Neuroprotective Effect Of Musk On Rat Brain Ischemic Model

Radnaa Gochoo (radnaa2004@gmail.com)
Mongolian National University of Medical Sciences https://orcid.org/0000-0001-7017-3765
Oyuntsetseg Namsrai
Mongolian National University of Medical Sciences
Dagvatseren Begzsuren
Institute of Traditional Medicine and Technology
Bat-Erdene Jargalsaikhan
Institute of Traditional Medicine and Technology
Chimedragchaa Chimedtseren
Institute of Traditional Medicine and Technology

Research article

Keywords: rat, BDNF, TrkB, musk, brain ischemia

Posted Date: August 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-37442/v1

Abstract

Background

Stroke is leading cause of morbidity and mortality in worldwide. Despite valuable progresses in understanding neurological deficits after stroke, its therapeutic options are remaining limited. We aimed to study the neuroprotective effect of musk on cerebral ischemia/reperfusion injury model rats.

Methods:

In our experiment, we used 180 whore meal breed Wistar rats which weigh 180-220 g and divided these rats into 50 mg/kg of musk, 100 mg/kg of musk, 10 mg/kg of nimodipine, the ischemic-reperfusion groups by filling the midriff of the brain and take the drugs for 7 days in each group.

Cerebral ischemia/reperfusion was induced in rats by temporary middle cerebral artery occlusion-reperfusion (MCAO/R) followed by treatment with musk at 50 mg/kg and 100 mg/kg doses. On days 1, 3 and 7 after MCAO/R, TGF-β, BDNF, TrkB and NGF mRNA expressions in the rat brain tissue were quantitatively analyzed using RT-PCR.

Results:

Musk 50 and 100 mg/kg treated groups brain stroke size were significantly decreased compared with the experimental group at 1, 3 and 7 days. Moreover, brain BDNF, TrkB, NGF and TGF-β mRNA express were not significant difference between experimental and control group. Also 3^{rd} and 7^{th} day, the data indicate that Musk 50 and 100 mg/kg were significantly ($p<0,05$) effective increasing rats brain BDNF, TrkB, NGF and TGF- β mRNA express in rats with ischemic stroke induced by MCAO/R.

Conclusions:

The 50 and 100 mg/kg doses of musk lead to increase neuro-protective factors BDNF, TRkB, NGF and TGF- β expression of mRNA in ischemic-reperfusion rat model. It implies that the Mongolian musk supports the neurogenesis of neuronal cell.

Background

Cerebrovascular disease is common among the adult population around the world. Mongolia is considered to be one of the countries with high rate of cerebrovascular disease prevalence.[1]

In the world, Mongolia is in the second place by number of stroke mortality rate, (1026.63 person/per year) [2] and stroke incidence and death rate has been increased by 30% among working age group population in the last 20 years and this tends to increase further.[3,4]

There many studies that to develop new drugs, have been conducted with aim of declining the rate of stroke morbidity and mortality rate and decreasing ischemic reperfusion injury after the cerebral blood flow recovery treatment.[5,6,7]

Stroke is a disease that demon stemmed, brain blood vessels trembled and descent to white channels and severe progressive aforementioned in Mongolian Traditional Medicine,[8,9] and thousand years ago musk of musk deer has been used as the main ingredient to treat white channels disease in south east Asia (Pepeira, 1957).

Musk has relaxing and refreshing functions and therefore by its these natures, it is used for neural, cardiovascular, respiratory and sexual dysfunction (Kun-Ying Yen, 1992; Pharmacopoeia Commission of the Ministry of Public Health, 1996 and Zuh, 1989). Recent research studies show that musk has pain reliefing and anti inflammatory effects and this effect has confirmed with inhibition of the arachidonic acid byosynthesis (Cheng G, 1992).

Brain-derived neurotrophic factor (BDNF) was discovered in 1982, originally described as a small dimeric protein [10] BDNF is also considered to be potent modulator, beneficial to neurononal functions. Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophic factor in brain tissue and serves a role in inhibiting apoptosis, promoting neuronal survival, activating neural stem cells (NSCs) following ischemic brain injury, and regulating neural plasticity. BDNF belongs to a class of proteins that are synthesized by brain tissues and are widely expressed in the central nervous system (CNS); it serves a significant role in CNS development and neuronal growth and differentiation. [11,12]

BDNF is broadly expresses in the developing and adult mammalian brain, synthesized in several areas of hypothalamus, including the paraventricular, ventromedial and dorsomedial nuclei, as well as the lateral hypothalamic area. BDNF are believed to be stored or secreted from non-neuron cells, when attacks occur, such as human platelets. It was also found to be present in the ependimal, microglial and endothelial cells of cerebral arterioles and astrocytes, respectively. Peripherally, BDNF accumulates in the vascular endothelium, neuromuscular synapse, muscle and liver tissue, which is essential for neuronal repair when stroke occurs [13-16] Mature BDNF binds with high specificity to the tropomyosin-related kinase receptor type B (TrkB) and to the low-affinity neurotrophin receptor p75.[17] The Trk receptor tyrosine kinase family includes TrkA and TrkC, which are receptors for NGF and NT3, respectively. The family also includes TrkB, which mediates the effects of BDNF and NT. Similar to BDNF, TrkB is widely expressed in the adult brain, including the cortex, hippocampus, multiple brain stem and spinal cord nuclei. BDNF binding to TrkB triggers autophosphorylation of the tyrosine residue in its intracellular domain, leading to ligand-induced dimerization in each receptor, which activates several intracellular signalling pathways with various functions. NGF is a group of proteins that have an adaptive protective response against the pathological changes that occur during cerebral ischemia.

