IN-VITRO AND *IN- VIVO* COMPARATIVE NEURO PROTECTIVE EFFECT OF PLANT EXTRACTS ON ETHIDIUM BROMIDE INDUCED DEMYELINATION

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ABSTRACT

In this study, the role of *Clitoria ternatea, Cucurbita maxima, and Artemesia vulgaris* extract(s) on remyelination in the prevention of demyelinated disorder such as Multiple sclerosis has been carried out. *In-vivo* and *in-vitro* investigation has been done to assess the defensive impact of ethidium bromide-induced demyelination on the rat model. The methanol and petroleum ether extracts of the plants were assessed for anti-inflammatory and antioxidant activity on neuronal cell lines and the assessment of protective effect extracts was conducted on an ethidium bromide induced rat model. Results disclosed that the *Cucurbita maxima* petroleum ether extract has a substantial influence on ethidium bromide-induced demyelinated rats due to the existence of fatty acids.

Keywords: Artemesia vulgaris, Remyelination, Cucurbita maxima, Demyelination, Clitoria ternatea, Neuronal cell lines.

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INTRODUCTION

Plants are always the main source of drugs or treatment strategies in various traditional medicinal systems. In recent years, many people either alone or in conjunction with other medications or herbal products to enhance their health or as a remedy. According to the WHO, a large number of population groups use herbs or herbal products for basic health care. Herbal medicine consists of herbs, herbal preparations, or materials, finished, and processed herbal products, and active plant ingredients [1]. In the 21st century, unhealthy lifestyles environmental toxins, and pollution has become a reason for the increase in the risk of disease. Side effects, allopathic medicine misuse/overuse is also a big problem. In 2013, the WHO established and released the "WHO Traditional Medicine Strategy 2014-2023", emphasizing the integration of conventional and complementary medicine in order to promote universal medical care and promote the quality, safety, and efficacy of this medicine. As a result, the globe is seeking low-cost, readily accessible, more physiologically appropriate traditional medicinal systems and a holistic approach to providing basic health care for all [2]. Nerve impulses while demyelination may create major disorders like MS ("Multiple Sclerosis")[3]. MS is acknowledged as a demyelinating, inflammatory, autoimmune, and neurodegenerative disorder that affects the central nervous system, and more than two million persons were being affected by the disease [4]. It is described by progressive clinical drop owing to axonal loss from chronic demyelination [5]. At present, it is generally accepted that 40-70% of MS patients experience cognitive dysfunction [6]. The most frequent cognitive deficits are memory disorders and spatial perception disorders [7,8]. Recent studies, in particular using MRI ("Magnetic Resonance Imaging"), have shown that MS patients experience structural disorders of the cerebral cortex as well as the hippocampus (an important site of memory consolidation) [9,10]. Although several factors could lead to neurodegeneration and demyelination in MS, in recent years it has become apparent that oxidative stress has an important role in this [11,12]. The brain is considered to be especially susceptible to free radical damage due to its high amount of PUFAs ("Polyunsaturated Fatty Acids"), high oxygen levels utilization, and inadequate antioxidant defenses compared to other organs. Therefore, it is apparent that oxidative stress has a role in the development and MS lesions persistence [13].

Cucurbita maxima, generally familiar be called pumpkin belonging to the Cucurbitaceae family. The chemical components of these seeds contain unsaturated fatty acids such as (linoleic, oleic) triterpenoids, flavonoids, saponins, vitamins, minerals, amino acids with a high content of carotenoids, including lutein β-carotene. It has been suggested that the phytosterols present in the seed may play an important role in the antioxidant, anti-inflammatory, and immunomodulatory activity. The pulp is used for burns, scalds, inflammations, abscesses, and boils and is a remedy for migraines, neuralgia, and hemorrhages. *Clitoria ternatea*, commonly known as butterfly pea, has traditionally been used to treat various diseases and the roots are used in oxidative stress, epilepsy, psychosis, neurotonic, and inflammation. It has been shown to have various pharmacological effects, such as antidepressants, anti-stress anti-inflammatory, and pain reliever. It improves memory, the ability to learn, increases the apical and basal dendritic branches. Artemisia vulgaris from the Asteraceae family, commonly known as mugwort in traditional herbal medicine, is used as an antiseptic, antidiabetic, antiepileptic, antidepressant, and anti-inflammatory. Artemisia vulgaris contains essential oils, flavonoids; Polyphenolic compounds have shown free radical scavenging activity. The primary goal of this study was to assess the role of extracts in remyelination to avoid demyelinated diseases such as multiple sclerosis.

