EVALUATION OF TAURINE AND TRYPTOPHAN AGAINST CUPRIZONE INDUCED MULTIPLE SCLEROSIS MODEL ON SWISS ALBINO MICE

A Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,

CHENNAI -600 032

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

BRANCH VI – PHARMACOLOGY

Submitted By

HALA K. SALIM

REGISTERNO:261725009

Under the guidance of

Dr.P.MURALIDHARAN,M.Pharm.,PhD.

Department of Pharmacology

DEPARTMENT OF PHARMACOLOGY,

C.L.BAID METHA COLLEGE OF PHARMACY ,JYOTHI NAGAR,

OLD MAHABALIPURAM ROAD, THORAPAKKAM, CHENNAI – 600097

April – 2020

C.L. Baid Metha College of Pharmacy

An ISO 9001:2008 approved institution

Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Dr.P.Muralidharan, M.Pharm., Phd

Prof & Head

Department of Pharmacology

CERTIFICATE

This is to certify that Project entitled **EVALUATION OF *TAURINE* AND *TRYPTOPHAN* AGAINST CUPRIZONE INDUCED MULTIPLE SCLEROSIS MODEL ON SWISS ALBINO MICE** submitted by Register No: **261725003** in partial fulfillment of the course for the award of the degree of **Master of Pharmacy in Pharmacology** was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97 under my guidance during the academic year 2019-2020.

(Dr .P. MURALIDHARAN)

Place : Chennai

Date :

Prof. Dr. GRACE RATHNAM,

M. Pharm.,Ph.D.

Principal

CERTIFICATE

This is to certify that Project entitled **EVALUATION OF TAURINE AND TRYPTOPHAN AGAINST CUPRIZONE INDUCED MULTIPLE SCLEROSIS MODEL ON SWISS ALBINO MICE** submitted by Register No: **261725009** in partial fulfillment of the course for the award of the degree of **Master of Pharmacy in Pharmacology.** It was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97.under the supervision of **Professor Dr.P.Muralidharan** during the academic year 2019-2020.

Place : Chennai

Date :   **(Prof. Dr. GRACE RATHNAM)**

.

DECLARATION

Register No: **261725009** hereby declare that this dissertation entitled **EVALUATION OF TAURINE AND TRYPTOPHAN AGAINST CUPRIZONE INDUCED MULTIPLE SCLEROSIS MODEL ON SWISS ALBINO MICE** submitted by Register No: **261725009** has been originally carried out by me under the guidance and supervision of **Prof. Dr.P.Muralidharan, M.Pharm,. PhD,** Head of the Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai-97 for the academic year 2019-2020. This work has not been submitted in any other degree at any other university.

Place : Chennai   **HALA K. SALIM**

Date :   Register No:**261725009**

ACKNOWLEDGEMENT

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

ABBREVIATIONS

5-HT	5 – hydroxy tryptamine
Ab	Antibody
ANOVA	Analysis of variance
APC	Antigen presenting cells
BBB	Blood Brain Barrier
BDNF	Brain derived neurotrophic factor
CC	Corpus callosum
CMV	Cytomegalovirus
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CPZ	Cuprizone
EBV	Epstein Barr virus
FDA	Food and Drug Administration
HHV	Human Herpes Virus
HLA	Human leukocyte antigen
IFN-y	Interferon gamma
IL	Interleukin
LP	Lumbar puncture
mAB	Monoclonal antibody

MAG	Myelin Associated Glycoprotien
MAOI	Monoamine oxidase inhibitor
MAP	Mycobacterium Avium Paratuberculosis
MBP	Myelin basic protein
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
NAD	Nicotinamide adenine dinucleotide
NAS	N – acetyl serotonin
NK	Natural killer cells
NMDA	N-methyl-D-aspartate
OS	Oxidative stress
PML	Progressive multifocal ukoencephalopathy
PP	Proteolipid protein
PPMS	Primary Progressive Multiple Sclerosis
PRMS	Progressive Relapsing Multiple Sclerosis
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RRMS	Relapsing/Remitting Multiple Sclerosis
SEM	Standard error mean
SPMS	Secondary Progressive Multiple Sclerosis
TAU	Taurine
TCR	T cell receptor

TFH	Follicular helper T cells
TH	T helper cell
TLR	Toll like receptor
TRP	Tryptophan
Treg	Regulatory T cells
UVR	Ultraviolet radiation

INTRODUCTION

1.1. MULTIPLE SCLEROSIS

Multiple sclerosis (MS), the most prevalent neurological disability, is an autoimmune-mediated disorder that affects the central nervous system (CNS) and often leads to severe physical or cognitive incapacitation as well as neurological problems in young adults. Multifocal zones of inflammation due to focal T-lymphocytic and macrophage infiltrations, and oligodendrocyte death are the primary causes of myelin sheath destruction that result in the formation of CNS plaques composed of inflammatory cells and their products, demyelinated and transected axons, and astrogliosis in both white and gray matter. These lesions can cross-talk with the correct transmission of nerve impulses and lead to neuronal dysfunction such as autonomic and sensorimotor defects, visual disturbances, ataxia, fatigue, difficulties in thinking, and emotional problems[1].
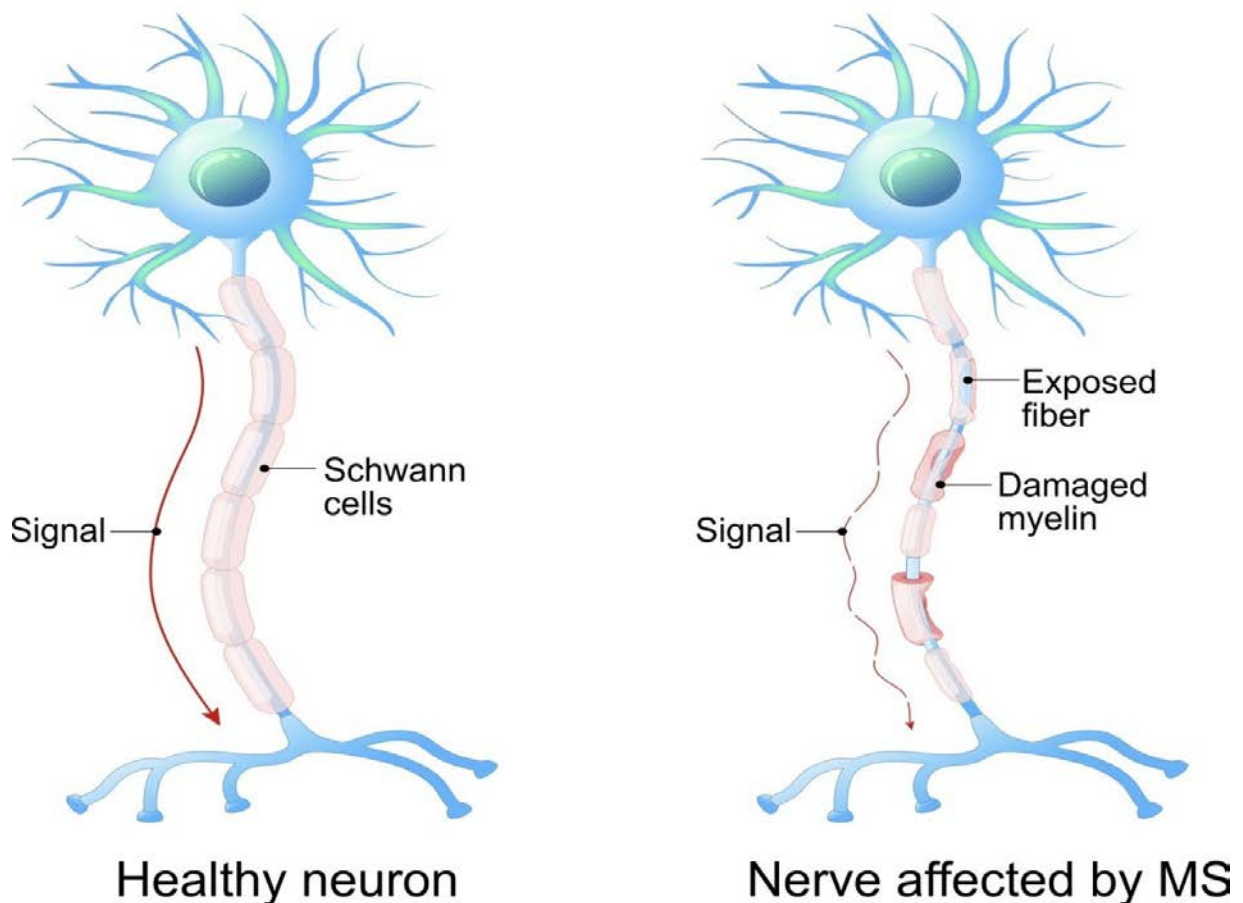
Figure 1.Nerve fibres in MS

