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ORIGINAL ARTICLE

Bee Venom–Derived BBB Shuttle and its Correlation with Oligodendrocyte Proliferation Markers in Mice Model of Multiple Sclerosis

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Abstract

Multiple sclerosis is a chronic demyelinating disease with a functional disturbance in the immune system and axonal damages. It was shown that Apamin as a blood–brain barrier shuttle acts as a Ca2+ activated K+ channels (SK channels) blocker. In this study, the effects of Apamin on oligodendrocyte differentiation markers were evaluated on an induced model of MS. Briefly, C57BL/6 male mice (22 ± 5 g) except the control group were fed with 0.2% (w/w) cuprizone pellets for 6 weeks. After cuprizone withdrawal, mice were divided randomly into six groups. Apamin (100 µg/kg/BW) was administered intraperitoneally as a co-treatment during phase I (demyelination) or post-treatment phase II (remyelination) twice a week. Mice were anesthetized, perfused with phosphate-buffered saline, then fixed brains were coronally sectioned and the changes in oligodendrocytes markers such as Olig2, PDGFR-α, and BrdU incorporation were assessed by immunohistochemistry assay. Apamin administration increased Olig2+ cells in phase I as compared to the control group ($p < 0.0001$). Also, a decreasing trend in PDGFRa+ cells observed after cuprizone withdrawal ($p < 0.001$). 5-Bromo-2′-deoxyuridine (BrdU) incorporation test was confirmed stimulation of oligodendrocyte progenitor cell proliferation in phase I in the Apamin exposed group ($p < 0.0001$), especially at the subventricular zone. This study highlights the potential therapeutic effects of Apamin as a bee venom–derived peptide on oligodendrocyte precursor proliferation and elevation in myelin content in an oxidative induced multiple sclerosis model due to cuprizone exposure.

Keywords Cuprizone · Apamin · PDGFR- α · Olig-2 · OPC · Myelin · Immunohistochemistry

Introduction

Multiple sclerosis (MS) is a demyelinating disease that causes chronic inflammation in the central nervous system (Olsson et al. 2017). MS especially affects young adults and is specified by lots of inflammatory cells with demyelinated lesions in the white matter (Lassmann et al. 2007). MS is widely spread all over the world from higher prevalence regions such as North America and Europe (> 100/100,000 population) to low prevalence in Eastern Asia and sub-Saharan Africa (2/100,000) (Leray et al. 2016; Aliomrani et al. 2019; Aliomrani et al. 2017).

The pathophysiology of MS is related to a functional disturbance in the immune system which leads to axonal damage (Gold and Wolinsky 2011). Although the exact mechanism of disease has not been determined yet (Greenstein 2007), as oligodendrocytes are involved in repairing and regenerating myelin in MS lesions (Reynolds et al. 2001),

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it is likely that failure of myelin restoration of chronically demyelinated neurons caused by the lack of sufficiently formed new oligodendrocytes (Dawson et al. 2000).

Oligodendrocyte progenitor cells (OPCs) encompass around 3–4% of cells in the grey matter and 7–8% in the white matter which is the fourth largest subtype of glial cells after astrocytes, microglia, and oligodendrocytes. OPCs are differentiated to adult oligodendrocytes and provide axonal remyelination by insolation with a myelin sheath. These are highly migratory and proliferative cells characterized by expression of the various markers including platelet-derived growth factor receptor A (PDGFRα), Chondroitin sulfate proteoglycan 4 (CSPG4), and ion channels. With this regard, it was proved that the oligodendrocyte transcription factor (OLIG2), a basic helix-loop-helix (bHLH) transcription factor encoded by the Olig2 gene, acts as a neurogenic factor and help OPC differentiation during the remyelination procedure.

In recent years, the use of bee venom has become common as a medicinal purpose (Hauser et al. 2001). Bee venom is a clear liquid that is soluble in water with a pH of around 5 to 5.5 (Eze et al. 2016). It contains various compounds such as peptide components, enzymes, biogenic amines, and phospholipids. Melittin, the most prevalent substance of bee venom, acts as an anti-inflammatory and cytotoxic peptide (SEYED et al. 2009). Apamin is the second most common peptide in bee venom (2–3% of dry bee venom) which is characterized by its neural effects because of the ability to cross the blood–brain barrier (Shimpi et al. 2016; Paknejad et al. 2019; Eze et al. 2016; SEYED et al. 2009). It selectively blocks axonal potassium channels named SK channels, a type of Ca2+ activated K+ channels that are especially expressed in CNS (Shimpi et al. 2016). Furthermore, Apamin inhibits Kv1.3 channels that are expressed in various tissues such as immune cells (Voos et al. 2017).