We aimed to study the neuroprotective potential of musk investigating the expressions of TGF-β, BDNF, TrkB, NGF mRNAs in rat brain tissue using MCAO/R (*Middle Cerebral Artery Occlusion/Reperfusion*–

MCAO/R) model.

Methods

Sample. The sample was taken from the musk deer *(Moschus Moschiferus* Linnaeus), which raised at the Musk Research station of The Institute of Traditional Medicine and Technology, located in the Shar Khooloi of Gachuurt by milking method.

The musk preparation was prepared out of dried musk, 96% ethanol, and dried coat liver in proportion of 1:5:49, respectively.

Then, by dissolving the prepared musk in carboxymethyl cellulose (CMC-Na-1%), a suspension was prepared for the study.

Subjects. Total of 180, adult, healthy, male, weighing 180-220 g Wistar rats were brought from the Shenjian animal breeding department of the Liáoníng province of the People's Republic of China and were used in the study.

During the experimental period, the rats were housed and maintained under consistent temperature (23 ± 1^0C) and humidity (55 ± 10%) on a 12-h light/dark cycle and food and water were provided *ad libitum*.

In vivo experimental cerebral ischemia/reperfusion model. Focal cerebral

Ischemia/reperfusion (IR) was induced in rats by occlusion of the MCA using the technique as described by Longa EZ et al. (1989) except for a normal group. The experimental group's rats were anaesthetized under 2% Isoflurane in a mixture of N_2O/O_2 (7:3) throughout the surgery. Focal cerebral ischemia was confirmed by the presence of characteristic behavioral deficits, including paralyzed forelimb flexion, torso twist, and spontaneous circling after reperfusion. All rats failed to meet this criterion were excluded from the study.

Treatment. The suspension that was prepared from musk was used in the study. Experimental animals were divided into 5 groups randomly; i.e. normal (n=30), model (n=30), musk 50 mg/kg, musk 100 mg/kg and nimodipine 10mg/kg (positive control). The medicines were given by orally after the IR in experimental groups for 7 days.

Quantification of RNA. The mRNAs expressions level of TGF-β, BDNF, TrkB and NGF were analyzed at the 1^{st}, 3^{rd} and 7^{th} days after the treatment.

The rats (each group n=8) were decapitated with cardiocentesis method, the brains were removed and then washed with PBS and 20 mg tissue taken from the left hemisphere were frozen immediately and stored at – 80ºC for use each times.

Total RNA was extracted from rat brain tissue with RNAprep Pure Tissue Kit (Tiangen Biotech Co.Ltd, Beijing, China) using specific primers (Supplemental 1) according to the manufacturer's instructions.

RNA extraction qualification was tested by gel electrophoresis as shown in Figure 1.

Statistics. Statistical difference between study groups was analyzed by Two way RM ANOVA followed by Tukey post hoc. Statistical significance was accepted at $p<0.05$ in Tukey test. The study data was expressed as mean ± standard deviation.

Results

The results of mRNA expression of BDNF

There was not revealed statistical significant difference in the mRNA expressions of BDNF between the normal and model at the first, third and seventh experimental days (Figure 2).

Also at the first day after MCAO/R modelling on rat brain, there was not statistical significant difference ($p<0.05$) between the experimental groups (musk 50mg/kg, musk 100mg/kg and nimodipine 10mg/kg).

On the other hand, there was significant statistical difference ($*p<0.05$) at the third and seventh days of the treatment in the mRNA expression of rat brain's BDNF among the groups of musk 50 mg/kg, musk 100 mg/kg and nimodipine 10mg/kg, which increased from 1.62 to 2.61.

The results of mRNA expression of TrkB

At the first, third and seventh days of the experiment, mRNA expression level of TrkB receptor was not changed significantly ($p>0.05$) between the normal and model groups.

Between experimental groups there was not difference at the first day while from the third day, there was an 1.75, 2.52 and 2.2 folded increase with statistical significance ($*p<0.05$) of mRNA expression level of TrkB receptor in the groups of musk 50mg/kg, musk 100 mg/kg and nimodipine 10mg/kg, respectively.

At the seventh day of the experiment, there was 2.0-2.79 folded increase with statistical significance ($*p<0.05$) in the mRNA expression of TrkB receptor in the groups of musk 50mg/kg and musk 100 mg/kg in comparison with the model group (Fig 3).

When comparing the nimodipine 10 mg/kg group with musk 100 mg/kg, mRNA expression level of TrkB receptor was 1.28 times lower with statistical significance ($^{\Delta}p<0.05$) than the musk group at the seventh day.

The results of mRNA expression of NGF

We analyzed mRNA expression level of NGF which increases nerve cell growth, at the first, third and seventh days after the MCAO/R modeling by RT-qPCR. There was no difference noticed ($p>0.05$) in the

mRNA expression of brain cells' NGF between normal and model groups at the three time points (Figure 4).

Between experimental groups, there was no significant difference at the 1st day.

When comparing musk 50mg/kg and the model group, brain NGF's mRNA expression were increased by 1.98 times and 2.34 times with statistical significance (*p<0.05) at the 3rd and 7th days, respectively.

Moreover, musk 100 mg/kg dose treatment increased the level of brain NGF's mRNA expression by 2.21 times and 2.84 times with statistical significant difference (*p<0.05) at the 3rd and 7th days, respectively.

When comparing the group of nimodipine 10mg/kg with model group, brain NGF's mRNA expression was increased by 1.8-2.3 times with statistical significance (*p<0.05) at the 3rd and 7th days, respectively.

And comparing the groups of musk 50 mg/kg, musk 100 mg/kg and nimodipine 10mg/kg there was no difference at the first and third days. But, musk 100 mg/kg group's mRNA expression of brain NGF was higher than the group of nimodipine 10mg/kg with the statistical significance ($^{\Delta}p$<0.05) at the 7th day.