METHODS

Extraction procedure

All selected parts of the plant were dried and sprayed in the shade. The 1 kg coarse powders from each of the three dry weighed plants were subjugated to cold maceration using petroleum ether and methanol for 72 hours. The extract was filtered at the conclusion of each extraction, and the filtrate was concentrated using the rotary evaporator under decreased pressure at 30°C (Büchi, Switzerland). The concentrated extracts were further dried in desiccators to produce dry extracts.

In-vitro cell line studies

Antioxidant activity

Determination of Superoxide Dismutase (SOD) Enzyme

N2a cells (neuroblastoma cells kindly provided by NCCS, Pune) were maintained at 37°C in "Dulbecco's Modified Eagles" Media in a CO2: air (1:19) atmosphere at a relative humidity of 90

to 100 %. The cells were incubated for 24 hours at 37°C. SOD was prepared using the technique reported by Paoletti et al. N2a cells were homogenized in 4 volumes of PBS with 2.0µM phenylmethylsulfonyl fluoride (Sigma) with Wheaten tissue grinder. The homogenate was centrifuged for 15 minutes at 4°C at 30,000 Xg. A G-25 column was used to collect the supernatant. After adding 10 mM mercaptoethanol/mg protein, the NADH oxidation was monitored spectrophotometrically at 340 nm. The quantity of SOD that can inhibit the NADH oxidation rate by mercaptoethanol by 50 % is defined as one unit of enzyme activity. Findings are represented as the mean + SEM [14].

Determination of lipid peroxidase by measuring MDA level

N2a cells were gathered in a 1µl cell culture medium. The cells were homogenized and sonicated on ice before being employed in the test. The cell lysates do not require to be diluted prior to the assay. A 100 µl specimen and 1000 µl SDS solution were placed in a 5 ml vial. 4 µl of color reagent was applied to the side of the vial and inserted in a floating foam tube holder. After a 10-minute ice bath, the vial was placed in boiling water. The process was performed as per prepared protocol and readings were noted at 530 nm to 540 nm [15].

Anti-inflammatory activity

Maintenance of cell lines

RAW264.7 macrophage cells were kept at 37°C in a "Dulbecco-modified Eagles" medium comprising 20 % (v/v) fetal bovine serum, 1 mM glutamine, 1 percent non-essential amino acids, 10,000 U/ml penicillin along with 10 percent. 000µg/ml streptomycin in a CO_2 atmosphere: air with a relative humidity of 90-100 % (1:19).

Determination of anti-inflammatory activity (TNF – α)

TNF-α production (a stable TNFα oxidation product from RAW264.7 macrophage cells) was determined as a biomarker for the inflammatory response. Cells were plated at a density of 2x105 cells/well in 96-well culture plates and incubated for 34 hours at 37°C. Seeded cells were treated with lipopolysaccharide (LPS, 1 mg/ml) to stimulate TNF- α production treated with each sample at the indicated concentrations, followed by 24, 48, and 72 hours of incubation. The Griess reaction was used to assess LPS-stimulated TNF-α generation in macrophage cells. In short, 100 ml of every supernatant was combined with 100 ml of the Griess reagent (1 percent sulfanilamide in 5 percent phosphoric acid as well as 0.1 percent N1-naphthyl ethylenediamine dihydrochloride in distilled water), and the combination was measured at the absorbance of 540 nm [16]