Recently, it was suggested that treatment with bee venom on experimental autoimmune encephalomyelitis (EAE) could be effective through anti-inflammatory properties (Shimpi et al. 2016). Considering the above-mentioned factors in this research, we studied the mechanistic effects of Apamin administration on PDGFR-α and Olig-2 markers as downstream factors affecting the myelination process in an animal model of cuprizone-induced MS model.

Material and Methods

Animals

C57BL/6 mice were obtained from Royan institute (Isfahan, Iran) and were kept in ventilated cages under standard conditions (12-h light/12-h dark cycles with controlled humidity) with ad libitum access to food and water. All of the experiments were approved by IRAN National Committee for Ethics in Biomedical research (IR.MUI.RESEARCH.REC 1397.387) and were carried out following the Guidelines for Care and Use of Laboratory Animals (Golmohammadi et al. 2019; Aliomrani et al. 2021). Possible efforts were made to decrease animal number and distress.

Cuprizone Exposure

Male Eight-week-old C57BL/6 mice were randomly divided into six groups. Group 1 (negative control, $N = 6$) was received regular food pellets during the study and serves as a healthy group. The rest of the subjects were fed with pellets containing 0.2% (w/w) cuprizone (Sigma-Aldrich, USA) for 6 weeks to induce MS model of demyelination in corpus callusom following 5 weeks' normal diet feeding for remyelination. Group 2 ($N = 12$) was performed to evaluate the effects of cuprizone after 6 weeks' exposure on demyelination and 5 weeks remyelination without any treatment. Group 3 ($N = 6$) received Apamin (100 µg/kg BW) twice a week at the first 6 weeks intraperitoneally and the sacrificed. Group 4 ($N = 6$) received Apamin (100 µg/kg BW) twice a week intraperitoneally for 5 weeks after cuprizone secession. Group 5 received phosphate-buffered saline (PBS) as a vehicle. At the end of each phase, mice were deeply anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and perfused. In all groups, Bromodeoxyuridine (BrdU) was administered via intraperitoneal injection at 100 mg/kg once per week.

Cardiac Perfusion

Mice were laid on the dorsal surface; then, about 2-cm lateral incision was made just below the sternum; after diaphragm diagnosis, it was cut away from ribs to see a thoracic cavity. The chest was exposed by pulling the hemostatic forceps carefully without any damage to the heart or lungs. The heart was grabbed with blunt forceps at the apex and perforated with the needle at the left ventricle. Starting with the 10 ml normal saline perfusion to clear the blood from the circulatory system and continued with 10 ml of 10% neutral formalin in PBS. At the end of perfusion, the subject was decapitated and the brain was removed immediately from the cranium and store in cold fixative for 24 h.

Tissue Processing

The samples were cut into 3–5–mm–thick slices coronally and placed inside the histology cassettes. After that placed in a tissue processor (Sakura Tissue Tek VIP E150) and dehydrated with growing concentration alcohol baths followed by a toluene clearing step to replace trapped alcohols inside the tissue. Finally, tissue samples solidified through

hot wax (44–60 °C). Embedded samples were sectioned to 5–10-µm slices with a microtome (LEICA RM2255) and after deparaffinization was used for pathological imaging.

Immunohistochemistry

Slides were dewaxed in xylene for 20 min 3 times and rehydrated in graded alcohol. Tissues were pretreated with Tris–EDTA buffer (TE buffer, 1 M, pH 9) or citrate buffer (1 M, pH 6) 10 min for antigenic retrieval. Rabbit monoclonal anti-olig2 (ab109186) and rat monoclonal to PDGF receptor alpha (ab5460) were used as primary antibodies, and staining was performed following the supplier instructions (Abcam, UK). Briefly, sections were incubated with the primary antibodies Olig-2 (1:100) and PDGFR-α (1:50) for 1 h at room temperature. Then washed 3 times with PBS, and blocked with 0.03% hydrogen peroxide blocking solution for 10 min. After that slides were incubated with the secondary antibody for 10 min at room temperature. Finally, 3–3′diaminobenzidine (DAB) was used to visualize the immunoreactivity of the samples. Also, the slides were counter-stained using hematoxylin then dehydrated and mounted for microscopic observation.

For BrdU incorporation, slides were subjected to acid hydrolysis using 2 M HCL in 0.1% PBS-Tween for 30 min at room temperature to denature the DNA. They were then permeabilized with 0.1% Triton X-100 for 5 min and blocked with 1% BSA/10% normal goat serum/0.3 M glycine in 0.1% PBS-Tween for 1 h. The slides were then incubated overnight at 4 °C with Anti-BrdU antibody (Alexa Fluor® 488) at 1/100 dilution and then quantified with a fluorescent microscope (excitation 495 nm, emission 519 nm).

