Effect of honey bee venom on Lewis rats with experimental allergic encephalomyelitis as regards changes of GABA and glutamate

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Abstract: Multiple sclerosis (MS) is a progressive, neurodegenerative disease of central nervous system (CNS). Experimental allergic encephalomyelitis (EAE) is a widely accepted animal model for MS. Honey bee venom (Apis mellifera) contains a variety of different low and high molecular weight peptides and proteins including melittin, apamin, adolapin, mast cell degranulating peptide and phospholipase A2. Bee venom (BV) also could exert anti-inflammatory and antinociceptive effects on the inflammatory reactions. Glotamic acid and γ-aminobutyric acid (GABA) are among neurotransmitters of central nervous system and participated in excitatory and inhibitory processes. In EAE the amount of GABA reduces and the level of glutamate will increase. Tracing them in brain could useful in monitoring the influences of drugs. In this research, hematoxylin and eosin methods for inflammation, ELISA to study tumor necrosis factor-alpha (TNF-α) and HPLC, to study the amount of GABA and glutamate were used for assessment. In this study, we showed that in EAE level of GABA has reduced and the amount of glutamate has increased and bee venom decreases pathological changes and the level of serum TNF-α, and level of glutamate and increases the level of GABA in EAE rats induced by spinal cord of guinea pig.
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1. Introduction

Multiple sclerosis (MS) is a progressive, neurodegenerative disease of central nervous system (CNS).This disease recognized by symptoms like the inflammation, demyelination and the destruction of neurologic actions (Urbach-Ross and kusnecov, 2007). Experimental allergic encephalomyelitis (EAE) is considered as a valuable animal model for MS research and the scientists who use it both for the evaluation of the process and treatment of diseases (Hedruel, Gillett, Olsson, Jagodic and Harris, 2009).

EAE is created in animals by injecting the tissue of myelin basic protein (MBP), CNS, or myelin oligodendrocyte glycoprotein (MOG) along with the adjuvant. EAE and MS are similar diseases pathologically and immunologicaly. In EAE certain symptoms are observed such as paralysis, inflammation, ataxia, elevated interferon-γ (IFN-γ), the brain-blood barier damage, and the penetration of CD4+T cells and macrophages to CNS (Ferguson, Sarlieve and Vincendon, 1990; Mao, Lu, Wang and Xiao, 2007).

The venom of bee (Apis Melifera) consists of different types of peptides light and heavy chain and proteins such as melittin, apamin, adolpin, phospholipaseA2 (Mirshafiey, 2007).

The healing bee poison is largely effective in treating chronic anti-inflammatory disease (Han, Lee, Yeo, Kweon, Woo, Lee, Beak, Kim and Park, 2007). Moreover, anti-inflammatory specifications of bee venom in rat model having infused arthritis has been reported, and it is observed that the injection of bee venom suppress leukocytes migration and reduces the level of TNF-α (Mirshafiey, 2007). Bee venom contains several bioamines, such as apamin, histamine, procamine, serotonin, and norepinephrine, which facilitate nerve transmission and healing in a variety of nerve disorders. This gives BV the ability to travel along the neural pathways from the spine to various trigger points and injured areas to help repair nerve damage and restore mobility (Son, Lee, Lee, Song, Lee and Hong, 2007).

Changes in the level of glutamate, GABA is accompanied by neurological disorders such as Alzeighmer's, Schizophrenia, and Epilepsy (De freitas saliva, Ferraz and Riberio, 2009)

Increase in the amount of glutamate was seen in the cerebrospinal fluid of patients with a range of central nervous system diseases from MS to meningitis and acute encephalitis. Activated macrophage and microglia produce and release significant amount of glutamate (Werner, Barn-Schieber and Raine, 2004).

MS disease may be associated with deletes the serum levels of GABA and its synthetic enzyme glutamic acid decarboxylase (Bhat, Axtell, Mitra, Miranda, Lock, Tsien and Steinman, 2010).