Multiple sclerosis can be grouped into four major categories based on the course of disease:

1. RELAPSING–REMITTING MS:

It is the most common form, affecting about 85% of MS patients. It is marked by flare-ups (relapses or exacerbations) of symptoms followed by periods of remission, when symptoms improve or disappear.[2]

During RRMS, inflammatory attacks on myelin and nerve fibers occur. Activated immune cells cause lesions in the CNS which generate symptoms of visual impairments, tingling and numbness, episodic bouts of fatigue, intestinal and urinary system disorders, spasticity, and learning and memory impairment.[1]

2. SECONDARY PROGRESSIVE MS:

It may develop in some patients with relapsing–remitting disease. For many patients, treatment with disease-modifying agents helps delay the progression. The disease course continues to worsen with or without periods of remission or leveling off of symptom severity (plateaus).[2] Nearly 65% of patients with RRMS will subsequently develop SPMS which is considered the second phase of this disease. Many individuals experience increased weakness, intestinal and urinary system disorders, fatigue, stiffness, mental disorders, and psychological impairment.

3. PRIMARY PROGRESSIVE MS:

Approximately 10-15% of MS patients are diagnosed with PPMS which largely affect the nerves of the spinal cord. PPMS patients tend to have fewer brain lesions. Induced symptoms include problems with walking, weakness, stiffness, and trouble with balance[1]

4. PROGRESSIVE-RELAPSING MS:

 A rare form,affecting fewer than 5% of patients. It is progressive from the start, with intermittent flare-ups of worsening symptoms along the way. There are no periods of remission[2].

1.2 .RISK FACTORS

Up to date, there are no reasons for MS incidence, however, there is a combination of environmental and hereditary factors including pollution, viral and bacterial infections, and stress may be included as risk factors [3].

ENVIRONMENTAL FACTORS

Environmental factors, including exposure to viral and bacterial agents such as Epstein Barr virus (EBV), human herpes virus type 6, and mycoplasma pneumonia, in addition to smoking , vitamin deficiency, diet, and exposure to UV radiation are associated with the onset of MS.

The foreign agents may have a nuclear antigen that is structurally homologous with myelin sheet components such as proteolipid protein, myelin basic protein, and myelin-associated glycoprotein.Thus, when immune cells are activated by these pathogens, myelin sheath lesions are formed. Currently, evidence suggests that smoking, due to nitric oxide (NO) and carbon monoxide (CO) production, plays an important role in MS. NO is a toxic soluble gas that in pathological concentrations can damage neurons and oligodendrocytes .Lipid peroxidation and mitochondrial damage that result from NO can lead to oligodendrocyte apoptosis,axonal degeneration, and demyelination .

Vitamin deficiency (especially vitamins D and B12) are considered risk factors for MS. Vitamin D comprises a group of fat-soluble secosteroids that include vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol).

Vitamin B_{12} is an important factor in the generation of myelin shell components. Thus, deficiency of this vitamin can be a major cause for neurological diseases such as MS. Beyond vitamin deficiency, low-term sunlight exposure has been identified as a potential risk factor for MS. In justifying this relationship, it can be said that sun light is a principal source of vitamin D3 and via induction of T regulatory (Treg) cells and anti-inflammatory cytokines such as IL-10 and TNF-α, it may have immunomodulatory effects in MS.

According to previous reports, diet could be an environmental factor involved in MS .Studies reported a significant negative association between MS risk and high fish intake , a positive significant association between high animal fat-based caloric intake and MS risk , a non-significant lower risk between incidence of MS and a higher intake of linoleic acid, and a positive significant association between obesity in adolescent girls and MS risk .

Figure 2.Risk factors of MS

GENETIC SUSCEPTIBILITY

A genetic predisposition may be involved in MS. Studies show that the risk of MS in family members of a patient depends on the amount of genetic information they share. Thus, the risk rate in monozygotic-twins that have 100% genetic similarity is approximately 25%. In all individuals who have 50% genetic similarities such as dizygotic twins and first degree relatives, this risk is 2-5%. In addition, the risk in second degree relatives with 25% genetic similarity is 1-2%, whereas in third degree relatives with 12.5% genetic similarity, this risk is less than 1%[1] .

MICROBIAL INFECTION

Microbial infections have been implicated in initiating and enhancing severity of autoimmune diseases including the demyelinating disease multiple sclerosis (MS). Nevertheless, the incidence of both acute and persisting viral infections without evidence of autoimmune sequelae suggests that this process is well controlled [4]. Many studies have suggested that there is an association between episodes of MS exacerbation and concomitant viralor microbial infections. It is thus reasoned that other antigens, such as those that are constituents of infective organisms, including viruses, could be cross-reactive with the major autoantigens of MS and as such, these mimics should be considered as potential T-cell targets. This view is supported by a number of other studies that have shown that T-cells reactive to brain antigens other than those associated with myelin can cause a breakdown of the BBB. In this regard, autoantigens from several types of microorganisms may be involved. For example, the 70-KDa heat shock protein of Mycobacterium tuberculosis and Mycobacterium leprae have been considered to be potential autoantigens in MS. In addition, a 28-KDa protein surface molecule of mycobacteria has been shown to mediate neuronal Schwann cell myelin forming cell entry or invasion [5,6].

Although T-cells specific for MBP have been derived from MS brain tissue, the evidence for MBP to serve as the sole autoantigen in MS is lacking. Moreover, because T cells from MS patients reactive to MBP also react strongly to peptides from a number of common viruses, it is not clear whether the primary antigen source is MBP or another viral peptide. The possible viral/microbial-associated etiology for MS is considered to be based on

    i.   epidemiological evidence of childhood exposure to infectious agents and increase in disease exacerbations with viral infection;

   ii.   geographic association of disease susceptibility with evidence of MS clustering;

  iii.   evidence that migration to and from high risk areas influences the likelihood of developing MS;

   iv.   abnormal immune response to a variety of viruses; and

   v.   analogy with animal models and other human diseases in which viruses can cause diseases with long incubation periods, relapse, and demyelination.

Many of these studies involve the demonstration of increased antibody titers to a particular virus, whereas some describe isolation of virus from MS material. Recently, the human herpes virus HHV-6 and HHV-8 have been reported to be present in active MS plaques. HHV shares some homology with cytomegalovirus CMV, and HHV has been considered as a possible viable etiologic agent in MS for several reasons, including

i. primary infection with HHV usually occurs during the first few years of life and is consistent with epidemiological evidence for MS, suggesting childhood exposure to an etiologic agent;

ii. HHVs, in general, are highly neurotropic and HHV proteins have been shown to be preferentially expressed by oligodendrocytes within MS lesions;

iii. a fundamental property of HHV is its ability to reactivate, and the same factors that lead to reactivation stress, infections have also been associated with MS exacerbations; and

iv. HHV has been found to infect cells of both lymphoid and non lymphoid origin. Therefore, it has been reasoned that a pleiotropic virus such as HHV that infects both the immune and the nervous systems could explain abnormalities in both[7].

The infectious disease paratuberculosis affects wild and domestic ruminants. This disease induced by Mycobacterium Avium Paratuberculosis (MAP), is correlated to MS incidence. In a study, 56.57% and 66.60% of cheese tested showed positive results for MAP and these can lead to increased incidence of MS in human[8].

1.3 .EPIDEMIOLOGY

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) and the most common cause of non traumatic disability in young adults. The incidence of MS is low in childhood and increases after the age of 18, reaching a peak between 20 and 40 years (mean age of 30 years) with women being affected approximately 2–5 years earlier than men[9]. After wards, the incidence declines, becoming rare at ages above 50 years.