Imaging and Quantification

Images were taken using Nikon Eclipse 55i light microscope (Nikon, Japanese) with a × 40 objective magnification. IHC slides stained cells with olig2 or PDGFRα positive brown dye were quantified with NIH Fiji software (National Institute of Health, Bethesda, Maryland, USA) and expressed as cells/mm^2. All of the mentioned quantifications were done on three slides for each brain sample and analyzed in a blinded coding system to omit any disturbance of confounding factors.

Statistical Analysis

Statistical differences between various groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc tests using GraphPad Prism software (San Diego, CA). Values of $p \leq 0.05$ were defined as statistically significant. Data are expressed as mean ± SD.

Results

As can be seen in Fig. 1, the number of cells with Olig2 marker in the group receiving cuprizone after 6 weeks had a significant increase compared to the control group ($p < 0.01$). Also, mice receiving Apamin at this stage had a multiplication in the number of positive cells for this marker compared to the control group ($p < 0.0001$) and the group receiving cuprizone alone ($p < 0.01$). It was observed that in the remyelination phase following cuprizone withdrawal, the positive cells for this neurogenic factor were almost higher than in healthy controls. Interestingly, there was a significant reduction in the number of these cells in the group receiving Apamin peptide compared to 6 weeks of cuprizone administration and also compared to the healthy control group in the same phase ($p < 0.01$).

As can be seen in Fig. 2, the examination of the number of cells in terms of having PDGFRα marker in the first phase, which was exposed to cuprizone and caused demyelination, showed that despite the higher number of cells counted, there was not a statistically significant difference. Also, there is no difference between the carrier and cuprizone groups and the healthy control group. While mice in which Apamin was administered at the same time as cuprizone were significantly different from the healthy control group ($p < 0.001$) and the cuprizone group ($p < 0.01$). Interestingly, this trend was reversed in the second phase of recovery following discontinuation of cuprizone, and the number of cells with this marker decreased significantly in the Apamin group ($p < 0.001$). In both phases, no significant difference was observed between the drug-receiving group and the cuprizone group.

To confirm the changes observed in the studied markers regarding cell proliferation, a thymidine analog was used, which is incorporated in the proliferating cells and can be observed with the help of specific antibodies. Exposure to cuprizone was found to reduce the number of proliferating cells compared to the healthy control group ($p < 0.01$), while administration of Apamin at this stage was able to increase the number of proliferating cells several times in the comparison cuprizone group ($p < 0.0001$). Interestingly, administration of this peptide in the second stage after cuprizone withdrawal did not cause a statistically significant change in the number of proliferating cells compared to the healthy control group. However, the increase in the number of positive cells for this marker in the cuprizone discontinuation phase indicates an increase