The concentration of GABA and glutamate are important biomarkers for disease states and drug effect (Eckstein, Ammerman, Reveles and Ackermann, 2008).

The aim of this study is examination the effects of anti-inflammation of bee venom on the rat with EAE and in addition to studying these effects has paid special attention to the level of GABA and the glutamate in brain of the rat EAE, and the groups under treatment to observe associate bee venom with the change levels of GABA and glutamate in brain of the rats with EAE.

2. Material and Methods

After anesthetizing guinea pigs, their spinal cords were extracted and mixed with equal volume of water 4 ºC to acquire homogeneous mixture. The guinea pig spinal cord homogenated (GPSCH) were emulsified in 1:1 ratio of complete Freund's adjuvant (CFA), consisting of 1mg/ml mycobacterium tuberculosis (Sigma-Aldrich, F5881).

EAE was created by subcutaneous injection of 0.2 ml of GPSCH-CFA to the adult female Lewis rats (weight 180-200 g, Laboratory of Animal Center Darupakhsh pharmaceutical company. Tehran. Iran). Also, for the control group, 0.2 ml of CFA was used.

The animals used in this research were kept under standard situation and fed with water and food adlibitum. The experimental procedures were done in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Academy Press (Washington, D.C. 1996).

EAE was induced in 30 rats, randomly placed in 3 groups of 10:

Group 1: Named E-S which received normal saline every day.

Group 2: Named E-BV which received 0.2 mg/kg bee venom every day.

Group 3: Named E-BV which received 0.5 mg/kg of bee venom everyday.

It is worth mentioning that the treatment started from the first day post of immunizing by GPSH-CFA and lasted until the tenth day.

After 19 days post of immunizing, rats were anesthetized after injection combinatory of ketamine and xylazine (Alfasan, Holand). Then their brain and spinal cords were removed. They were kept under the process of histotechnique for 24 hours in 10% formalin as a fixative.After that, the sections of brain and spinal cord were stained with hematoxylin and eosin (H & E) for inflammatory cell infiltration The intensity of inflammatory cell infiltration was assessed according to the protocol Okuda, Sakodo, Fujimura, Saeki, Kishimato and Yanagihara (1999) and classified according to the obtained scores as follows: Score zero: the absence of inflammation, score one: the penetration of cells around blood vessels and meninges, score two: subtle penetration of cells in parenchyma (1- 10/section), score three: average penetration of inflammatory cells in parenchyma (1-100/section) (Okuda, Sakodo, Fujimura, Saeki, Kishimato and Yanagihara, 1999).

The rate of serum TNF-α was specified by using rat TNF-α ELISA KIT (Abcam, UK).

The brains of rats were taken out of skull and put them on ice-cold plate so as to separate the brain stem and then weighed and placed inside tubes of microcentrifuge (1.5 ml) .The samples were homogenized in 15 vol. of methanol/water (85/15, v/v) then centrifuged under conditions (7800×g, for 15 min at 4° C) and stored at -20 centigrades until derivatisation for GABA/glutamate analysis. To determine the amount of GABA and glutamate we use HPLC method which was used by De Freitas Silva (De Freitas Saliva, Ferraz and Riberio, 2009).

Data were analyzed using the SPSS statistical program (version 17 for windows). In all of the cases for comparison between groups Mann-Whitney U test was used. Significance level was set at $p<0.05$ throughout the experiment.

3. Results

Effect bee venom on the penetration of inflammatory cells central nervous system of EAE rats

Following the staining sections and analyzing the samples, in tissue samples related to control group, no penetration of inflammatory cells to brain parenchyma and spinal cord was observed. The sample of tissue which contains signs such as penetration mononuclear inflammatory cells in parenchyma, the existence of inflammatory cells around blood vessels and meninges belonged to tree-label groups. These signs were observed with varying intensity in the specified groups. The intensity of pathological changes and the penetration of inflammatory cells both in brain tissue and spinal cord of group E-S was noticeable. The intensity of pathological changes in groups E-BV1, E-BV2 had significantly decreased. This decrease was seen both in brain parenchyma and in tissue of spinal cord (Figures 1 and 2).