The results of mRNA expression of TGF-β

mRNA expression level of TGF-β was analyzed by RT-qPCR from rat brain tissue, among the study groups. There was not change (p>0.05) in the TGF-β mRNA expression between normal and model groups' at the 1st, 3rd and 7th days (Figure 5).

Between experimental groups there was no difference in TGF-β's mRNA expression at the 1st day. Then at the third day of the experiment, comparing the groups of musk 50 mg/kg, musk 100 mg/kg and nimodipine 10 mg/kg with the model group, there was a significant 1.56-2.17 folded increase of the TGF-β's RNA expression, comparing the model group with the experimental medicine groups.

At the 7th day of the study, when comparing the groups of musk 50 mg/kg and musk 100 mg/kg with model group, the brain TGF- β mRNA expression increased by 2.38-3.36 times significantly (*p<0.05). Also, when comparing the groups of musk 100 mg/kg and nimodipine 10 mg/kg, mRNA expression of brain TGF- β increased by 1.71 times significantly ($^{\Delta}p$<0.05), at 7th day of the study.

Conceiving occlusion reperfusion ischemic stroke in rat brain, it was treated by musk suspension with different doses and compared the results with normal, model and between medicine groups.

Then we obtained results that rat brain BDNF, NGF, TrkB and TGF- β receptors mRNA expression level were increased with statistical significant difference at the third and seventh days of the study.

Discussions

There is an assumption that cerebrovascular disease, especially the stroke will become the second most leading cause of death by 2030.[18]

Therefore, treatment and prevention of stroke has become one of the vital issue not only in medical science but also in socio-economic sector. Our study findings demonstrate that the musk is effective during ischemic-reperfusion model in way of fostering nerve cell growth factors.

Ischemic-reperfusion model was created by Longa EZ (1989) method[19] on rat brain and neuroprotective effect of 10% musk suspension was evaluated on rat brain cells mRNA expression level of BDNF, TrkB, NGF and TGF-β proteins by Real-time reversed transcription polymerase chain reaction (RT-qPCR) method.

BDNF protein connects with TrkB receptor in a unique way and also binding with p75 receptor by low affinity. [17,20]

TrkB has the function to protect brain cells, via activating following three ferments; MARK, PI3K, PLCγ and triggers the three signal transduction. [21-23]

NGF is a neurotrophic cluster protein, which has neuro cells protection reaction, during brain ischemic. NGF has nerve cells protection effect, through connecting with TrkA receptor in a unique way.[24]

When comparing the groups of musk 50 mg/kg and musk 100 mg/kg with model group, the mRNA expression of BDNF and NGF increased.

The results show that oral administration of musk 50 mg/kg and 100 mg/kg, BDNF-TrkB's neuro cells signal transduction and mRNA expression of NGF are improved, which further explains that musk has effect of nerve cells protection, anti-inflammation, against apoptosis, which supports nerve cells' survival and preventing nerve poisoning.

Moreover, our study results prove that the above function of the musk has the most efficient effect in the third and seventh day of the treatment.

Specially, in the group of musk 100 mg/kg, BDNF-TrkB and NGF are increased by 1-2 times, comparing to the group of musk 50 mg/kg. This explains that we need to do more detailed study on musk dose.

The research studies of Kowianski P and Miao JT (2018), shows that BDNF has important function in rehabilitation of nerve function and sustaining neuron structure.[11,25]

There is a similar result of study on Shinjian Tonshuan pill for reducing thrombosis, obstructing aggregation, improving brain blood circulation, which has the ingredients of musk, saffron, and ginseng etc. in the Chinese traditional medicine.[26]

Researchers Khaidav Ts (2000) Injection of musk 25-100 мг/кг and muscone 0.02-0.5 мг/кг into mice visceral cavity and administration of musk 200 мг/кг and muscone 5 мг/кг orally to rat, it shows the reaction of shortening the duration of sleeping period, which triggered by phenobarbital, in both cases. However, high dose of musk (100-1000 мг/кг) lengthened the duration of sleeping period, which triggered by phenobarbital. This explains that the musk has two directions effect in central nervous system. In other words, less dose of musk has refreshing effect, contrary high dose of musk has retarding effect on CNS.[26]

By contemporary, Sampilnorov pill has been investigated and revealed that the has nerve cells protection function, during IR in rat model, by improving interrelated NTFs (Neurotrophic factors) such as BDNF (brain derived neurotrophic factor), NGF (nerve growth factor), GDNF (glial cell-derived neurotrophic factor), bFGF (Basic fibroblast growth factor- basic fibrablast growth factor), TGF-β (transforming growth factor β) and PDGF (platelet-derived growth factor – thrombosis related growth factor). Also, the Sampilnorov pill medicine has the effects of increasing IGF-1 (Insulin-like growth factor 1), reducing cerebral infarction, raising a volume of Nogo-A etc. in the occlusion reperfusion ischemic stroke modelling rats.[27-31]

Researchers Jiang T et.al. (2016) studied muscone effects as nasal nebulizer on IR model. The study results showed that the brain tissue's BDNF and NGF rate has reduced in control group, while in the group of rats which had treatment of muscone, brain swelling declined and BDNF, NGF rate has increased. Moreover, it proves the similar results with our research study that the muscone has increased the BDNF and NGF exertion.[32]

The effect of the results of our study on the increase of neuroprotective factors in the brains of animals in experimental musk can be considered in connection with its main active ingredient, muscone. This is because the following studies have shown that musculature penetrates the blood-brain barrier. Researchers such as Chen WK (2004) injected musk into a rat's tail vein and chromatographed the amount of musk contained in the brain and other organs, revealing high levels in the brain, suggesting that musk penetrates the blood-brain barrier.[33] In his research, Wang GY (2015) concluded that muscone reduced the ability of P-gp efflux and inhibited the MMP-9 on the decomposition of the basal lamina of the blood-brain barrier. Muscone can alter the permeability of BBB model in vitro, which is related to reduce the expression of P-gp and MMP-9.[34]

Investigating the musk effects in protecting nerve cells from apoptosis in cerebral ischemia/reperfusion model of rat, the results have provided great opportunity to use musk in stroke treatment.