IL-6 Assay

The premixed standard was reconstituted in 0.5 ml of medium to provide a stock concentration of 50,000 pg/ml for every cytokine in the IL-6 assay. The sample was carried out on the 96-well plate that was included with the kit. Each well of the filter plate was filled with premixed beads (50 µl) coated with the target capture antibodies and rinsed twice with BioPlex wash buffer. A premixed standard solution or sample (50 µL) was applied to every well comprising the washed beads. The plate was stirred for 30 seconds before being kept at room temperature for 30 minutes with slow shaking. After washing and incubation, a premixed detection antibody (50 µl) was applied to each well. After shaking at room temperature for 10 minutes, the incubation was completed. The beads were resuspended in 125 µl BioPlex assay buffer after being rinsed 3 times. The beads were scanned by the "BioPlex Suspension Array" System and the data were examined with BioPlex Manager™ software [17].

***In-vivo* studies**

Animals

Wistar rats (male and female) (220g to 250g) were employed in the research. Animals were bred in a 12-hour light-dark cycle under humidity (55±15percent) and standard temperature (22±5°C) conditions. Animals were randomly split into six groups, each with six animals, and were provided standard laboratory food and water. All processes were conducted as per the guidelines of CPCSEA (Committee for the Management and Supervision of Animal Experiments) of the Animal Welfare Department of the Government of India in New Delhi ("JSSCP/IAEC/PH.D/PH.COG/03/2016-2017").

Experimental procedure

Male, as well as female Wistar rats (twelve weeks old, weighing 200 g to 250 g), were selected and kept over diet. After two weeks of dietary manipulation, the animals were randomly grouped and deeply anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (20 mg/kg). The rat was put in a rat stereotaxic device with the skull flat. The rat skull was exposed after the surface of the skull was shaved and an incision was created. The matching

coordinates were used to drill two holes in the skull. Two guiding cannulas were inserted and secured with dental cement into the holes. A dummy medial cannula was put into the guide cannula after surgery and remained in place until the injection was given. All animals were recovered for a week before starting microinjection. The experimental MS model was bilaterally induced by a single direct single injection of 0.01 percent ethidium bromide in sterile 0.9 % physiological saline. Animals were treated with extract after demyelination and examined for behavioral tests. The animals were slaughtered for histopathological and biochemical investigation after twenty-eight days[18,19].

Grouping of animals

The research was conducted in nine groups, with each group consisting of five Albino Wistar rats of both sexes (200 gm to 250 gm).

- Group(G_1) - Only 0.3 percent CMC (2 ml) was given orally and daily as solvent control.
- Group(G_2) – intracerebrally, 1 µg/kg body weight of EB ("Ethidium Bromide") in 0.03 ml PBS (sterile) was given.
- Group(G3) - In 0.3 percent CMC, Fingolimod 0.3 mg/kg body weight was administered orally and daily in the form of EB.
- Group(G4) - *Cucurbita maxima* dried petroleum ether extract and EB at a dose of 200 mg/kg body weight in 0.3 percent CMC were administered daily and orally
- Group(G5) - Received EB along with dried methanol extract of *Clitoria ternatea* root 200 mg/kg body weight in 0.3 percent CMC daily and orally
- Group(G6) - In 0.3 percent CMC, EB and dried methanol extract of *Artemesia vulgaris* leave 200 mg/kg body weight were given to the patients orally and daily.

Behavioural studies

This study was used to monitor rat behavioral changes when animals were exposed to a new environment and were used to identify anxiolytic and anxiogenic activity under the same conditions. A conventional rat's device was used, including a huge black area (96 x96 cm) with wall height (96 cm). The floor was split into 16 squares with white lines and the device was positioned in a dimly lit room. Individual mice were put in the corners of the device and examined for the next 10 minute to 15 minute.

The parameters that were mentioned were:

- Defecation (number of fecal pellets).
- Freeze (immobility periods)
- Rearing (number of times animal stand on its rear paws)
- Ambulation (number of squares crossed)

Grip strength

This test evaluates the strength and neuromuscular function of rats. These may be affected not only by sedatives and muscle relaxants, but also by toxic substances. Female or Male rats are placed on a horizontal thin thread or metal wire hanging about 10 cm in the air and immediately grabbed by the forelimbs. Ordinary animals can grab the wire with hind legs and climb in 5 seconds. Animals that cannot touch the wire are considered disabled. Animals treated with the extract were subjected to grip strength measurements [20].