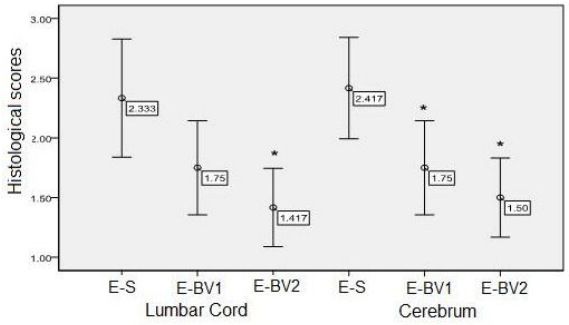

Figure 1: The treatment with bee venom reduces the penetration of inflammatory cells in cerebrum and lumbar cords of EAE rats induced by GPSCH-CFA. Pathologic changes in rats assessed with using of semi-quantitative score as described in experimental procedures section. (*p<0.05 compared with E-S group and n = 4 for all groups.)

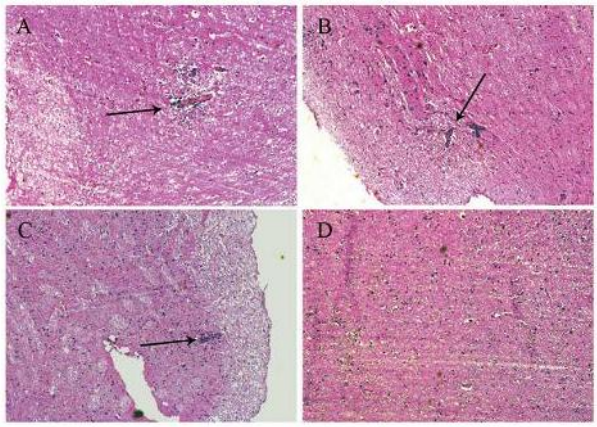

Figure 2: The treatment with bee venom reduces the penetration of inflammatory cells in brain of EAE rats induced by GPSCH-CFA. (A) E-S group, (B) E-BV1 group, (C) E-BV2 group, (D) Control group

Result show that the bee venom can meaningfully decrease penetration inflammatory cells into CNS in rat EAE induced by GPSCH.

The amount of TNF-α had decreased in under treatment groups in comparison with E-S and this reduction process in group and E- BV2 is salient (Figure3).

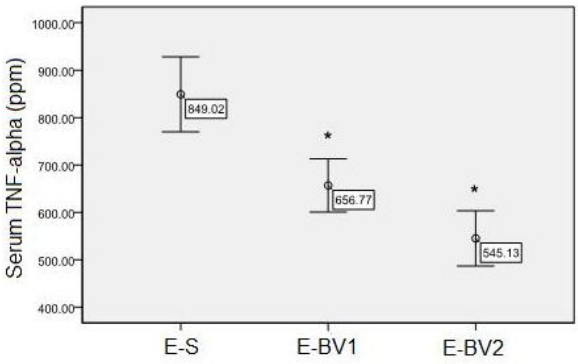

Figure 3: The treatment whit bee venom reduces the rate of TNF-α in serum of rats induced by GPSCH-CFA. ELISA method for TNF-α was used for assessment. The level of TNF-α in different groups compared to E-S (*p<0.05 compared with E-S group)

These results show that bee venom decreases the amount of TNF-α in the serum of rats which induced with GPSCH-CFA considerably.

The amount of glutamate in E-S group rats increased as compared to control group but the amount of GABA in E-S group had meaningfully decreased compared to control group. The amount of glutamate in E-BV2 decreases significantly in comparison with E-S group. But instead the amount of GABA in E-BV1 and E-BV2 in comparison with E-S had increased significantly (Figures 4 and 5).