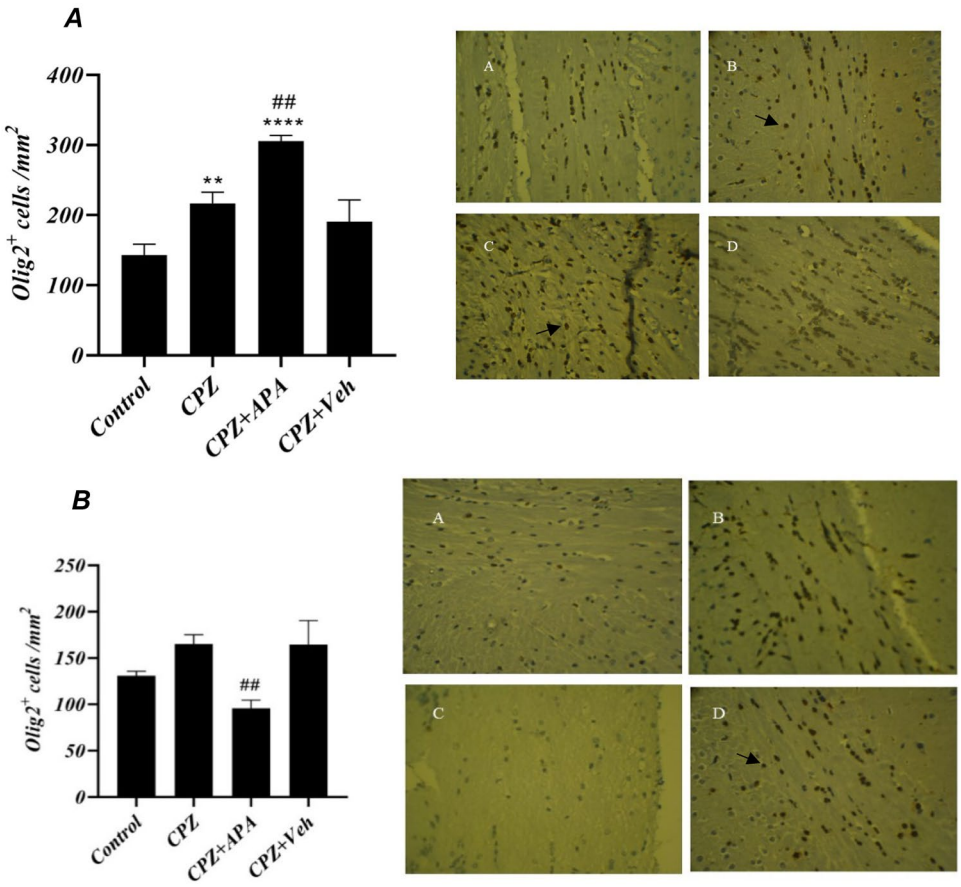

Fig. 1 Immunohistochemistry staining microscopic images of $Olig^{2+}$ marker for healthy control group **a**, Cuprizone (CPZ, **b**), Apamin administered group (100 µg/kg) twice a week (CPZ+APA, **c**), and PBS injected group (CPZ+Veh, **d**). Graph **a** shows the mean of $Olig^{2+}$ cells number per mm^2 during the 6 weeks of cuprizone exposure and Graph **b** during the 5 weeks of cuprizone withdrawal phase with × 40 magnification. #Comparison of groups with CPZ group; *comparison of groups with control group. ($^{\#\#}p < 0.01$, $^{**}p < 0.01$, $^{****}p < 0.0001$, $n = 6$)

in proliferation and delayed remyelination compared to the healthy control group (Fig. 3).

Discussion

About 5 to 10% of the population in developed countries are affected by autoimmune diseases (Davidson and Diamond 2001). These diseases are the leading cause of death and rising medical costs. In terms of cause, autoimmune diseases can be caused by a combination of different factors such as genetic, immune, hormonal, and environmental factors. Multiple sclerosis is a chronic demyelinating disease of the central nervous system that is accompanied by autoimmune manifestations (Aliomrani et al. 2017, 2016). For unknown reasons, the immune system is stimulated and specific lymphocytes are activated against myelin. The entry of these cells into the brain plays a key role in the immunopathology of the disease and the exacerbation of inflammatory reactions in the brain (Hemmer et al. 2002; Davidson and Diamond 2001).

Modern studies of bee therapy, particularly the use of bee venom, began in 1888 with the efforts of the Austrian physician Philipp Terc (VENOM n.d.). In his reports, he cited "a special link between bee stings and rheumatism." Bee venom is a complex mixture of proteins (phospholipase A2, phospholipase B, hyaluronidase, alpha-glucosidase), peptides (Melittin, Apamin, Adolapin, protease inhibitors, Tretiapine, MCD, and Procamine A, B), and low molecular weight components such as biologically active amines (histamine, dopamine noradrenaline), pheromones (a set of ethers), sugars, and amino acids (alpha-amino acid, aminobutyric acid). Apamin and melittin are found only in Apis, while Mastoparan is found in many species, including Vespa, Parapolybia, Protonectarina, Polistes, and Protopolybia (Carpena et al. 2020; Pakzad et al. 2021; Sharifan et al. 2021; Aliomrani et al. 2021).

Apamin is a neurotoxic peptide containing 18 amino acids in the H-Cys-Asn-Cys-Lys-Ala-Pro-Glu-Thr-Ala-Leu-Cys-Ala-Arg-Arg-Cys-Gln-Gln-His-NH_2 sequence. This peptide has two disulfide bonds (binding position 1 to 11, 3 to 15) and 7 hydrogen bonds and is highly stable at different pH, temperatures and is resistant to serum proteases (Gholami et al. 2020). Recently, Apamin has been shown that inhibits the Kv1.3 channels. These channels are widely expressed at the surface of immune cells such as T cells, macrophages, dendritic cells, and neurons. Oligodendrocytes express different types of potassium channels on their surface, which