Conclusion

We conclude this study, reported that musk of musk deer at 50mg/kg and 100 mg/kg doses increased the BDNF, NGF, TGF-β neuroprotection factors and RNA expression of the TrkB rreceptor, which indicates that the Mongolian musk has the beneficial effect to support the neurogenesis of brain cells. This protective

effect may have related to the reduction of oxidative stress, inhibition of NO production and anti-inflammation.

Abbreviations

MCAO/R: Middle cerebral artery occlusion-reperfusion; TGF-β: Transforming growth factor beta; BDNF: Brain-derived neurotrophic factor; TrkB: Tropomyosin receptor kinase B; NGF: Nerve growth factor; RT-PCR: Real time polymerase chain reaction; mRNA: Messenger RNA; IR:Ischemia/reperfusion; NSCs: Neural stem cells; CNS: Central nervous system;

Declarations

Acknowledgements

We would like to express my sincere gratitude to the International School of Mongolian Medicine, MNUMS, Institute of Traditional Medicine and Technology, Inner Mongolia University for Nationalities for providing us with the laboratory to conduct experimental research.

Authors' contributions

R.G. designed the study and carried out the experiment by collecting data and analyzing the results to the writing of the manuscript. B.J. assisted with drafting and revised the manuscript with input from all authors. C.C. and O.N. supervised the study. D.B. worked as a advisor. All authors read and approved the final manuscript.

Funding

This research done within the framework of Comparative research of reproduction and genetics of Mongolian and Chinese musk deer joint-project funded by Science and Technological Fund of Mongolia.

Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animal study was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. Formal approval to conduct the experiments was obtained from the Ethical Committee of the Mongolian National University of Medical Sciences (Protocol No.2018/3-11). All efforts were made to minimize the number of animals used and their suffering.

Consent for publication

All authors have approved of the manuscript and agree with its submission

Competing interests

The authors declare that they have no competing interests.

Reference

1. Tsagaankhuu G, Delgermaa Ts. Clinical Neurology. Ulaanbaatar: Admon Printing; 2019. p.328-384.
2. worldlifeexpectancy.com/ cause-of-death/stoke/by- country/.
3. Baasanjav D. Stroke Epidemiology Surveys in the Population of Ulaanbaatar: Trend for the last 30 years, Ulaanbaatar, 2004. p.1-81
4. Baasanjav D. Сосудистые заболевания мозга в Монголии: (эпидемиологическое исследование) Дисс. Докт. Наук, Москва, 1993.
5. Munkhtuya Ts. The Pharmacologycal study of Spiraea aqiolegifolia. A Monograph for a Doctoral Dissertation. Ulaanbaatar. MNUMS; 2009
6. Uranchimeg D. The structure of the Mongolian spleen, age characteristics of vascularity. A Monograph for a Doctoral Dissertation. Ulaanbaatar. MNUMS; 2009
7. Tulguur R. Pharmacology study of traditional compound “Renchinnida”. A Monograph for a Doctoral Dissertation. Ulaanbaatar. MNUMS; 2016
8. Seesregdorj S, Chimedragchaa Ch, Khishigjargal S, Tserendagva D, Chuluunchimeg B. The healing power of traditional Mongolian medicine. Ulaanbaatar. 2005.p. 295-304
9. Tumbaa Kh. Medical four Tantra. Ulaanbaatar. 1991.p.39-47
10. Lessmann V and Brigadski Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. *Neuroscience Research.* 2009;65(1):11-22
11. Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A, Moryś J. BDNF: a key factor with multipotent impact on brain signaling and synaptic plasticity. Cellular and molecular neurobiology. 2018;38(3):579-593.
12. Li ZC, Jia YP, Wang Y, Qi JL, Han XP. Effects of dexmedetomidine post- treatment on BDNF and VEGF expression following cerebral ischemia/reperfusion injury in rats. Molecular medicine reports. 2018;17(4):6033-6037.
13. Tapia-Arancibia L, Rage F, Givalois L, Arancibia S. Physiology of BDNF: focus on hypothalamic function. Frontiers in neuroendocrinology. 2004;25(2):77-107.
14. Yamamoto H, Gurney ME. Human platelets contain brain-derived neurotrophic factor. Journal of Neuroscience. 1990;10(11):3469-3478.
15. Béjot Y, Prigent-Tessier A, Cachia C, et al. Time-dependent contribution of non neuronal cells to BDNF production after ischemic stroke in rats. Neurochemistry international. 2011;58(1):102-111.