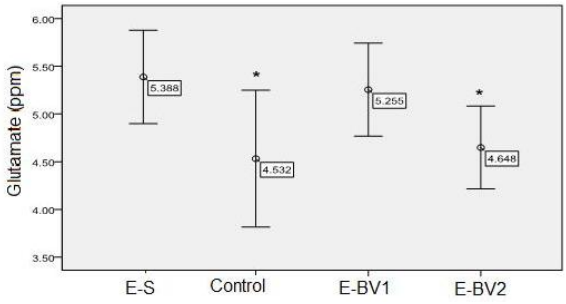

Figure 4: Level of glutamate in brain stem of EAE rat increases in compared to control group and bee venom in dose 0.5 mg/kg decreases amount of glutamate in brain stem of EAE rats. The level of glutamate (ppm) in different groups compared to E-S (*p<0.05 compared with E-S group)

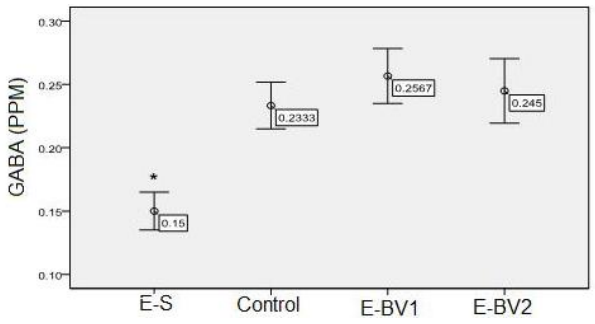

Figure 5: Level of GABA in brain stem of EAE rat decreases in compared to control group and bee venom increases amount of GABA in brain stem of EAE rats. The level of GABA (ppm) in different groups compared to E-S (*p<0.05 compared with E-S group)

The results show that bee venom can increase the amount of GABA and decrease glutamate in the brains of EAE rats induced with GPSCH.

4. Discussions

Multiple Sclerosis (MS) is an autoimmune disease of CNS which shows pathology characteristics like the penetration of macrophages and lymphocytes into CNS, demyelination, axonic damage and ect. Etiology of this disease in is an unknown yet, but it is a kind of disease in which T cells and B cells attack on myelinated parts of nervous system (Chen, Chen, Wang, Wu, Yang, Xu, He, Wang, Chen and Zheng, 2010).

Medical properties of bee products have been known from ancient times and today the bee venom is used extensively for the treatment of arthritis and other inflammatory, autoimmune and destructive diseases (Han, Lee, Yeo, Kweon, Woo, Lee, Baek, Kim and Park, 2007). Bee venom includes some kinds of peptides, enzymes, and active amines and other composites which can be effective in the treatment of various diseases. For example the melitin (main substrate of bee venom) is one of the most effective anti- inflammatory factors which are known. Also adolapin is another effective anti-inflammatory substrate that suppresses the activity of cyclooxygenase (COX) (Son, Lee, Lee, Song, Lee and Hong, 2007). It is reported that bee venom presents the production of Interleukin -1ß (IL- 1ß) from macrophages in rats in response to inner stimulation by bacterial lipopolysaccharides (Kwon, Lee, Han, Mar, Kang, Yoon, Beitz and Lee, 2002).

Primary allergic compounds of bee venom such as histamine and phospholipase A2 induced the producing of the Interleukin -10 (IL-10) by the T-helper cells and suppress the proliferation of T-cell and also they can be effective in the reduction of inflammation and finally the reduction of demyelination (Jutel, Akdis and Blaser, 2007).

The Immune suppression and anti-inflammatory effects of bee venom are reported in MS disease, rheumatoid arthritis and their laboratory models (Mirshafiey 2007).

