expression of NGF and NF-kB of the brain. (A) Representative images from NGF and NF-κB proteins by Western blotting analysis of brain in all experimental groups, (B) The respective graphical representation of their relative protein level in the brain. All samples were standardized to GAPDH which was used as loading control. ***$p<0.001$ denote a significant difference with sham group. $^{###}p<0.001$ denote a significant difference with CCI group. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; CCI; chronic constriction injury, CCI+TC; chronic constriction injury+ Terminalia Chebula.

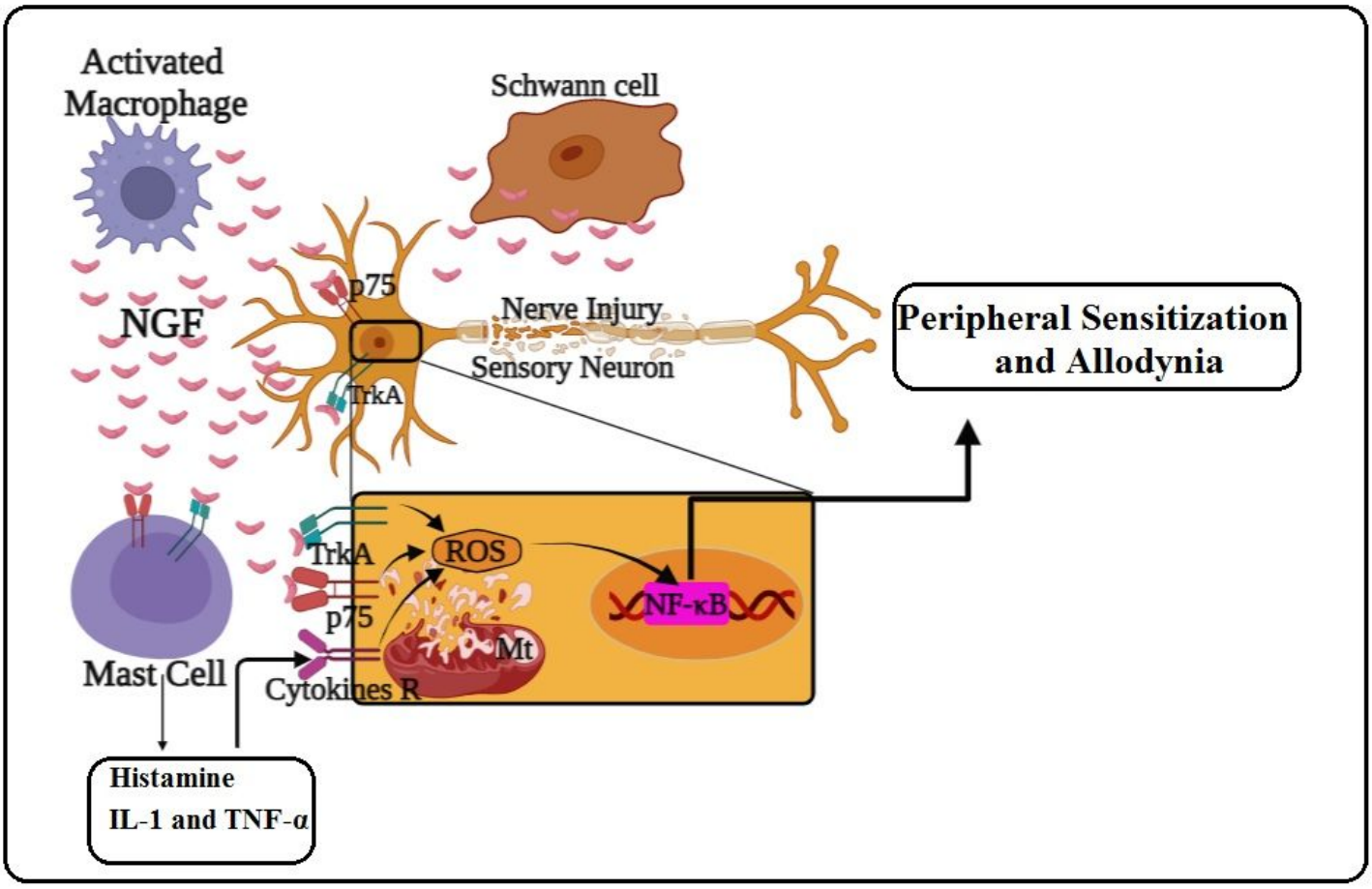

Figure 6

Figure 6

Nerve injury induced-allodynia. Nerve injury induces activation of peripheral Schwann cells, mast cells, and macrophages, leading to generation and release of NGF in the site of injury. Additionally, NGF can activate mast cells, leading to generation and release of different kinds of inflammatory markers; including TNF-α and IL-1. Furthermore, excess level of NGF induce bursting of mitochondria in the injured neuron, leading to high production of ROS and subsequently oxidative stress. Aditionally, high levels of the NGF, ROS, and also different types of cytokines, along with together, over-activate the expression of the transcription factor NF-κB in the sensory neuron. All of these events initiate peripheral sensitization, and allodynia. Furthermore, high levels of pro-algesic cytokines leading to propagating the inflammatory response over time, which induces central sensitization and chronic pain perception.