16. Cassiman D, Denef C, Desmet VJ, Roskams T. Human and rat hepatic stellate cells express neurotrophins and neurotrophin receptors. Hepatology. 2001;33(1):148-158.
17. Massa SM, Yang T, Xie Y, et al. Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. The Journal of clinical investigation. 2010;120(5):1774-1785.
18. Organization WH. Global status report on noncommunicable diseases 2014. World Health Organization;2014.
19. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. stroke. 1989;20(1):84-91.
20. Teng HK, Teng KK, Lee R, et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. Journal of Neuroscience. 2005;25(22):5455-5463.
21. Zampieri N, Chao M. Mechanisms of neurotrophin receptor signalling. Portland Press Ltd.; 2006.
22. Skaper SD, Floreani M, Negro A, Facci L, Giusti P. Neurotrophins rescue cerebellar granule neurons from oxidative stress-mediated apoptotic death: selective involvement of phosphatidylinositol 3-kinase and the mitogen- activated protein kinase pathway. Journal of neurochemistry. 1998;70(5):1859- 1868.
23. Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. Annual review of biochemistry. 2003;72(1):609-642.
24. Numakawa T, Suzuki S, Kumamaru E, Adachi N, Richards M, Kunugi H. BDNF function and intracellular signaling in neurons. Histology and histopathology. 2010.
25. Li ZC, Jia YP, Wang Y, Qi JL, Han XP. Effects of dexmedetomidine post- treatment on BDNF and VEGF expression following cerebral ischemia/reperfusion injury in rats. Molecular medicine reports. 2018;17(4):6033-6037.
26. Chimedragchaa Ch. Musk deer and study of musk. Ministry of Education, Culture, Science and Sports. Institute of Traditional Medicine and Technology. Ulaanbaatar 2019.
27. [illegible]. [illegible] MCAO/R [illegible]. 2014.
28. [illegible]. [illegible]. 2014:29:2596-2598.
29. [illegible]. [illegible]. 2013.:238-239.
30. [illegible]. [illegible] Nogo-A [illegible]. 2015.
31. [illegible], [illegible]. [illegible] c-fos [illegible] 2013:136-140.
32. Jiang T, Huang LF, Zhou SJ, Cui JJ, Ye Q. Brain Protection of Muscone in Rats with Brain Injury. Chinese journal of integrated traditional and western medicine. 2016 Jun; 36 (6):724-8.
33. Chen WK, Huang YF, Wang HD. An experimental study on distribution of musk into the brain through blood brain barrier. Zhong Xi Yi Jie He Xue Bao. 2004 Jul;2(4):288-91.
34. Wang GY, Wang N, Liao HN. Effects of Muscone on the Expression of P-gp, MMP-9 on Blood-Brain Barrier Model in Vitro. Cell Mol Neurobiol. 2015 Nov;35(8):1105-15. doi: 10.1007/s10571-015-0204-8. Epub 2015 May 15