Across Europe, the prevalence rate of MS is about 83:100,000, and the mean annual incidence rate is about 4.3 : 100,000 [10] . MS is more common in women than in men, and has increased over the last decades from a female-to-male ratio of 1.4 in 1955 to 2.3 in 2000. This corresponds to a lifetime risk of 2.5% in women compared to 1.4% in men[11]. The increasing overall prevalence seen in longitudinal studies is due to the longer life expectancy of MS patients and advances in diagnosing MS rather than an increased overall disease risk. Life expectancy in MS patients is reduced by 7–10 years. The standardized mortality ratio is increased threefold, but this has improved over the last decades[12].

The role of genetics and environmental factors in the susceptibility of MS remains complex and poorly understood. It appears that factors such as geographical location, ethnic background, and clustering in temperate climates all contribute indirectly to MS susceptibility. In particular, individuals with a north European heritage appear to be statistically more susceptible to MS than those from a more tropical environment and it is more common in women. It has been estimated that approximately 1 in 1000 persons of northern European origin who reside in temperate climates are more likely to develop MS.

Epidemiological data from multiple whole-genome screens in multiplex families indicated that, in all probability, MS is not a single-gene disorder and, in addition, environmental factors contribute to the syndrome. It has been suggested that interactions between different genes could contribute to an increase in susceptibility. Therefore, the contribution of genetic factors are complex. Strong evidence for potential genetic contributions comes from studies on families with twins. The risk of MS was found to increase with the degree of shared genetic information within a family and that multiple genes were thought to interact to increase susceptibility. In a

comparative analysis of monozygotic and dizygotic twins, the risk for dizygotic twins and siblings was smaller but still increased 20-40-fold compared with that of the general population. Data from concordance rate analysis of individual twins indicated that genetic background as well as exogenous or somatic events appear to be essential for disease development[7].

Countries with the highest prevalence rates of multiple sclerosis as of 2015 (per 100,000 people)

Country	Cases per 100,000 people
Canada	291
San Marino	250
Denmark	227
Sweden	189
Hungary	176
Cyprus	175
United Kingdom	164
Czech Republic	160
Norway	160
Germany	149

Cases per 100,000 people
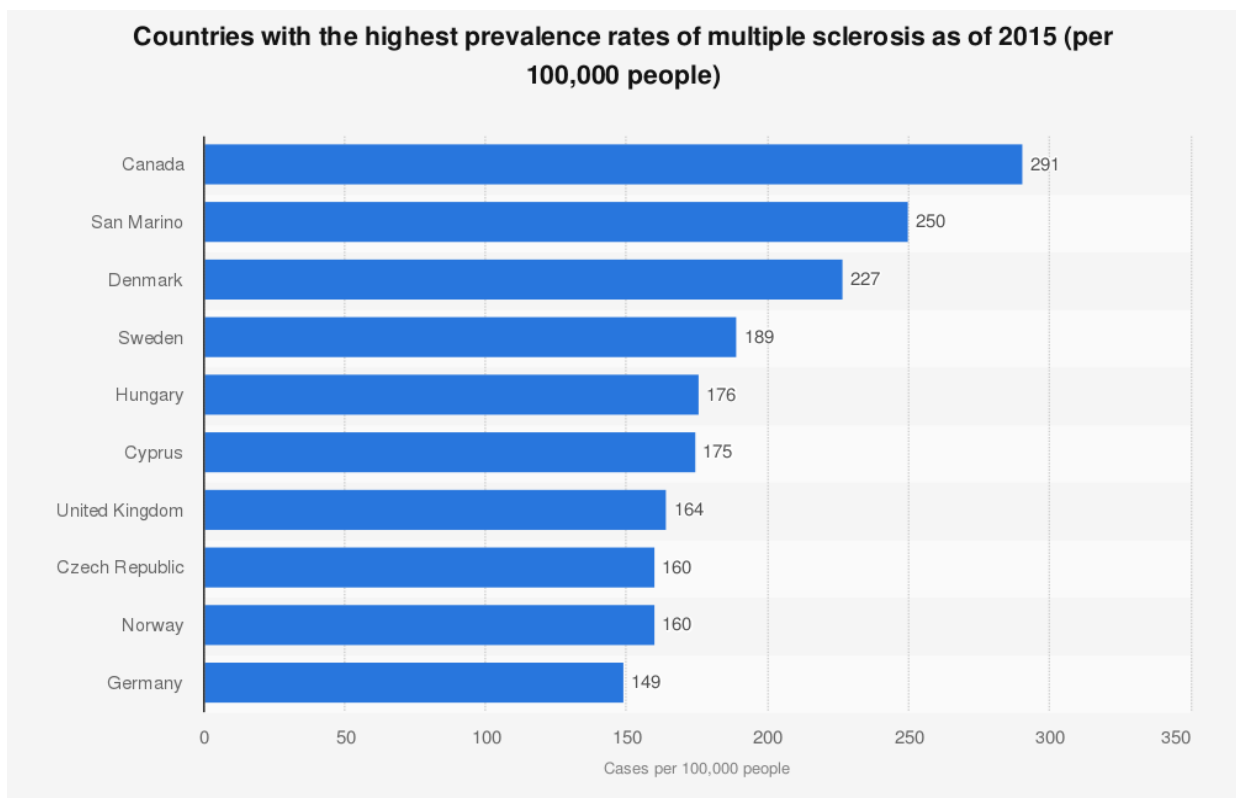
Figure 3. Highest MS prevalence countries

1.4. PATHOGENESIS OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a chronic, inflammatory demyelinating disease of the CNS, characterized by infiltration of T-lymphocyte, B-lymphocytes, macrophages, NK cells, demyelination, and axonal damage[12].Inflammation of central nervous system is the primary cause of damage in MS. The specific elements that start this inflammation are still unknown. Studies have suggested that genetic, environmental and infectious agents may be among the factors influencing the development of MS. Many immunological studies have been done on the animal model for human MS.Based on this and observations of MS in humans, roles of several immunological pathways involved in MS are being explored. To understand these pathways it is important to first understand some basic points of the immune system in MS.

There are two general types of immune response. These are the innate and adaptive immune responses. The innate immune response is initiated by microbial products that activate specific receptors, mainly toll-like receptors (TLRs) in an antigen nonspecific manner. Activation of specific subsets of TLR is done by pathogen-associated molecules that are unique for different groups of pathogens. Binding of these molecules to TLRs results in the production of cytokines that modulate the adaptive immune response .The innate system plays a role both in the initiation and progression of MS by influencing the effector function of T and B cells.For example, when activated through TLRs, dendritic cells (DCs) become semi- mature and induce regulatory T cells to produce inhibitory cytokines such as IL-10 or TGF-β . As the dendritic cells continue to mature, they start to polarize CD4+ T cells to differentiate into either Th1, Th2 phenotypes or Th17 phenotypes. When T cells differentiate to a Th1 phenotype, inflammation is promoted.

The adaptive response is initiated by the presentation of a specific antigen to T lymphocytes by antigen presenting cells (APCs). These antigen presenting cells include B cells, dendritic cells, microglia and macrophages. The interaction between the APC and T cell is a chief component to initiate the adaptive immune response. Several types of T cells including CD4+ and CD8 + phenotype can be activated by APCs. Th1, Th2 cells and Th17 are CD4 + effector cells that are polarized in response to exposure to specific interleukins. Once polarized to Th1, Th2 or Th17, these effector T cells secrete specific cytokines. The cytokines produced by Th1 cells are

proinflammatory cytokines such as interferon gamma, whereas Th2 cells secrete anti –inflammatory cytokines such as IL-4 and IL-13. Th17 is a newly recognized CD4 + T cell subset that produces IL-17, IL-21, IL-22 and IL-26. Like Th1 cells, Th17 cells promote inflammation in MS. IL-17 receptors are seen in acute and chronic MS plaques.Th1 cells and Th17 cells migrate to the central nervous system after being primed in the periphery. Subsequent demyelination and axonal loss is then seen.

Regulatory T cells (T reg) are another CD4+ T cell type involved in the pathogenesis of MS. The role of T reg cells is to regulate effector Th1, Th2 and Th17 cells. The number of T reg cells is the same between MS patients and controls, however patients with MS have reduced T reg function . IFN-γ enhances CD4+ regulatory T-cell function . Besides the involvement of CD4+ T cells in MS pathogenesis, studies have shown that CD8+ T cells are present in MS lesions and may have regulatory function in the progression of disease. CD8+ cells mediate suppression of CD4+ T cell proliferation through the secretion of perforin, which is cytotoxic on CD4+ T cells, leading to their inactivation.