Rotarod test

This test is conducted to assess the activity of medicines that disrupt motor coordination. The device contains a horizontal metal rod with a diameter of 3cm linked to the motor at a speed of 2025 revolutions/min. A wooden box divides the stick into five pieces. Five rats can be tested at the same time and the sticks are 50cm above the tabletop to prevent the animals from falling. The box is made of cardboard to prevent the animal from escaping if it falls off the bar. Test animals were observed while falling from the rollers.

Beam walks Test

This test is conducted to assess an animal's ability to walk on a beam. The device contains a horizontal metal rod with a diameter of 1cm and is sustained by 2 side stands with a height of 30cm. The test animal was held in the center of the bar so that it could walk on the beam. The parameters assessed were fecal pellets, walking distance, immobility time, and fall time [21].

Biochemical parameters

After the behavioral study was completed, the animals were sacrificed for biochemical examination due to cervical dislocation. The cerebral cortex, as well as midbrain, were immediately separated and prepared for different biochemical studies after the entire brain was dissected and rinsed with regular ice-cold saline.

Antioxidant activity

MDA (Malondialdehyde)

MDA levels in brain tissue were used to assess lipid peroxidation. MDA was analyzed by measuring the reactive thiobarbituric acid species. Substances that react with thiobarbituric acid to

make a red complex with peak absorption at 532 nm.

SOD (Superoxide dismutase)

Enzyme activity is expressed as a unit/mg of protein. The BSA ("Bovine Serum Albumin") was applied to prevent the solution from precipitating when BCS ("Basokproin Disulfonic Acid") was applied. Both BCS (electron transport chain-related free radical production inhibitor) and diethylenetriamine penta acetic acid (DETAPAC) were applied to inhibit the iron-related redox cycle and free radical production. Different amounts of protein were applied to the tubes till the reduction in NBT was maximally suppressed. A colored complex showing peak absorption at 532 nm was reported.

Anti-inflammatory activity

TNFα and IL6 were measured as per guidelines using an enzyme-bound immunoadsorption measurement kit. These specific test kits were chosen due to their high specificity, sensitivity, inter-assay and intra-assay accuracy, and the minimal quantity of tissue samples necessary to perform the test.

Histopathological study

Tissues from the midbrain and cerebral cortex were isolated, fixed with 10% formalin, treated according to conventional methods, embedded in paraffin, cut to a thickness of 45 µm, and stained with heamotoxylin and eosin. The tissue was examined under a light microscope.

Statistical analysis

One-way ANOVA ("Analysis of Variance") was utilized to examine the statistical data presented as Mean±SD using Turkey Posts trial. Statistical significance was defined as a value of $p<0.001$. For the graphical and statistical assessments, Graph Pad Prism Version 5.0 was employed.

RESULTS

In-vitro cell line studies

Anti-oxidant activity

a) SOD (Super Oxide Dismutase)

SOD activity was performed using the post-confluence N2a cell line. SOD is an enzyme that converts superoxide anion to H_2O_2. SOD activity increased significantly ($P < 0.0001$), during the first four days after confluence, remained high, and dropped on day 16. SOD enzyme activity showed that petroleum ether extracts from Cucurbita maxima were more effective than methanol extracts from Artemisia vulgaris and Clitoria ternatea, comparable to the standard medication fingolimod. The amount of SOD that can inhibit 50 percent of the NADH oxidation rate by mercaptoethanol is one unit of enzyme activity. Findings are presented as mean±SD. The SOD activity of *Artemesia vulgaris*, *Cucurbita maxima*, and *Clitoria ternatea* revealed at the end of the day 16^{th} was 8.9±1.01, 7.9±1.89, & 8.5±1.98, as well as the standard drug, has demonstrated 6.5±1.98 IU/mg/protein. The results are presented in Fig 1.

b) Lipid Peroxidation

The current study's findings demonstrated a strong dose-dependent rise in MDA generation in response to hydrogen peroxide therapy. Lipid peroxidation is caused by ion radicals created during oxidative stress. After 24 hours of H_2O_2 treatment, a clear dose-dependent and substantial rise in MDA concentration were seen, although the mean absorbance values dropped after 48 hours of treatment. This might be owing to the decreased H_2O_2 short half-life or owing to the cell's internal protection mechanism. When contrasted to all other extracts and the conventional drug Fingolimod, the petroleum ether extract of Cucurbita maxima showed high efficacy (0.2338±0.001) after 48 hours at 200m concentration.