Figures

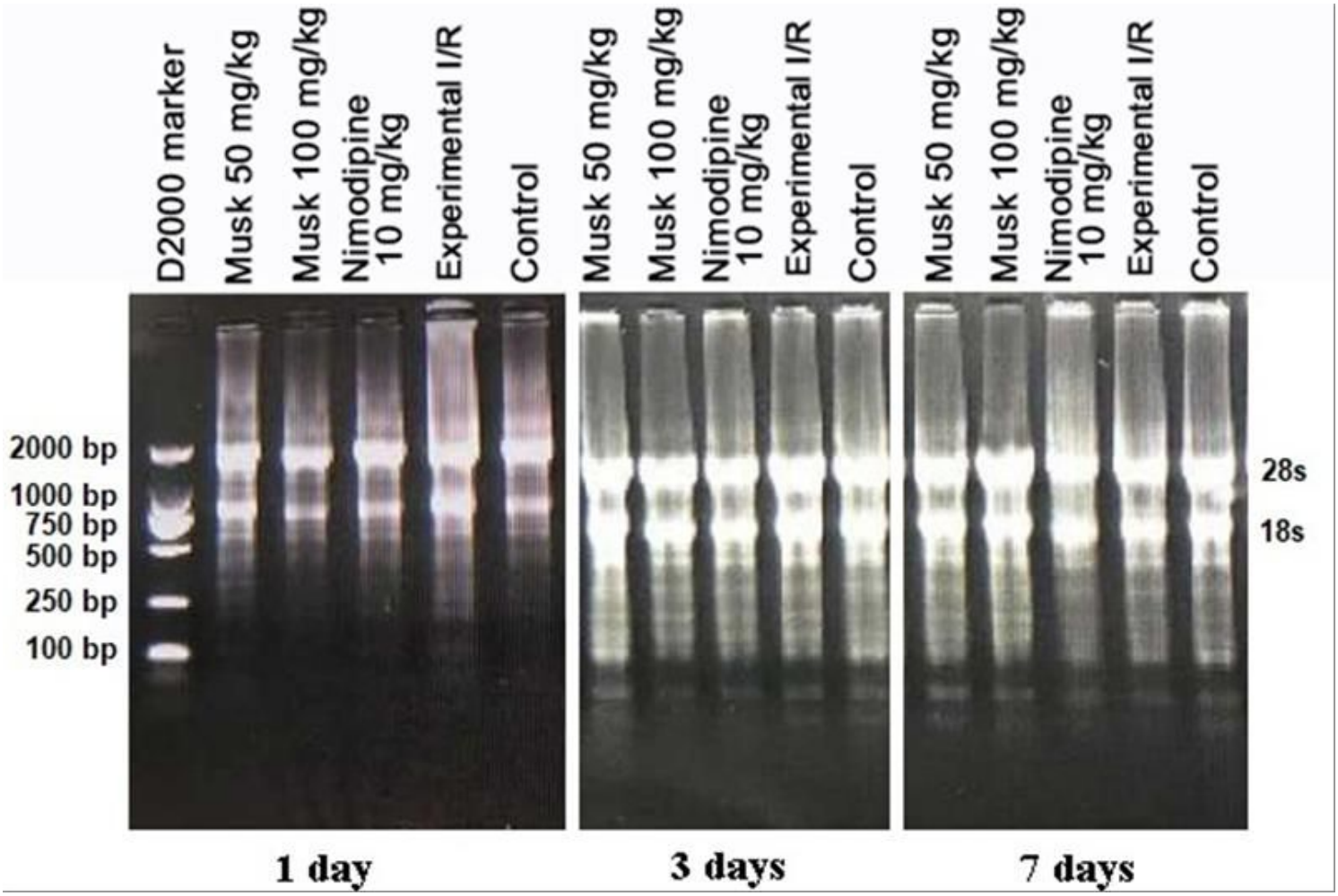

Figure 1

RNA gel electrophoresis Note: At the 1st, 3rd and 7th days of the experiment 150 voltage electricity transmitted in the 1.3% agarose gel and TBA buffer solution environment for 15 min and 28s pRNA, 18s pRNA is separated by standard D2000 RNA in comparison with Fusion FX ultra-ray. cDNA was created out of 50 ng of total RNA using FastQuant RT Kit with gDNAse (Tiangen Biotech Co.Ltd, Beijing, China) as per protocol. Real-time quantitative reverse transcription–PCR was performed using the SuperReal PreMix Plus (SYBR Green No.FP205) (Tiangen Biotech Co.Ltd, China) and specific primers.

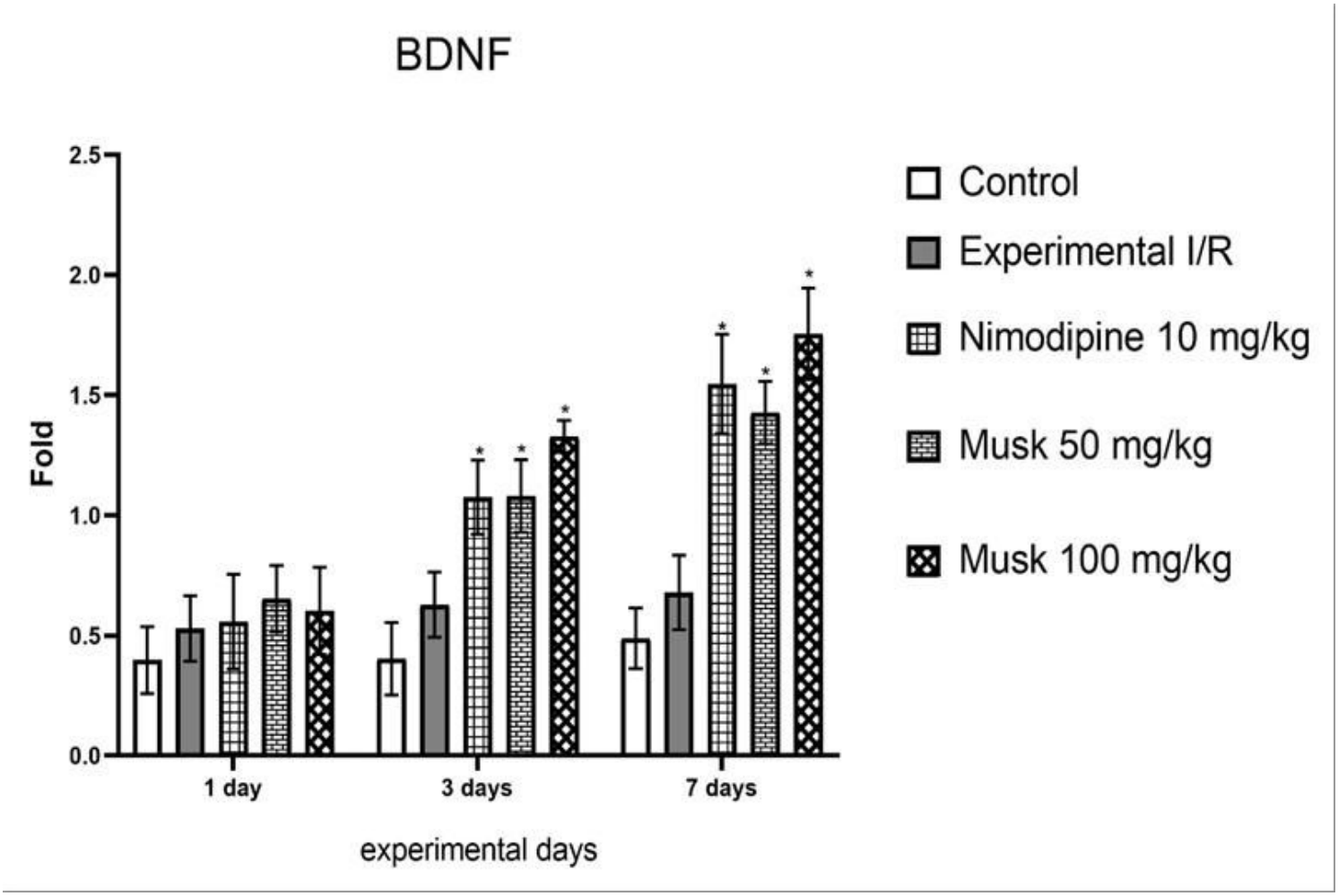

Figure 2

The effect of musk in the mRNA expression of rat brain's BDNF Note: *p<0.05 vs model; Two-way RM ANOVA post hoc Tukey