Also, CD8 +cells kill glial cells, leaving axons exposed. Moreover, CD8+ T cells transect axons, promote vascular permeability and activate oligodendrocyte death. All of these events are seen in MS lesions. In addition to T cells, B cells and their products are involved in the pathogenesis of MS. It has long been recognized that B cells become plasma cells that make antibodies. The presence of these polyclonal antibodies in the cerebrospinal fluid of MS patients is known as oligoclonal bands. The target of these antibodies is not yet known. Besides the production of antibodies, B cells are now known to produce proinflammatory (lymphotoxin, TNF-alpha produced by memory B cells) and anti-inflammatory (IL-10) cytokines (produced by naïve B cells)[13]. A schematic overview of the roles of immune cells in MS pathogenesis is represented in Figure 4.

THE MITOCHONDRIAL DYSFUNCTION THEORY IN MS PATHOGENESIS

Mitochondria play a significant role in synthesizing adenosine triphosphate and providing energy to the cells. They possess their own DNA and are genetically independent organelles. Moreover, they are involved in apoptosis and metabolism of fatty acids. An oxidative energy metabolism is required for the lifespan of neurons

while the large amount of adenosine triphosphate is produced during oxidative phosphorylation. In this reaction, the greatest amount of harmful ROS and RNS is formed. In the case of the disturbed mitochondrial antioxidant production, the following are observed: decreased adenosine triphosphate synthesis, impaired Ca2+, and elevated ROS and RNS. Mitochondrial dysfunction plays a particular role in inflammatory processes. In the case of mitochondrial dysfunction, an overproduction of toxic ROS and RNS is observed.It plays a pivotal function in myelin and oligodendrocyte loss which is detrimental to neurons and glia. Mitochondrial disturbances cause many neurodegenerative processes, including DNA damage, insufficient mitochondrial enzyme activity, abnormal mitochondrial gene expression, and defective DNA repair mechanism. As a result, mitochondrial damage in MS was considered to play an important role in disease progression.OS leads to mitochondrial damage, thus disrupting transport of adenosine triphosphate along axons, resulting in neurodegeneration. Faulty mitochondrial DNA was reported as the consequence of oxidative and nitrosative stress. In addition, recent findings in EAE suggest that mitochondrial dysfunction occurs in the early stage of MS. Interestingly, mitochondrial damage seems to develop before the inflammatory process in the disease[14].
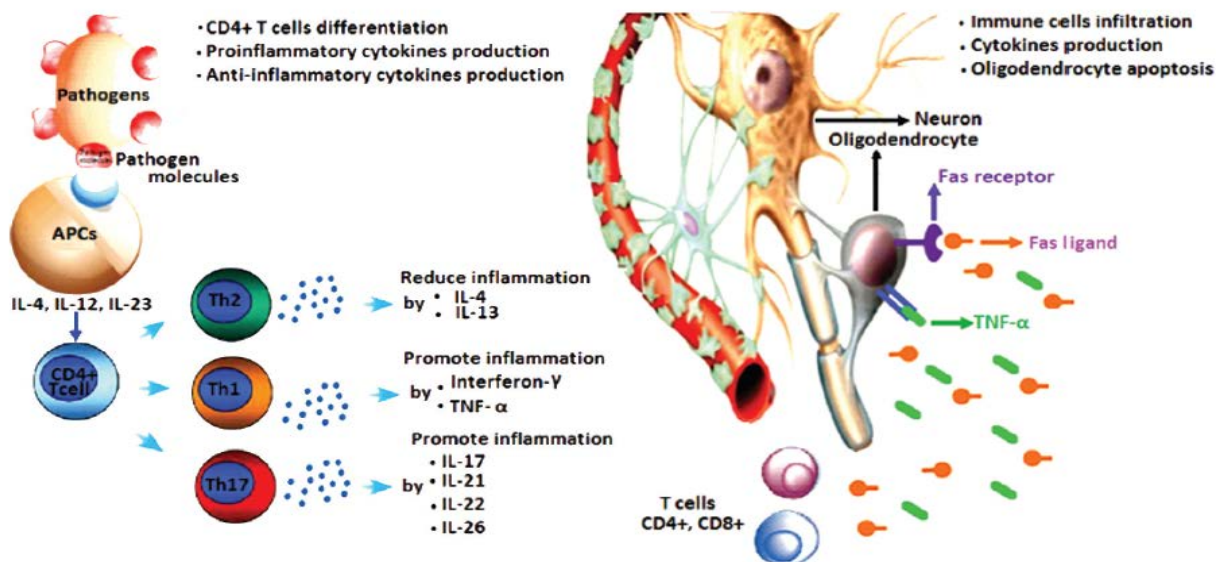
Figure 4. Role of immune cells in MS pathogenesis

1.5.CLINICAL MANIFESTATIONS

Usually, MS symptoms are unpredictable and uncertain. Since this disease can affect any region of the CNS, it can generate almost any neurologic symptom. In addition, symptoms vary greatly among patient and within one patient over time.During the course of MS, some abnormalities appear to be more dominant or have a greater effect on functional ability. Fig 3 depicts the more common symptoms of MS that may appear during different courses of the disease.

More than 30% of MS patients have moderate-to severe spasticity, mostly in the legs. Initial clinical findings in MS patients are often sensory disturbances, the most common of which are paresthesias (numbness and tingling), dysesthesias (burning and "pins and needles"),diplopia, ataxia, vertigo, and bladder (urinary sphincter) disturbances. A common manifestation of MS is unilateral numbness affecting one leg that spreads to involve the other leg and rises to the pelvis, abdomen, or thorax. Sensory disturbances usually resolve but sometimes evolve into chronic neuropathic pain. Trigeminal neuralgia also occurs. Another common presenting sign of MS is optic neuritis, highlighted by complete or partial loss of vision.

Bladder dysfunction occurs in more than 90% of MS patients and results in weekly or more frequent episodes of incontinence in one-third of patients. At least 30% of patients experience constipation. Fatigue occurs in 90% of patients and is the most common work-related disability associated with MS. Sexual problems are often experienced as well.
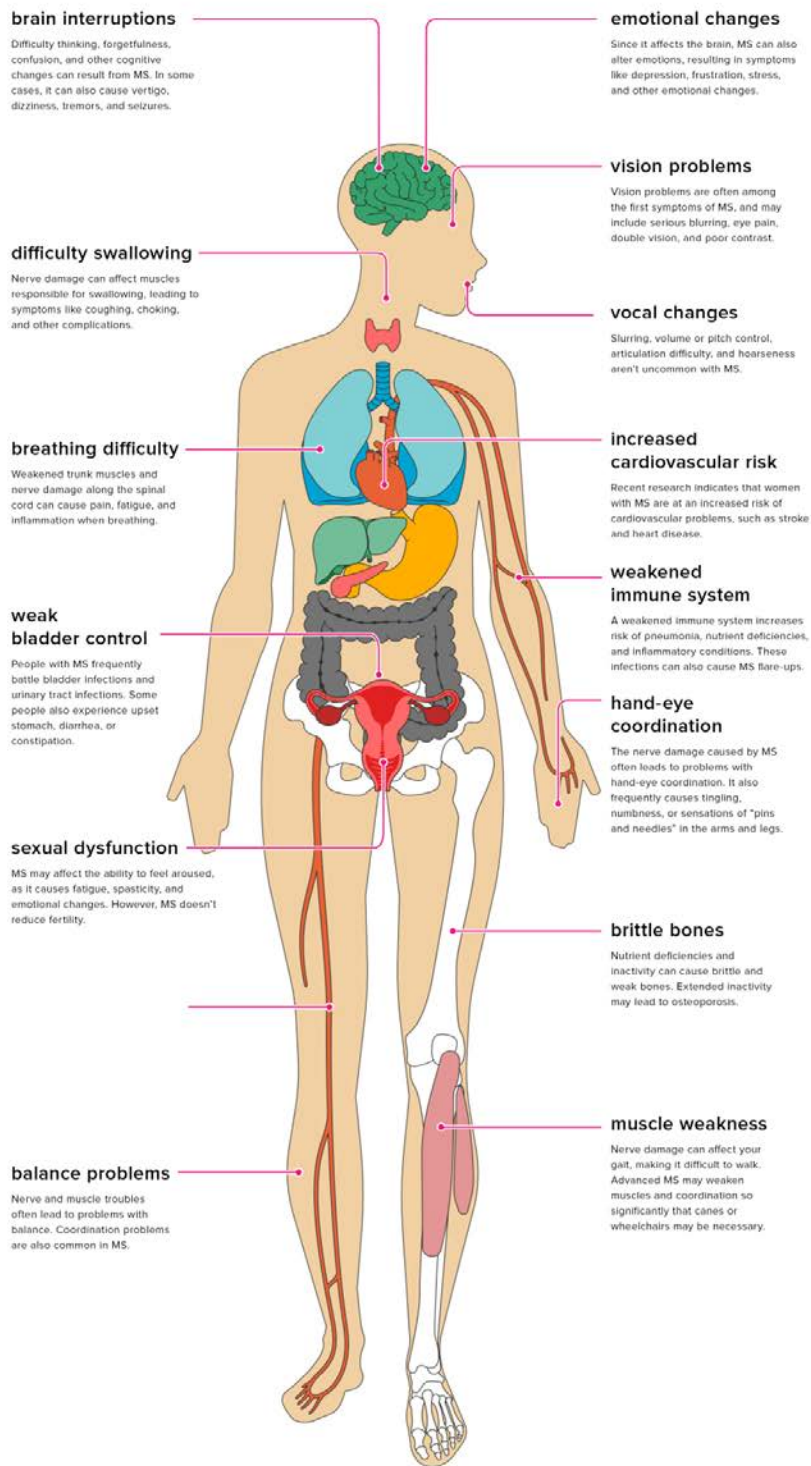
Figure 5.Clinical features of MS