The findings of this research show a clear dose-dependent rise in MDA production when treated with hydrogen peroxide. Lipid peroxidation is caused by ionic radicals produced during oxidative stress. In this research, a significant dose-dependent and substantial rise in MDA concentration were achieved after H2O2 treatment after 24 hours, but mean absorbance decreased after 48 hours of treatment. This might be owing to a short half-life reduction of $H2O_2$ or a cell internal protective mechanism. After 48 hours, the Cucurbita maxima petroleum ether extract at a concentration of 200 µm showed strong activity (0.2338±0.001) comparison to standard drug fingolimod and all other extracts. The results are presented in Fig 2.

Anti-inflammatory activity:

The extracts were shown to be capable of reducing a variety of inflammatory mediators, according to the findings of the current investigation. LPS-induced TNF-α has been suppressed by the extracts. These cytokines act as mediators of proinflammatory. The phytoconsituents found in the extracts may have controlled the process of cell-mediated inflammation by inhibiting cytokines. The IL-6 and TNF-α production in RAW264-7 macrophage cells was reduced after

treatment with the *Cucurbita maxima* petroleum ether extract and were 42.89±0.051 and 40.15±0.002 respectively. The activity was strong and equivalent to the "standard drug Fingolimod". The impact of various extracts TNF- α inhibition. The impact of various extracts TNF- α inhibition and IL-6 inhibition were presented in Fig 3 and Fig .4 respectively.

In-vivo studies

Behavioural tests

The methanol extracts of *Clitoria ternatea* root and petroleum ether extract of *Cucurbita maxima* seeds and *Artemesia vulgaris* leaves have revealed their protective impact on ethidium bromide induced demyelinated rats. The consequence was an increase in muscular strength and coordination. When compared to standard, the protective impact of *Cucurbita maxima* seed at 200 mg/kg has revealed a protective impact in regulating motor neuron disorders.

Methanol extracts from Clitoria ternatea roots, and petroleum ether extracts from Cucurbita maxima seeds and Artemesia vulgaris leaves have revealed protective effects against ethidium bromide-induced demyelinated rats. This improves muscle and strength coordination. The protective impact of Cucurbita maxima seeds at 200 mg/kg shows a protective impact in the control of motor neuron disease compared to standard. The behavioural studies of the extracts on demyelinated rats were presented in Fig 5.

Estimation of biochemical parameters

Anti-oxidant activity

To examine the anti-oxidant impact of the extracts, the levels of MDA and SOD were assessed using tissue homogenates of the midbrain as well as cortex in the biochemical investigation. In comparison to other extracts, it was discovered that the petroleum extract at 200 mg/kg dosage level has a protective impact.

In biochemical studies, MDA and SOD levels were determined using midbrain and cortical tissue homogenate to assess the antioxidant activity of the extract. Petroleum extract has been observed to have a protective effect at a dose of 200 mg/kg compared to other extracts.The results are shown in Fig. 6.

Anti-inflammatory activity

The IL-6 and TNF- α levels were measured to determine the impact of plant extracts on neuronal cells inflammation prevention.The results presented in Fig.7.

Histopathology result

In the histopathology analysis, the less protective impact was found in groups V and VI treated with methanol extracts of *Clitoria ternatea* root as well as *Artemesia vulgaris* leaves. In comparison to the toxic control group, group IV treated with 200 mg/kg petroleum ether extract of *Cucurbita maxima* seeds showed a good protective impact.

In this work, histopathological abnormalities in the rat brain were detected when repeated doses of all three plant extracts (200 mg/kg) were administered over 28 days.