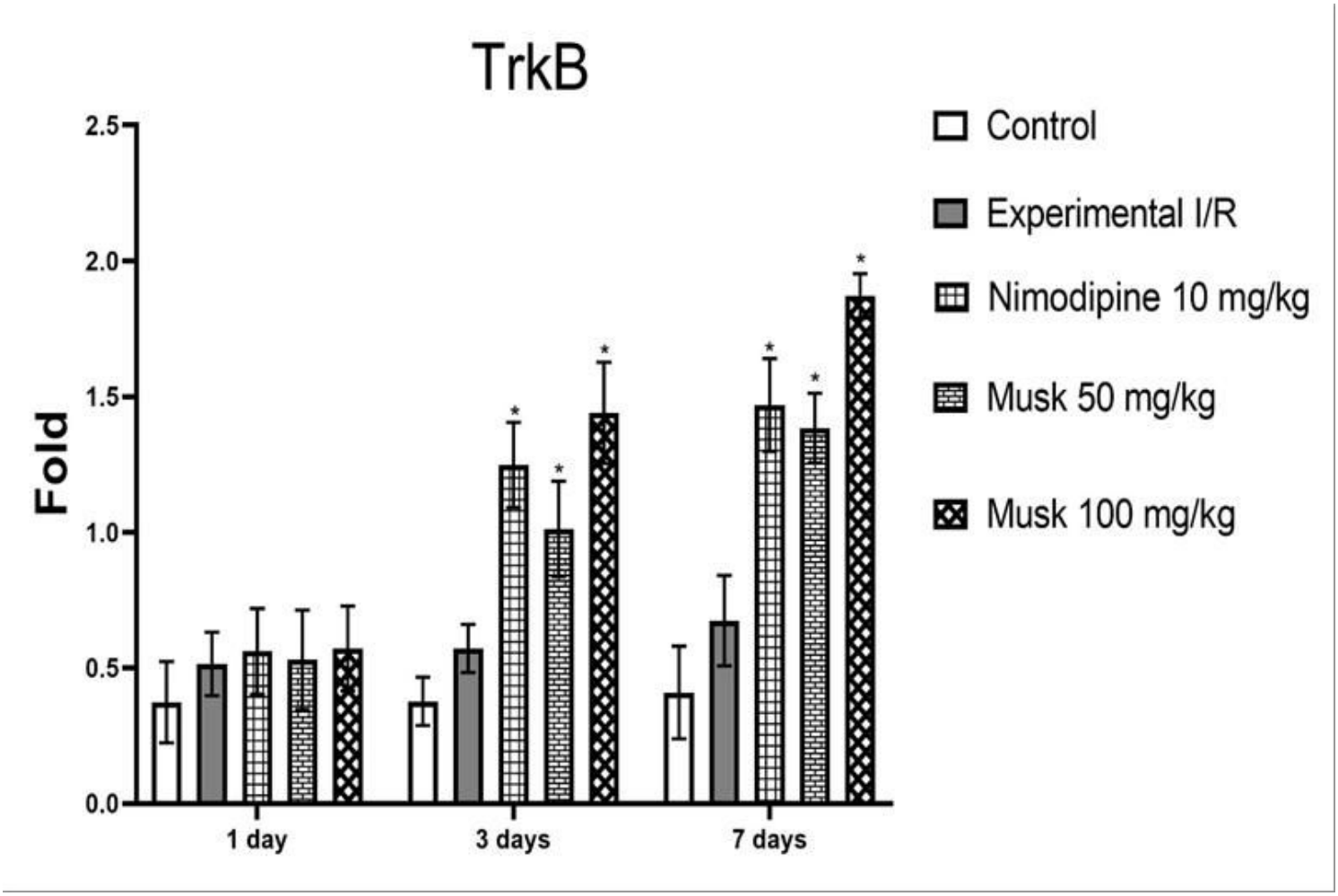

Figure 3

Effect of musk in mRNA expression of brain TrkB receptor Note: *p<0.05 vs model group; Two-way RM ANOVA post hoc Tukey