1.6. DIAGNOSIS OF MULTIPLE SCLEROSIS

Early detection of MS is important because it gives us the opportunity to seek treatment and plan for the future. An exact diagnosis of MS is based on medical history and neurological examination using imaging techniques such as magnetic resonance imaging (MRI), lumbar punctures (LP) for cerebrospinal fluid (CSF) analysis, evoked potentials, and blood samples analysis. Obtaining a history about the onset of the first symptoms, any neurological disorders as well as illnesses such as diabetes and thyroid diseases, food habits, geographic locations, and history of medications taken and substance abuse is important. In addition, an eye examination and evaluation of Babinski's reflexes can be useful. MRIs can identify any scar tissue formation and damage in the CNS. Evoked potentials test that include visual, brain stem auditory, and somatosensory evoked potentials offers information about demyelination in the optic nerve and CNS. In addition, CSF analysis for myelin basic protein and immunoglobulingamma (IgG) determinations and blood sample analysis for detect of vitamin deficiencies may be diagnostically helpful.[1]

1.7. DRUG THERAPY FOR MS

There are currently 8 FDA approved agents for relapsing forms of MS. No agents are FDA approved for the primary progressive version of MS. FDA approved agents include four preparations of interferon-beta , glatiramer acetate, mitoxantrone, and natalizumab and the recently approved first oral medication fingolimod. Many other immunologically active agents are used off label and others are nearing study completion and FDA application. The differing types and durations of immunologic effects of these agents will increase the complexity and likely risks of future MS care.

i.   INTERFERON-BETA

Interferon beta (IFN-beta) was first approved by the FDA for MS treatment on 1993. It has been shown to reduce relapse rate, decrease disability progression, and MRI evidence of disease activity. The clinical efficacy of IFN-beta is greater in RRMS than in SPMS. The exact of mechanism of how IFN-beta affects MS is uncertain, however several potential pathways have been postulated. Among these mechanisms, inhibition of T cell activation and proliferation as well as reduction in matrix metalloproteinase activity may be important. IFN-beta has numerous other immunologic effects. It reduces the production of proinflammatory cytokines and induces the production of anti-inflammatory cytokines by increasing suppressor T cell activity. The most common adverse effects of interferon beta are injection site inflammation, headache, and flu like symptoms (fever, myalgia and rigors), fatigue and possibly depression. Other adverse reactions include lymphopenia,thrombocytopenia and elevated liver transaminase levels. Prior to starting IFN-beta therapy and periodically thereafter patients should have a complete blood cell count and hepatic function tests.

ii.   GLATIRAMER ACETATE

Glatiramer acetate (GA), formerly known as copolymer 1, is a random polymer of glutamic acid, lysine, alanine and tyrosine, the most common amino acids in myelin basic protein. The exact mechanism of action is not clear yet.GA inhibits response of various antigen- specific murine T cell hybridomas. It also blunts human myelin basic protein-specific T cell lines from lysing targets in the presence of three human

leukocyte antigen-DR types associated with MS. Further studies have shown that GA increases cytokine levels such as IL-10, TNF-alpha, and IL- 4, thus altering the cytokine population to a more regulatory type [24]. It also increases the expression of Foxp3 in $CD4^+$ $CD25T^+$ regulatory cells, thus increasing anti-inflammatory action. Therefore it appears that GA shifts the GA-reactive lymphocyte population from a proinflammatory Th1 state to an anti-inflammatory Th2 state. Further, GA has been shown to act upon CD8+D T cells by correcting their regulatory deficit.

There is one formulation of GA marketed as *Copaxone*, and it is currently approved for the treatment of RRMS. It has proven effects on exacerbation rate and MRI clinical assessment. It is given subcutaneously on a daily basis. Side effects may include erythema, induration, or lipoatrophy at the sites of injections. An immediate post-injection systemic reaction can occur seconds to minutes after injection. The symptoms consist of chest tightness, dyspnea, tachycardia,flushing and palpitations. This reaction lasts 10-20 minutes,and has not been determined to be dangerous to patients. There are no specific blood tests that need to be monitored while the patient is taking GA.

iii.   MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAbs) have been studied since the 1980's. There are three different types of monoclonal antibodies differentiated by their structural similarity to human antibody structure. Humanized antibodies consist of more than 90% human components with the balance from the original murine structure. These antibodies include natalizumab, alemtuzumab and daclizumab. Chimeric antibodies are at least 66% human structure and structure, and rituximab belongs to this class of antibodies. Fully human antibodies have no murine structural components.Each mAb is developed to bind to a specific target molecule. mAb mechanism of action depends on the distribution of the targeted molecule, efficacy of the antibody in reaching the target, the interaction between the antibody and target, and the effector functions of the interaction.To date the four monoclonal antibodies most studied for MS are natalizumab, daclizumab, alemtuzumab and rituximab.

Natalizumab

Natalizumab (*Tysabri*) is a humanized monoclonal antibody with an IgG4 framework. It was specifically designed for the treatment of MS and was FDA

approved on 2004. It was temporarily withdrawn from the market in 2005 after several cases of fatal progressive multifocal leukoencephalopathy were reported in patients treated with natalizumab. It was reapproved on 2006 as a monotherapy for the treatment of RRMS . Its target molecule is CD49, the α 4 subunit of very late antigen-4 (VLA-4) receptor. VLA-4 interacts with vascular cell adhesion molecule-1 so that immune cells can migrate through the blood brain barrier. By binding to CD49, natalizumab prevents the adhesion between the endothelial cell and the immune cell, thus migration of leukocytes into the central nervous system is blocked. This agent has robust benefits on relapse rate, disability progression, and MRI activity. The risk of PML requires clinical surveillance for infection with JC virus while treated with this agent.

Alemtuzumab

Alemtuzumab (*Campath-1H*) is a humanized antibody that was initially approved for the treatment of B-cell chronic lymphocytic leukemia. It remains an investigational agent for MS. Its target molecule is CD52, a glycoprotein expressed widely throughout on T and B cells, natural killer cells, dendritic cells, monocytes, macrophages and granulocytes with the exemption of neutrophils. Alemtuzumab causes a complete depletion of CD52 bearing cells. It depletes cells that mediate Ab-dependent cellular cytotoxicity, i.e. natural killer cells. Studies have shown that the depletion of these immune cells is associated with a decrease in contrast enhancing lesions in MS, thus suggesting stabilization of the blood brain barrier. Despite its efficacy, a number of potentially dangerous adverse effects and complications may occur in patients treated with alemtuzumab and limit its use. Such side effects include idiopathic thrombocytopenic purpura, Graves' disease and Good pasture syndrome, all which are Ab-mediated autoimmune diseases.