Many pathological alterations were detected in the brain of the experimental animal. The current research examined the severity of histopathological alterations that repeated exposure to plant extracts had a protective effect(Fig 8)

DISCUSSION

For the bulk of the world's population, herbal treatments are still significant. Herbs as well as herbal-based compounds have received a lot of interest in recent years as an alternative source of treatments for a variety of disorders owing to their wide pharmacological properties and fewer side effects. Herbal remedies were utilized for the prevention and treatment of neurodegenerative illnesses for many decades as viable anti-inflammatory and anti-oxidant agents. Herbal medications are in high demand due to their effectiveness and lack of/minimal negative effects. Certain herbals might be utilized to ease and control symptoms depending on their active constituents as well as therapeutic activities. Phytochemical analysis reveals that all extracts of *Cucurbita maxima* seeds, *Artemesia vulgaris* leaves, and *Clitoria ternatea* root contain high levels of phytochemicals such as proteins, fats, flavanoids carbohydrates, and phenols.

The current work demonstrates that the extract of *Artemesia vulgaris* leaves, *Clitoria ternatea* root, and *Cucurbita maxima* seeds have contained different phytoconstituents. The existence of fatty acids, flavanoids, as well as phenolic compounds, among others, may be responsible for the antioxidant and anti-inflammatory characteristics, and effectiveness was assessed by behavioral experiments in terms of muscle strength and muscle coordination, which underline the protective impact. After *In-vivo* and *In-vitro* findings the petroleum ether extract of *Cucurbita maxima* seeds has demonstrated substantial action towards remyelination when compared to the *Artemesia vulgaris* leaves and *Clitoria ternatea* root. The various extracts of *Clitoria ternatea*

root, *Cucurbita maxima* seeds, and in *vitro* anti-oxidant as well as anti-inflammatory activity on RAW264.7 macrophage cells and N2a cell lines demonstrate that the methanol extracts and petroleum ether of three chosen plants have a protective impact. The petroleum ether extract of *Cucurbita maxima* seeds outperforms the other extracts in terms of antioxidant and anti-inflammatory properties. This might be due to the fact that it contains fatty acids.

In vivo studies employing Wistar rats, divided into Ethidium bromide + AV group, Ethidium bromide + CT group, Ethidium bromide group, Ethidium bromide + CM group, Ethidium bromide + Fingolimod group, and control group, each comprising six animals, for the behavior analysis over 28 days is revealing that the petroleum ether extract is having a protective impact among all the extracts. The levels of SOD and MDA, as well as the levels of inflammatory markers IL-6 and TNF-α, have all decreased in brain and cortex homogenate and cells, according to biochemical parameters tests. The petroleum ether extract of *Cucurbita maxima* has been revealed to have remyelination potential in histopathological research.

To conclude**,** the current study demonstrates that the extract of *Artemesia vulgaris* leaves, *Clitoria ternatea* root, and *Cucurbita maxima* seeds have contained different phytoconstituents. The antioxidant and anti-inflammatory properties may be due to the presence of fatty acids, flavanoids, and phenolic substances. Furthermore, effectiveness was assessed by behavioral experiments that focused on muscle strength and coordination, highlighting the protective impact. When compared to *Clitoria ternatea* root and *Artemesia vulgaris* leaves, the petroleum ether extract of *Cucurbita maxima* seeds showed substantial action towards remyelination in both *in vitro* and *in vivo* studies.