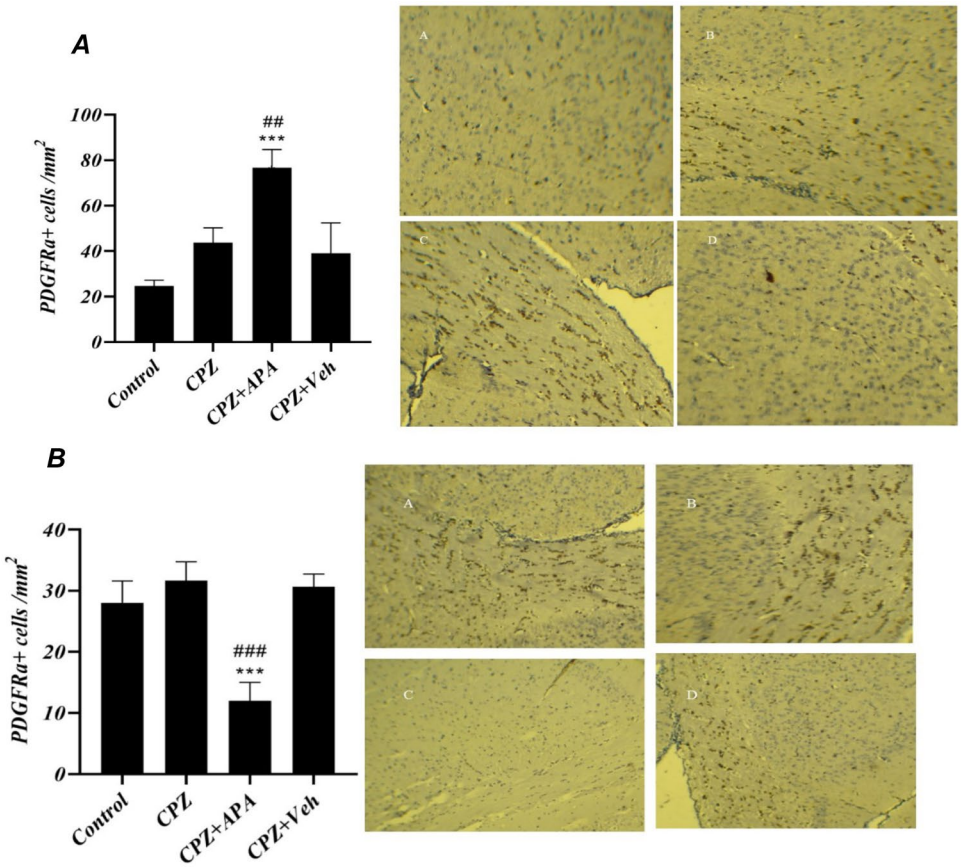

Fig. 2 Immunohistochemistry staining microscopic images of PDGFRα marker for healthy control group **a**, Cuprizone (CPZ, **b**), Apamin administered group (100 µg/kg) twice a week (CPZ+APA, **c**), and PBS injected group (CPZ+Veh, **d**). Graph **a** shows the mean of PDGFRα^{+} cells number per mm^2 during the 6 weeks of cuprizone exposure and Graph **b** during the 5 weeks of cuprizone withdrawal phase with × 40 magnification. #Comparison of groups with CPZ group; *comparison of groups with control group. (##$p < 0.01$, ###$p < 0.001$, $^{***}p < 0.001$, $n = 6$)

is essential for the synthesis of myelin structural protein (Marques et al. 2020; Park et al. 2020; Jung et al. 2020).

It was suggested that oligodendrocytes were begun apoptosis 3–7 days after exposure to cuprizone. During this time, large vacuoles, mega-mitochondria, and dense nuclear chromatin can be seen in these cells by electron microscopy. Also, mRNA expression of myelin-associated proteins such as proteolipid protein, myelin oligodendrocyte glycoprotein, and myelin basic protein is significantly reduced during the first week. In the second week, progenitor cells proliferate and accumulate in the subventricular zone and migrate to the demyelinated regions. In the sixth week, new oligodendrocytes are formed, resulting in the phenomenon of remyelination (Gudi et al. 2014). We have previously observed that administration of Apamin during the first 6 weeks caused a significant increase in myelin levels compared to the group receiving cuprizone (Mohammadi-Rad et al. 2019). This meant that Apamin prevented further degradation of myelin, whereas administration of Apamin 2 weeks after withdrawal of cuprizone had less effect on myelin levels. Therefore, Apamin increased myelin levels in both phases but was more effective in reducing the rate of demyelination than increasing the rate of remyelination. It is interesting to note that we observed that in the group receiving Apamin, the number of cells expressing this marker during the first phase was more than other groups and in the second phase was reduced to a greater extent. Olig2 is one of the most important members of the bHLH family, and the expression of this protein is mostly in the central nervous system, including motor neurons and oligodendrocytes. This transcription factor and its downstream protein, zinc finger protein, have been shown to play a key role in differentiating OPCs from adult oligodendrocytes. It is noteworthy that many OPCs accumulated at the site of acute or chronic demyelination do not differentiate into adult oligodendrocytes, so the importance of this differentiation and their activity in myelin regeneration is very important. These OPCs and their differentiation are used as markers for the effectiveness of treatment in models of induced demyelination/remyelination disease such as cuprizone to evaluate the therapeutic or destructive effectiveness (Mazloumfard et al. 2020; Tiane et al. 2019).