Rituximab

Rituximab (*Rituxan*) is a chimeric murine/human IgG1 monoclonal antibody. Its target is CD20, an antigen produced only on mature B cells and not on the Ab-producing plasma cells. This monoclonal antibody is FDA approved for the treatment of rheumatoid arthritis and B cell lymphoma and it remains an investigational agent for treatment of MS. Rituximab's primary mechanism of action is a complete depletion of

B cells. This depletion of B cells occurs within 2 weeks of initiating treatment and can last for more than 6 months. The exact mechanism of benefit with rituximab treatment remains uncertain. Cases of PML have occurred after the use of this agent as well.

Daclizumab

Daclizumab (*Zenapax*) is a humanized monoclonal antibody with an IgG1 framework. It was used initially for the prevention of allogenic tissue transplantation. Its target molecule is CD25, the IL-2 binding epitope of the β-chain of the IL-2 receptor. IL-2 plays an important role both in the regulation of lymphocyte expansion and contraction.

iv.   CYTOTOXIC AND OTHER AGENTS

Clinicians may use a variety of cytotoxic agents for control of MS despite only mitoxantrone having FDA approval for MS. Off label use of other cytotoxic agents is based on smaller studies, and agents most frequently used include cyclophosphamide, azathioprine, methotrexate, and mycophenolate mofetil. The main mechanism of action of all these agents appears to be a more broad immunosuppressive action and these agents not unexpectedly may increase the risk of infections and neoplasia. Other immunomodulatory agents used include intravenous immunoglobulin and corticosteroids. Overall the risks of cytotoxic drugs for treatment of multiple sclerosis are proportional to the length and intensity of immunosuppression. Data regarding their efficacy as monotherapy or in combination with FDA approved immunomodulatory is still limited. Thus, cytotoxic drugs are not the first line agents used in the treatment of MS.

Mitoxantrone

Mitoxantrone (*Novantrone*) is a synthetic anthracenedione that intercalates into DNA. It causes cross-linking and strand breaks and inhibits topoisomerase II, thus interfering with DNA repair. Besides causing generalized immunosuppression, mitoxantrone inhibits monocyte and lymphocyte migration, induces apoptosis of dendritic cells, decreases the secretion of proinflammatory cytokines such as tumor necrosis factor, interleukin-2 and interferon-gamma . It also inhibits B cell function, increases T cell suppressor function and inhibits macrophage mediated myelin

degradation. Mitoxantrone was approved by the FDA on 2000 for SPMS and worsening RRMS . It can be given intravenously every 3 months. Side effects include bone marrow suppression, mild alopecia, nausea and possible transient bluish discoloration of the sclera and urine.Risks also include vacuolar cardiomyopathy, treatment related leukemia, and sterility/ teratogenesis.

Cyclophosphamide

Cyclophosphamide is a synthetic chemical agent related to nitrogen mustards. It is transformed in the liver to active alkylating metabolites to then cross link DNA. In addition to generalized suppression, cyclophosphamide increases Th2 cells and targets selectively CD45+/CD4+/RA T cells. Its side effects include alopecia, nausea, vomiting, amenorrhea, myelosuppression, hemorrhagic cystitis, bladder carcinoma and other secondary cancers, and sterility/teratogenesis. Currently, cyclosphosphamide is used off label for RRMS.

Azathioprine

Azathioprine is a purine antagonist that blocks the de novo pathway of purine synthesis, thus depleting the production of lymphocytes that lack a salvage pathway. Azathioprine is sensitive to inactivation by thiopurine S-methyltransferase polymorphisms. Besides immunosuppression, the side effects include gastrointestinal and hepatic toxicity.

Methotrexate

Methotrexate inhibits the synthesis of DNA, RNA and protein by inhibiting dihydrofolic acid reductase, which is necessary for the synthesis of thymidylate. Through this reaction, lymphocyte production is inhibited, thus reducing inflammation. Side effects include myelosuppression, gastrointestinal toxicity, pneumonitis, and hepatic fibrosis. This agent is most often used orally in lower doses but has also been tried in high dose intravenous regimens.

Mycophenolate Mofetil

Mycophenolate mofetil causes lymphocyte depletion, inhibits T cell activation and B cell function, and inhibits B and T cell migration through the blood brain barrier.

Its mechanism involves the inhibition of inosine monophosphate dehydrogenase activity, which is needed for purine synthesis. Side effects include leukopenia, anemia, headaches and diarrhea.

v.   INTRAVENOUS IMMUNOGLOBULIN

Treatment with intravenous immunoglobulin (IVIG) has been reported as beneficial for treatment of patients with RRMS . The dose ranged widely from 0.2 mg/kg to 2 g/kg. No ideal dosage was determined.

vi.   CORTICOSTEROIDS

Corticosteroids inhibit lymphocyte proliferation and the synthesis of most pro-inflammatory cytokines. Because of their potent anti-inflammatory effects, corticosteroids have been used to treat MS for the last 50 years. Today corticosteroids are standard treatment for patients with acute relapses, however it is not yet clear if they are as efficacious as a long term treatment.The acute and long term risks of corticosteroids are well known and may be serious.

vii.   EMERGING TREATMENTS

To date all the FDA approved treatments for MS are given *via* injection or infusion. A significant amount of research has been done to develop oral agents in the hope of patient compliance and tolerability. Fingolimod is a new oral medication with a recently approved FDA application and is awaiting wider use .A recent placebo controlled trial was done using cladribine in patients with RRMS . Ongoing clinical trials are being conducted with three other oral agents: laquinimod, fumarate (BG00012) and teriflunomide.

Fingolimod

Fingolimod (FTY720) is a structural analogue of sphingosine. It targets receptors of sphingosine-1-phosphate (S1P). S1P is an important signaling lipid involved in the migration of lymphocytes from secondary lymphoid organs to the periphery. Studies have shown that with higher density of S1P, there are more lymphocytes released from secondary lymphoid organs.Other studies have shown that FTY720 inhibits mature dendritic cells migration to secondary lymphoid organs.

FTY720 also improves myelination in animal models of EAE. Risks include transient cardiac conduction changes as well as potentially severe viral infections.

Cladribine

Cladribine (2-chlorodeoxyadenosine) is an adenosine deaminase-resistant purine nucleoside. Cladribine induces apoptosis of lymphocytes by increasing deoxynucleotides levels in these cells. This immune cell depletion results in decrease of inflammation. Cladribine causes sustained reduction in CD4 +cells and CD8 +T cells and transient reduction in CD19+ B cells, without affecting other immune cells. Adverse effects included lymphopenia , and herpes zoster .

Laquinimod

Laquinimod is a derivative of immunomodulatory agent linomide (quinoline-3-carboxamide). Its target has not been identified yet. Studies have shown that laquinimod induces the release of TGF-beta and favors Th2 cytokine production. Preliminary studies have shown that laquinimod decreases MS disease activity without causing generalized immunosuppression.

Fumarate

Fumarate (BG0012) is an immunomodulatory agent whose exact mechanism of action is unknown. Studies have shown that it activates the nuclear factor-E2-related factor 2 (Nrf2) transcriptional pathway.

Teriflunomide

Teriflunomide is an active metabolite of leflunomide. It is currently approved for the treatment for rheumatoid arthritis.Its mechanism of action consists on inhibiting dihydroorotate dehydrogenase, which is a rate limiting step in pyrimidine synthesis .Other actions include suppression of pro-inflammatory cytokines, inhibition of tyrosine kinase activity and inhibition of interaction between APCs and T cells.[13]

1.8. MULTIPLE SCLEROSIS ANIMAL MODELS

Experimental Autoimmune Encephalomyelitis is the most frequently used animal model for human MS. This form of encephalomyelitis pictures an acute, chronic-relapsing, acquired, inflammatory demyelinating autoimmune disease.Animals are injected with proteins that are part of the myelin sheath surrounding axons. Myelin Basic Protien, myelin proteolipid protien, and Myelin Oligodendrocyte Glycoprotien are frequently used antigens. These proteins, when systemically introduced, induce an autoimmune response which, in consequence, leads to an attack on the myelin. EAE can be induced in different animal species including mice, rats, guinea pigs, rabbits, macaques, rhesus monkeys, and marmosets. For various reasons such as the number of immunological tools, availability, lifespan, fecundity, and similarity of the resulting pathology to human MS appearance, mice and rats are the most frequently used species. Other experimental systems for MS studies encompass cuprizone, lysolecithin, or ethidium bromide-induced demyelination and are often pooled together as "toxic demyelination models". Another animal model used for more than 30 years to investigate MS-related symptoms is Theiler's murine encephalomyelitis (TMEV). This model is based on virus-induced demyelination . The cuprizone model is characteristic for toxic demyelination and represents a reversible demyelination and remyelination system.