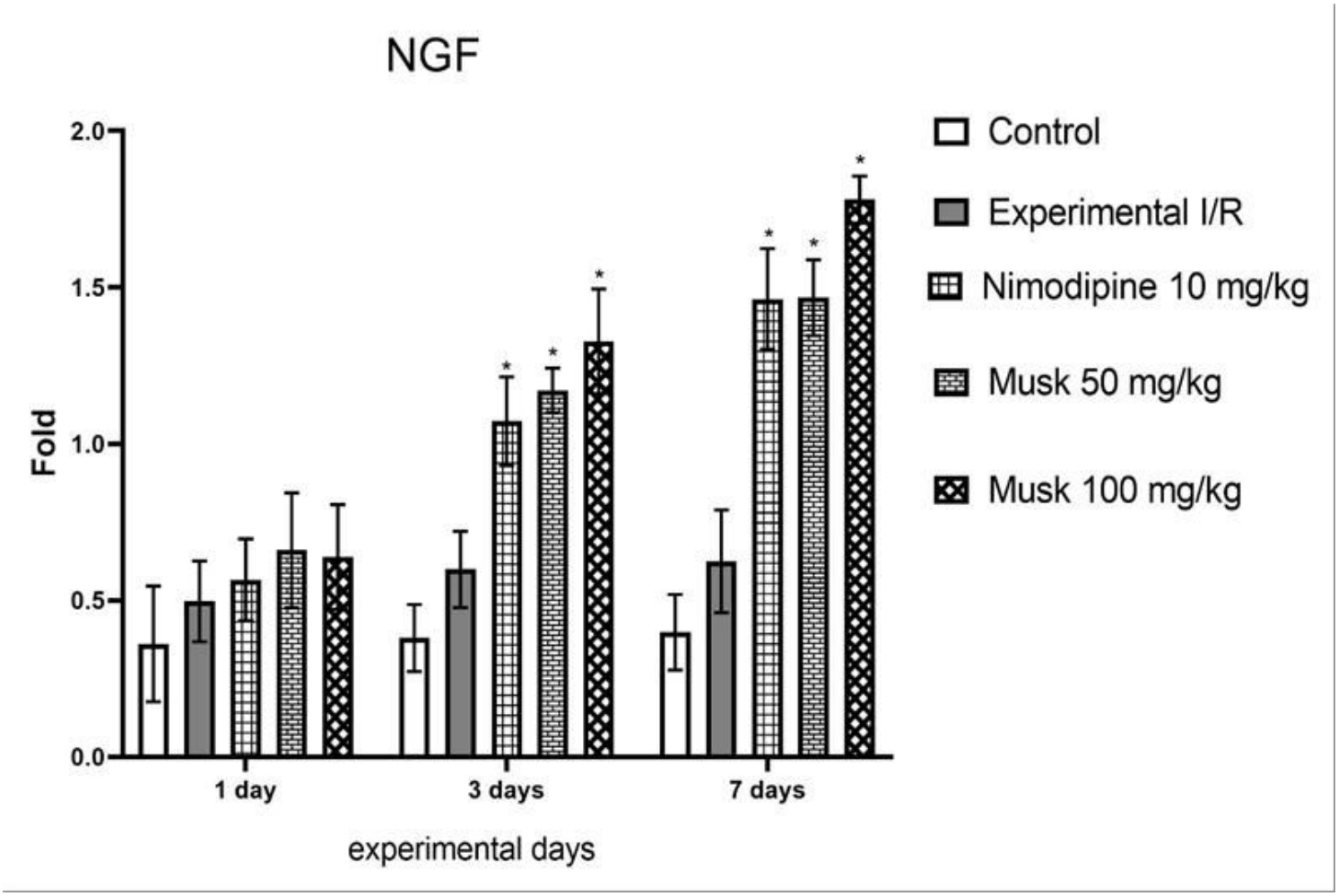

Figure 4

Effect of musk in mRNA expression of brain NGF Note: *p<0.05 vs model group; Two-way RM ANOVA post hoc Tukey

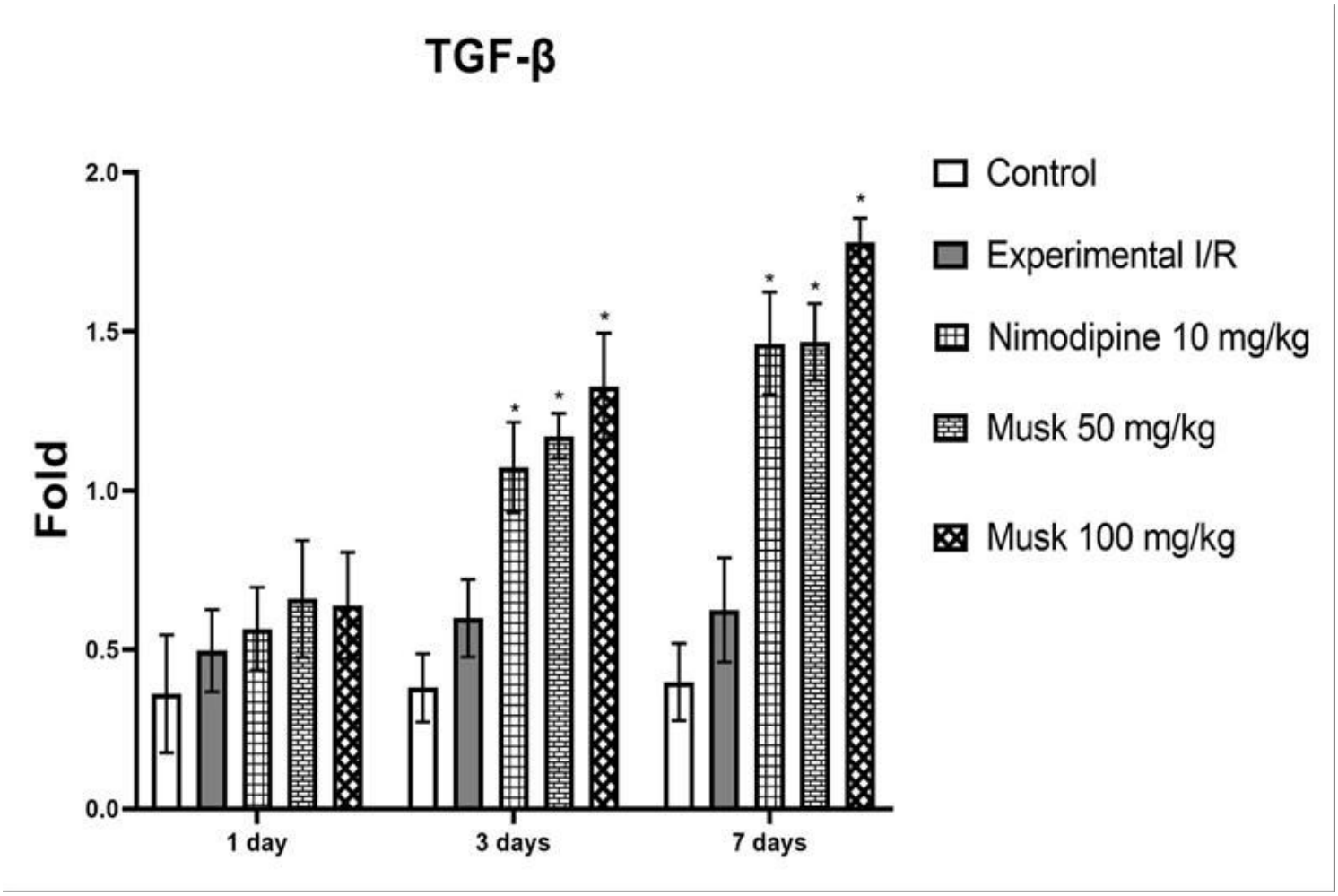

Figure 5

Effect of musk in mRNA expression of brain TGF-β Note: *p<0.05 vs model group; Two-way RM ANOVA post hoc Tukey