Several studies have shown the expression of PDGFRα on the surface of oligodendrocyte progenitor cells of parenchymal origin and its role in remyelination following induced demyelination in animal models. These specific receptors, which are essentially tyrosine kinases, are located to bind platelet-derived growth factors. If ligand binds, it phosphorylates itself and other involved proteins to transmit signals into the cell, ultimately the cellular response, which includes survival, evolution, migration, and differentiation

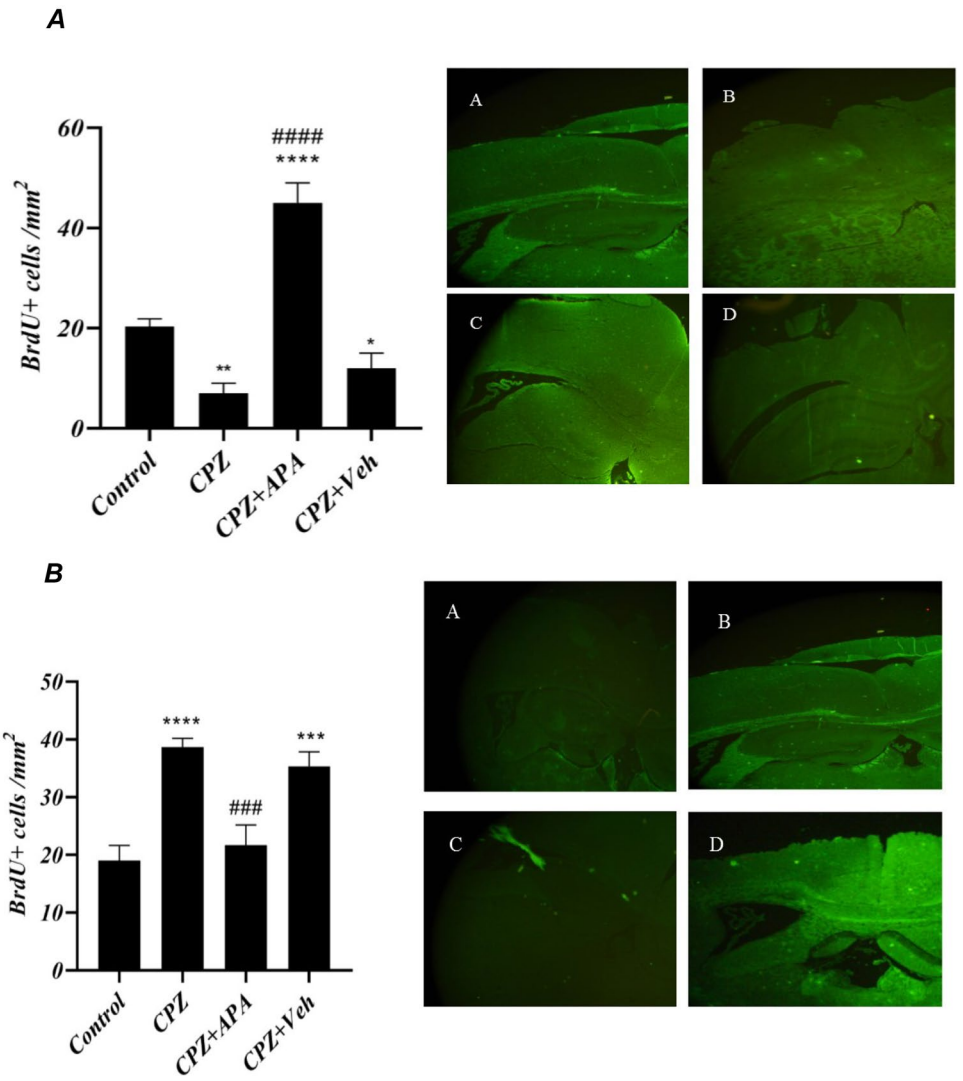

Fig. 3 Immunohistochemistry staining florescent microscopic images of 5-bromo-2'-deoxyuridine marker for healthy control group **a**, Cuprizone (CPZ, **b**), Apamin administered group (100 µg/kg) twice a week (CPZ+APA, **c**), and PBS injected group (CPZ+Veh, **d**). Graph **a** shows the mean of $BrdU^+$ cells number per mm^2 during the 6 weeks of cuprizone exposure and Graph **b** during the 5 weeks of cuprizone withdrawal phase with × 40 magnification. #Comparison of groups with CPZ group; *comparison of groups with control group. (###$p < 0.001$, ####$p < 0.0001$, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, $n = 6$)

(Đặng et al. 2019; Sugiarto et al. 2011; Crawford et al. 2013). Examination of positive cells for PDGFRα marker showed that in the first phase, the number of these cells in all groups increased compared to healthy controls, which indicates the stimulation of migration and proliferation of immature cells. This stimulation in the Apamin administered group was more than others. Examination of the number of positive cells in the second phase showed a significant decrease in the number of these cells, which can be related to the oligodendrocyte class due to cell differentiation and lack of expression of the relevant marker. These cells in the first phase in the Apamin group can be considered as a reason for overstimulating the migration of oligodendrocyte precursors from the subventricular region. Unlike other markers such as SOX10 and Olig2, which are expressed in both progenitor cells and oligodendrocytes, PDGFRα is specifically expressed in the immature OPC and subsequently not detectable in the differentiation stage (Sugiarto et al. 2011).