THE CUPRIZONE ANIMAL MODEL

Cuprizone ingestion in mice induces a highly reproducible demyelination of distinct brain regions, among them the corpus callosum (CC) which represents the most frequently investigated white matter tract in this animal model. After 5–6 weeks of cuprizone treatment, the CC is almost completely demyelinated, a process called "acute demyelination".

Acute demyelination is followed by spontaneous remyelination during subsequent weeks when mice are fed normal chow. In contrast, remyelination is highly restricted when cuprizone administration is prolonged (12 weeks or longer), a process called "chronic demyelination". During late stages of acute demyelination, spontaneous remyelination occurs partially but fails under a continued cuprizone

challenge. First experiments using cuprizone as a toxic compound date back to the late 1960s and were conducted by Carlton He reported that oxalic bis cyclohexylidene hydrazide, a chelator used as a reagent for copper analysis,induces microscopic lesions in the brain accompanied byedema, hydrocephalus, demyelination, and astrogliosis. He administered different doses of cuprizone mixed in basic chow ranging from 0.2 to 0.5%. Mice fed cuprizone showed signs of growth retardation in a dose-dependent manner. At higher concentrations (0.5%), cuprizone caused high mortality especially when introduced in the chow early during development.

Cuprizone induces distinct demyelination in several brain regions such as the hippocampus, the cerebellum, the caudate putamen, and the ventral part of the caudate nucleus. Interestingly, distinct grey matter regions are also affected in the cortex and hippocampus formation. Cuprizone-induced demyelination results from degeneration of supporting oligodendrocytes rather than a direct attack on myelin sheaths.The distal parts of oligodendrocytes appear to be primarily vulnerable before cytopathological changes in the oligodendrocyte cell body can be observed.

The extent of demyelination can be assessed by various laboratory methods including histological procedures (luxol-fast blue staining, LFB), immunohistochemistry (IHC) against the myelin components MBP and PLP, electron microscopy, and gene/protein expression studies.LFB is an alcohol-soluble counterpart of water soluble Alcian Blue. Staining is due to the presence of lipoproteins and an acid–base reaction with salt formation.

Mechanisms of cuprizone-induced demyelination

Cuprizone [oxalic acid bis(cyclohexylidene hydrazide)] is a well-known copper-chelating agent, discovered and described in the early 1950s. The resulting copper–cuprizone complex is characterized by a very intense and unusual absorption band in the visible region, centered at 595 nm. Due to the highly chromogenic reaction of cuprizone with copper (II) ions, cuprizone was largely exploited for the quantitative determination of copper in various samples. Starting from the early 1970s, a lot of interest focused again on cuprizone, mainly within the biomedical scientific community, as this compound was reported to possess unique neurotoxic properties

and, therefore, to serve as a valuable pharmacological tool for CNS demyelination and spongiosis in various laboratory animals.

The underlying mechanisms of cuprizone-induced oligodendrocyte cell death are not fully understood. It is well known that feeding of cuprizone causes the formation of mega-mitochondria in the liver. Liver tissue from cuprizone-fed animals was studied with respect to mitochondrial dysfunction. The formation of mega-mitochondria also termed ''giant mitochondria'' is linked to metabolic injury. Mega-mitochondria reveal short cristae that are restricted to the organelle periphery. The matrix is expanded and lacks membrane-like structures but is of normal density.

Such mega-mitochondria can emerge by two different mechanisms: Normal sized mitochondria are enlarged or mitochondria fuse. Early observations indicate that both mechanisms, mitochondrial fusion and fission, are involved in mega-mitochondria formation in this animal model . However, also the inhibition of mitochondrial division has been postulated as a putative mechanism Since cuprizone induces alterations of mitochondrial morphology, it is speculated that the neurotoxic properties of this copper-chelating compound are due to a disturbance of cellular respiration, a key function of mitochondria. Besides structural changes in mitochondrial morphology, it appears that the activity of a set of enzymes is disturbed prior to myelin loss during the first days or weeks of cuprizone exposure, a decrease in monoamine oxidase and cytochrome oxidase activities in the brain and liver following cuprizone treatment.

Carbonic anhydrase II (CA II) activity declines in the brain of cuprizone- exposed animals well before demyelination develops .This enzyme plays an important role in buffering acute changes in pH in the brain. Disturbance of the intracerebral pH (acidosis) might contribute to oligodendrocyte stress and, in consequence, to cell death. It is still not clear whether the copper chelating nature of cuprizone is implicated in its neurotoxic properties. Additional mechanisms such as inhibition of oligodendrocyte precursor (OPC) differentiation and secretion of pro-inflammatory cytokines from activated microglia/macrophages are also suggested.[15]

DRUG INTRODUCTION

AND

LITERATURE REVIEW
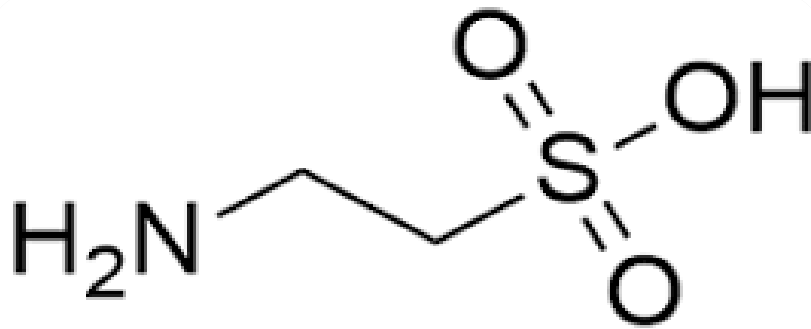
Taurine

2.1.TAURINE

Taurine is one of the most abundant amino acids in the brain and spinal cord, leukocytes, heart and muscle cells, the retina, and indeed almost every tissue throughout the body. It was first identified and isolated from the bile of the ox (Bos taurus), from which it derives its name . The chemical structure of taurine shown, reveals that it lacks the carboxyl group typical of other amino acids, but does contain a sulfonate group.

The major route for the biosynthesis of taurine, shown in Figure 3B is from methionine and cysteine via cysteine sulfinic acid decarboxylase (CSD), and typically requires oxidation of hypotaurine to taurine as the final step. CSD was initially cloned and identified by Reymond et.al 1996 as the rate-limiting enzyme in the biosynthesis of taurine and was later shown to be present in the kidney as well as the brain, where it is localized in glial cells. CSD levels are very low in humans and other primates, but the ingestion of meat and seafood , helps to maintain normal tissue concentrations of taurine. There is an increased incidence of pediatric problems in children being raised on the totally vegetarian diets of vegan communities due to this reason.
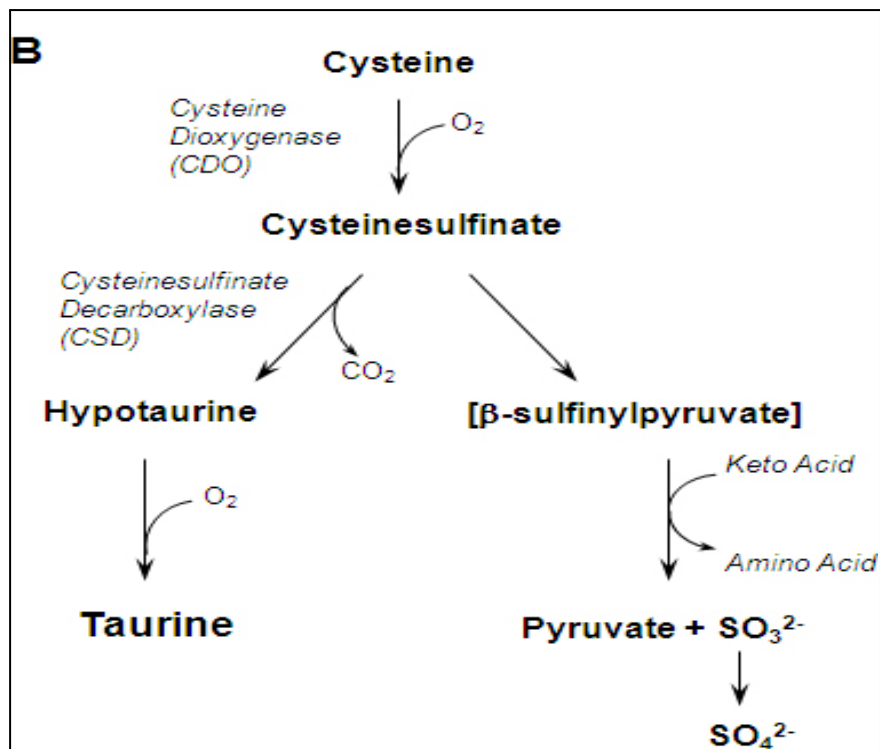
Figure 6.Synthetic pathway of taurine

Aside from the retina, every region of the brain that has been tested contains or takes up taurine; this includes the pineal, pons medulla, hypothalamus , striatum, and cerebellum. At each of these sites, there is evidence of taurine's ability to ameliorate certain forms of neuropathology.