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REFERENCES

1. Pan S, Litscher G, Gao S. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. Evid Based Complement Altern Med. 2014;1-20.
2. World Health Organization.WHO Traditional Medicine strategy 2014-23. Geneva: World Health Organization.2013.
3. Mozafari S, Sherafat MA, Javan M, Mirnajafi-zadeh J, Tiraihi T. Visual evoked potentials and MBP gene expression imply endogenous myelin repair in adult rat optic nerve chiasm following local lysolecithin induced demyelination. Brain Res. 2010;1351: 50-56.
4. Kouhsar SS, Karami M, Tafreshi AP, Roghani M, Jalali Nadoushan MR. Microinjection of L-arginine into corpus callosum cause reduction in myelin concentration and neuroinflammation. Brain Res. 2011; 1392: 93-100.
5. Patel JR, Klein RS. . Mediators of oligodendrocytes differentiation during remyelination. FEBS Lett. 2011;585: 3730-3737.
6. Sartori E, Edan G. Assessment of cognitive dysfunction in multiple sclerosis. J Neurol Sci.2006; 245: 169-175.
7. Glanz BI, Healy BC, Hviid LE, Chitnis T, Weiner HL. Cognitive deterioration in patients with early multiple sclerosis: a 5-year study. J Neurol Neurosurg Psychiatry. 2012;83:38-43.
8. Ziehn MO, Avedisian AA, Tiwari-Woodruff S, Voskuhl RR. Hippocampal CA1 atrophy and synaptic loss during experimental autoimmune encephalomyelitis, EAE Lab Invest 2010;. 90:774-786.
9. Goudarzvand M, Javan M, Mirnajafi-Zadeh J, Mozafari S, Tiraihi T. Vitamins E and D3 Attenuate demyelination and potentiate remyelination processes of hippocampal formation of rats following local injection of ethidium bromide. Cell Mol Neurobiol. 2010;30:289- 299.
10. Kim DY, Hao J, Liu R, Turner G, Shi FD, Rho JM. Inflammation-mediated memory dysfunction and effects of a ketogenic diet in a murine model of multiple sclerosis. Plo One.2012; 7:e35476-e35484.
11. Acar A, Cevik MU, Evliyaoglu O, Uzar E, Tamam Y, Arıkanoglu A. Evaluation of serum oxidant/antioxidant balance in multiple sclerosis. Acta Neurol Belg.2012; 112: 275-280.
12. Van Horssen J, Schreibelt G, Drexhage J, Hazes T, Dijkstra CD, van der Valk P. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. Free Radic Biol Med. 2008; 45:1729-1737.
13. Abdel-Salam OM, Khadrawy YA, Mohammed NA. Neuroprotective effect of nitric oxide

donor isosorbide - dinitrate against oxidative stress induced by ethidium bromide in rat brain. EXCLI J. 2012;11:125-141.
14. Susan S Baker, Robert D. Antioxidant enzymes in the differentiated CACO- 2 cell line. In vitro cell. Dev. Biol. 1992;28: 643-647.
15. Nayan NS, Yazid MA, Nallappan K, Amran AA, Zaidi NS, Zakaria F ,et al. In Vitro Modulation of Endogenous Antioxidant Enzyme Activities and Oxidative Stress in Autism Lymphoblastoid Cell Line (ALCL) by Stingless Bee Honey Treatment. Oxidative medicine and cellular longevity. 2020.
16. Tavakkol J Afshari, Ghomian N, Shameli A, Shakeri MT, Fahmidehkar MA, Mahajer E. Determination of Interleukin-6 and Tumour Necrosis Factor-alpha concentrations in Iranian- Khorasanian patients with preeclampsia BMC Pregnancy and Childbirth. 2005; 5:14.
17. Omar ME, Abdel-Salam. Neuroprotective effect of nitric oxide donor isosorbide-dinitrate against oxidative stress induced by ethidium bromide in rat brain EXCLI Journal.2012; 11:125-141.
18. Loy DN, Talbott JF, Onifer SM, Mills MD. Both dorsal and ventral spinal cord path way contributes to over ground locomotion in adult rat. Exp Neurology.2002; 7716.
19. Jaiswal AK, Bhattacharya SK. Effect of Enilagit as memory anxiety and brain monoamine in rats, Ind. J. Pharmacol. 1992; 24: 12-17.
20. Vogal HG, Wolf gang HV. Drug discovery and Evaluation, Pharmacological assay. Verlog Heidel berg. 1997; 211-212.
21. Omar ME, Abdel-Salam. Neuroprotective effect of nitric oxide donor Isosorbide-dinitrate against oxidative stress induced by ethidium bromide in rat brain EXCLI Journal .2012; 11:125-141.