In this study, BrdU, a thymidine nucleoside analog, was used to replace the strands of DNA being synthesized and used as a marker to represent proliferating cells. It was observed that despite the increase in the number of positive cells in terms of Olig2 and PDGFRα markers, during phase I cell proliferation increased only in the Apamin group, while other groups have shown delayed proliferation in the second phase after the withdrawal of cuprizone. Also, an increase in the number of proliferating cells in the SVZ region was observed in the Apamin-receiving group, which is consistent with the observations made in the previous study and is a reason for inducing proliferation and migration of oligodendrocyte precursors from this region. It has previously been suggested that administration of Apamin can potentially increase the activity of adult oligodendrocytes and decrease the activity of microglia cells by increasing the expression of miR-219 and decreasing the level of miR-155-3p, reducing myelin degradation in vivo (Gholami et al. 2020).

In summary, according to the observed results regarding the passage of this peptide through the blood–brain barrier and the increase in myelin content in the induced disease model following changes in the studied miRs, it seems that the increase in Olig2 transcription factor and stimulation in proliferation/migration of OPCs is one of the most important mechanisms involved in the effectiveness of Apamin. With these results, it seems that this peptide could be used as a therapeutic option in inhibiting the progression of multiple sclerosis.

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Declarations

Conflict of Interest The authors declare no competing interests.

References

Aliomrani M, Sahraeian MA, Khoshayand MR, Danesh Seta T, Sharifzadeh M, Ghahremani MH (2019) The prevalence of the genetic polymorphism of GSTM1, GSTT1 AND GSTP1 and its relationship with clinical criteria of multiple sclerosis (Ms) patients in Tehran. *Journal of Babol University of Medical Sciences* 21(1)

Aliomrani M, Mesripour A, Sayahpour Z (2021) AChR is partly responsible in mice depressive-like behavior after phosalone exposure. Neurotoxicol Teratol 84:106957

Aliomrani M, Sahraian MA, Shirkhanloo H, Sharifzadeh M, Khoshayand MR, Ghahremani MH (2017) Correlation between heavy metal exposure and GSTM1 polymorphism in Iranian multiple sclerosis patients. Neurol Sci 38(7):1271–1278

Aliomrani M, Sahraian MA, Shirkhanloo H, Sharifzadeh M, Khoshayand MR, Ghahremani MH (2016) Blood concentrations of cadmium and lead in multiple sclerosis patients from Iran. Iranian Journal of Pharmaceutical Research: IJPR 15(4):825

Carpena M, Nuñez-Estevez B, Soria-Lopez A, Simal-Gandara J (2020) Bee venom: an updating review of its bioactive molecules and its health applications. Nutrients 12(11):3360

Crawford AH, Chambers C, Franklin RJM (2013) Remyelination: the true regeneration of the central nervous system. J Comp Pathol 149(2–3):242–254

Đặng TC, Ishii Y, De Nguyen V, Yamamoto S, Hamashima T, Okuno N, Nguyen QL, Sang Y, Ohkawa N, Saitoh Y (2019) Powerful homeostatic control of oligodendroglial lineage by PDGFRα in adult brain. Cell Rep 27(4):1073–1089

Davidson A, Diamond B (2001) Autoimmune diseases. N Engl J Med 345(5):340–350

Dawson MRL, Levine JM, Reynolds R (2000) NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? J Neurosci Res 61(5):471–479

Eze OB, Nwodo OF, Ogugua VN (2016) Therapeutic effect of honey bee venom. *Proteins (Enzymes)* 1(2)

Gholami S, Mirian M, Eftekhari SM, Aliomrani M (2020) Apamin administration impact on MiR-219 and MiR-155-3p expression in cuprizone induced multiple sclerosis model. Mol Biol Rep 47(11):9013–9019

Gold R, Wolinsky JS (2011) Pathophysiology of multiple sclerosis and the place of teriflunomide. Acta Neurol Scand 124(2):75–84

Golmohammadi J, Jahanian-Najafabadi A, Aliomrani M (2019) Chronic oral arsenic exposure and its correlation with serum S100B concentration. *Biological Trace Element Research* 189(1). https://doi.org/10.1007/s12011-018-1463-2