The present study has also evaluated the result similar to those of studies on the effects of bee venom on inflammatory autoimmune diseases and anti-inflammatory and immune suppressing activities (Kwon, Lee, Han, Mar, Kang, Yoon, Beitz and Lee, 2002; Jutel, Akdis and Blaser, 2007).

TNF-α and interferon-γ are some kinds of proinflammatory cytokines each are mainly secreted by autoimmune T-cells and directly destruct blood-brain barrier and induce the apoptosis of oligodendrocytes also considered as a demyelination factor (Akassoglou, Bauer and Kassiatis, 1998). Plasma level of TNF-α has relationship with the severity of EAE and MS and it also explains the immune status (Polka, Ovadia, Orion, Weidenfeld and Yirmiya, 2003).

In the rats of an EAE model serum level of TNF-α has the increasing regulation in the beginning of disease and it shows that TNF-α has an important role in the commencement and spread of disease (Schneider, Shuetz and Zollner, 2009).

During the present study we have observed that TNF-α decrease in the groups treated with the bee venom. Bee venom can prevent the production of pro-inflammatory cytokines like TNF-α (Nam, Je, Lee, Han, Lee, Kang and Mar, 2003).

In our research we observed that the amount of glutamate in the brain of EAE rats increases substantially and instead the amount of GABA will decrease.

Death of neural cells are susceptible to expose with high amounts of excitatory amino acids (EAA) was for the first time discovered by Olney in 1978. Exciotoxicity emerges following imbalance between excitatory processes from one side which leads to increasing glutamate on the other side, inhibitory processes which are mediated by GABA. (Gonsette, 2008)

Glutamate excitatory is proposed as a pathological mechanism in MS and it still has a place for discussion. The T cells stimulated by antigens, macrophages, and microglias are become activated and attracted into white matter of central neural system. These glutaminase-positive cells produce high amounts of glutamate. In parallel, the capacity of the tissue to absorb the additional glutamate is reduced (Werner, Barn-Schieber and Raine, 2004).

Excitotoxicity damages myelin but it seems that axons are more resistant, excitotoxicity contributes to

blood brain barrier (BBB) dysfunction via endothelial cell EAA receptors damage (Gonsette, 2008).

GABA is the principal inhibitory neurotransmitter in the central neural system which is necessary for normal performance of the brain, neuronal activity, information processing, flexibility, network synchronization and in diseases. Prescribing GABAergic is used to treat anxiety, alcohol consumption and epilepsy and making soothing (De freitas saliva, Ferraz and Riberio,2009).

GABA treatment reduces production of inflammatory cytokines in the peripheral macrophages. GABAergic factors directly influence the antigen-presenting cells (APCs), and reduce MAPK messages and lead to deleting inflammatory responses to myelin proteins (Bhat, Axtell, Mitra, Miranda, Lock, Tsien and Steinman, 2010).

The results of work of Bjurstom, Wang, Ericsson, Martin, Liu, Kumar-Mendu, Issazadeh-Navikas and Birnir in 2008 specify that extracellular GABA protect the neurons from the potential damage caused by pathogenic T cells and low physiological concentrations of GABA do activate GABA channels on the T lymphocytes and do decrease activated T cell proliferation. (Bjurstom, Wang, Ericsson, Martin, Liu, Kumar-Mendu, Issazadeh-Navikas and Birnir, 2008).

The amount of GABA is available in the composition of bee venom up to 1% of its dry weight (Son, Lee, Lee, Song, Lee and Hong, 2007).

In this research we observed that bee venom has increased the amount of GABA in EAE rats' brains. After that we can remember it as a boosting factor of GABA. Regarding the role of GABA in preventing from inflammatory reactions, preventing from T cells propagation and its protective role in brain and it could be said that bee venom can potentially reduce the signs and effects of EAE. Regarding the role of bee venom in reducing inflammation and glutamate and increasing GABA we can use bee venom to prevent from EAE effects and finally treatment of MS.

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