Greenstein JI (2007) Current concepts of the cellular and molecular pathophysiology of multiple sclerosis. Dev Neurobiol 67(9):1248–1265

Gudi V, Gingele S, Skripuletz T, Stangel M (2014) Glial response during cuprizone-induced de-and remyelination in the CNS: lessons learned. Front Cell Neurosci 8:73

Hauser RA, Daguio M, Wester D, Hauser M, Kirchman A, Skinkis C (2001) Bee-venom therapy for treating multiple sclerosis: a clinical trial . Alternative & Complementary Therapies 7(1):37–45

Hemmer B, Archelos JJ, Hartung H-P (2002) New concepts in the immunopathogenesis of multiple sclerosis. Nat Rev Neurosci 3(4):291–301

Jung HS, Seo MS, An JR, Kang M, Heo R, Li H, Jung W-K, Choi I-W, Cho E-H, Park H (2020) The vasodilatory effect of gemigliptin via activation of voltage-dependent K+ channels and SERCA pumps in aortic smooth muscle. Eur J Pharmacol 882:173243

Lassmann H, Brück W, Lucchinetti CF (2007) The immunopathology of multiple sclerosis: an overview. Brain Pathol 17(2):210–218

Leray E, Moreau T, Fromont A, Edan G (2016) Epidemiology of multiple sclerosis. Revue Neurologique 172(1):3–13

Marques AA, Macedo CH, da Silva F, de Souza P, de Almeida CLB, Cechinel-Filho V, Lourenço ELB, Junior AG (2020) Nitric oxide and Ca2+-activated high-conductance K+ channels mediate nothofagin-induced endothelium-dependent vasodilation in the perfused rat kidney. Chem Biol Interact 327:109182

Mazloumfard F, Mina M, Seyed-Mehdi E, Mehdi A (2020) Hydroxychloroquine effects on MiR-155–3p and MiR-219 expression changes in animal model of multiple sclerosis. *Metabolic Brain Disease*, 1–9

Mohammadi-Rad M, Ghasemi N, Aliomrani M (2019) Evaluation of apamin effects on myelination process in C57BL/6 mice model of multiple sclerosis. Research in Pharmaceutical Sciences 14(5):424

Olsson T, Barcellos LF, Alfredsson L (2017) Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nat Rev Neurol 13(1):25

Paknejad B, Shirkhanloo H, Aliomrani M (2019) Is There any relevance between serum heavy metal concentration and BBB leakage in multiple sclerosis patients? Biol Trace Elem Res 190(2):289–294

Pakzad D, Vajihe A, Mohammad RS, Mehdi A (2021) Risk of neurodegenerative disease due to tau phosphorylation changes and arsenic exposure via drinking water. *Toxicology Research*

Park J, Jang KM, Park K-K (2020) Apamin suppresses LPS-induced neuroinflammatory responses by regulating SK channels and TLR4-mediated signaling pathways. Int J Mol Sci 21(12):4319

Reynolds R, Cenci I, di Bello M, Dawson, and Joel Levine. (2001) The response of adult oligodendrocyte progenitors to demyelination in EAE. Prog Brain Res 132:165–174

SEYED KNA, Sina A, Kamyar G, KHOUEI NAS, OMIDI YA (2009) Melittin and hyaluronidase compound derived from bee venom for the treatment of multiple sclerosis

Sharifan A, Etebari M, Zolfaghari B, Aliomrani M (2021) Investigating the effects of bark extract and volatile oil of pinus eldarica against cisplatin-induced genotoxicity on HUVECs cell line. *Toxicology Research*

Shimpi R, Chaudhari P, Deshmukh R, Devare S, Bagad Y, Bhurat M (2016) A review: pharmacotherapeutics of bee venom. Word J Pharm Pharm Sci 5(7):567–656

Sugiarto S, Persson AI, Munoz EG, Waldhuber M, Lamagna C, Andor N, Hanecker P, Ayers-Ringler J, Phillips J, Siu J (2011)

Asymmetry-defective oligodendrocyte progenitors are glioma precursors. Cancer Cell 20(3):328–340
Tiane A, Schepers M, Rombaut B, Hupperts R, Prickaerts J, Hellings N, van den Hove D, Vanmierlo T (2019) From OPC to oligodendrocyte: an epigenetic journey. Cells 8(10):1236
VENOM, BIOLOGICAL ACTION OF BEE (n.d.) Biological and therapeutic properties of bee venom
Voos P, Yazar M, Lautenschläger R, Rauh O, Moroni A, Thiel G (2017) The small neurotoxin apamin blocks not only small conductance Ca 2+ activated K+ channels (SK type) but also the voltage dependent Kv1. 3 channel. Eur Biophys J 46(6):517–523