Taurine is one of the few amino acids not used in protein synthesis, taurine is often referred to as a "nonessential" amino acid, or more generously as a "conditionally essential" amino acid. Considering its broad distribution, its many cytoprotective attributes, and its functional significance in cell development, nutrition, and survival, these are clearly misnomers. Taurine is undoubtedly one of the most essential substances in the body[16].

Ta mai et.al 1995 have shown that taurine is transported into neurons by taurine transporter (TAUT), which is also widely expressed in the CNS, including on the blood–brain barrier (BBB), indicating that peripheral taurine could be transported into the brain through the BBB and take effect there[17]. As a semi-essential amino acid of humans, taurine has an observed safe level of supplemental intake in normal

healthy adults at up to 3 g/day, and was approved to have no adverse effects for up to 1,000mg/kg/day by the European Food Safety Authority[18].

Rahmeier, F. L. *et al*. 2016 has reported that taurine could increase the emotional learning ability and memory of diabetic rats. Taurine is able to improve the performance of animals in memory tests, increase the exploration activity and interfere with apoptosis and neurodegeneration process. The environmental enrichment also modulate behaviour and animal physiology, increasing the curiosity and exploration. Thus, improved mechanisms of acquisition and memory consolidation, both in diabetic and non-diabetic animals is observed[19].

Altamura et.al 1995 has demonstrated an increase in the plasma concentration of taurine in depressed patients, while a deficiency in taurine is related to the development of depression, suggesting a close relationship between taurine and depression[20].

Gao-Feng Wu and co workers in 2017 have investigated the antidepressant effect of taurine in chronic unpredictable mild stress induced depressive rats. Taurine was found to inhibit the decrease of sucrose consumption and prevent the deficiency of spatial memory and anxiety in rats exposed to CUMS, suggesting a preventive effect of taurine on depression-like behavior. Furthermore, the decreased levels of 5-hydroxytryptamine, dopamine, noradrenaline; the increased levels of glutamate, corticosterone; and the decreased expressions of fibroblast growth factor-2, vascular endothelial growth factor and brain derived neurotrophic factor in depressive rats were hindered by taurine pre-administration. The results demonstrated that the anti-depressive effect of taurine may be involved in the regulation of hypothalamic-pituitary-adrenal (HPA) axis and the promotion of neurogenesis, neuronal survival and growth in the hippocampus[21].

TAURINE AND NEUROPROTECTION

Perhaps the most exhaustive body of experimental work on the neuroprotective properties of taurine was performed by Wu and colleagues 1998. These innovative studies provide convincing evidence that there are several avenues by which taurine

exerts its protective role. Using primary neuronal cultures from the fetal rat brain, these researchers showed that taurine suppresses glutamate-induced toxicity through several pathways:

- It inhibits calcium influx through L-, N- and P/Q-type voltage-gated calcium channels
- It prevents the downregulation of Bcl-2 and the upregulation of Bax, the protein products of which otherwise would translocate to the mitochondria and result in the release of the highly toxic cytochrome C (cyC)
- It protects neurons from oxidative stress
- It inhibits glutamate-induced calpain activation, thereby preventing the cleavage of Bcl-2[22]

The anti-neuroinflammatory activity of taurine has been confirmed by studies done by Y.Su et.al in 2014. Following traumatic brain injury (TBI) in rats, taurine significantly suppressed interleukin (IL)-1β levels and elevated the levels of RANTES (regulated on activation, normal T cell-expressed and secreted) . Moreover, a one week treatment with taurine noticeably reduced levels of 17 cytokines including IL-1α, IL-1β, IL-4, IL-5, IL-6, IL- 10, IL-12p70, IL-13, IL-17, tumor necrosis factor (TNF)-α, interferon gamma[23].

ROLE OF TAURINE IN DEVELOPMENT

In addition to its protective and therapeutic actions, taurine has proven essential for normal development. Taurine also plays a critical role in brain development.Taurine deficiency leads to a delay in cell differentiation and migration in cerebellum, pyramidal cells, and visual cortex in cats and monkeys.Taurine was found to be one of the most important amino acids in the development of immature brains, including maintaining the survival and development of neuronal proliferation, stem cell proliferation and differentiation, and protecting neural cells from excitotoxicity induced by excitatory amino acids by Li, X. W., Gao, H. Y. & Liu, J. in 2016[24].Moreover, Hernandez-Benitez et al. 2010 have shown that taurine promotes neural development not only in embryonic brain, but also in adult brain regions[25].

TAURINE AND OXIDATIVE STRESS:

It has become increasingly apparent that oxidative stress plays a major role in a broad range of human diseases. The overproduction of reactive oxygen species and the body's inability to stem the accumulation of highly reactive free radicals have been implicated in cardiovascular disease , diabetes, inflammatory disease, reperfusion injury and several of the major disorders of the CNS.

Chen G.et.al in 2012   demonstrated the protective effects of taurine against oxidative stress in the heart of MsrA knockout mice[26].

Das J and Sil PC in 2012 established that taurine ameliorates alloxan-induced diabetic renal injury, oxidative stress-related signaling pathways and apoptosis in rats[27].

Marcinkiewicz J, Kontny E in 2012 in their review "Taurine and inflammatory diseases"have pointed out that the fundamental role of taurine in the immune system is related to its antioxidant properties. Taurine protects tissues from oxidative stress associated with the pathology of various inflammatory diseases[28].

Schaffer SW, Azuma J, Mozaffari M in 2009 have reviewed the role of antioxidant activity of taurine in diabetes[29].

Jong CJ, Azuma J, Schaffer S. in 2012 have investigated and confirmed the mechanism underlying the antioxidant activity of taurine as prevention of mitochondrial oxidant production[30].

In each case, taurine, by virtue of its antioxidant activity, has been shown to play a crucial role as a cytoprotectant and in the attenuation of apoptosis. Despite this diversity of pathophysiology in so varied a group of seemingly unrelated disorders, there is a growing consensus that oxidative stress is linked to mitochondrial dysfunction and that the beneficial effects of taurine are a result of its antioxidant

properties as well as its ability to improve mitochondrial function by stabilizing the electron transport chain and inhibiting the generation of reactive oxygen species[16].

FUNCTIONS OF TAURINE

COGNITIVE FUNCTION:

IT CAN ACT AS A NEUROTRANSMITTER AND IMPROVE THE BRAIN FUNCTION

INCREASE THE GABA LEVELS

IT ENCOURAGES THE RELAXATION AND THE FEELING OF WELLNESS

IT HELPS TO DEAL WITH STRESS AND IMPROVES THE QUALITY OF SLEEP

CELL:

PRESERVES THE INTEGRITY OF THE STRUCTURE OF THE CELL MEMBRANE

PARTICIPATES IN THE CALCIUM HOMEOSTASIS

CELL OSMOREGULATION, IT MAINTAINS THE BALANCE BETWEEN SODIUM AND WATER

SPORT:

SUPPORTS THE CONTRACTION OF THE MUSCLES

REDUCES MUSCLE CRAMPS

FAVORS THE POST-WORKOUT RECOVERY

DEVELOPMENT:

NERVOUS SYSTEM

MAINTENANCE OF THE RETINE

BILE SYNTHESIS

HEALTH:

ANTIOXIDANT FUNCTION AND ANTI-INFLAMMATORY EFFECT

IT ENCOURAGES THE FUNCTION OF THE IMMUNE SYSTEM

CONTROL OF BLOOD PRESSURE BY REDUCING THE ANGIOTENSIN

Figure 7.Functions of Taurine
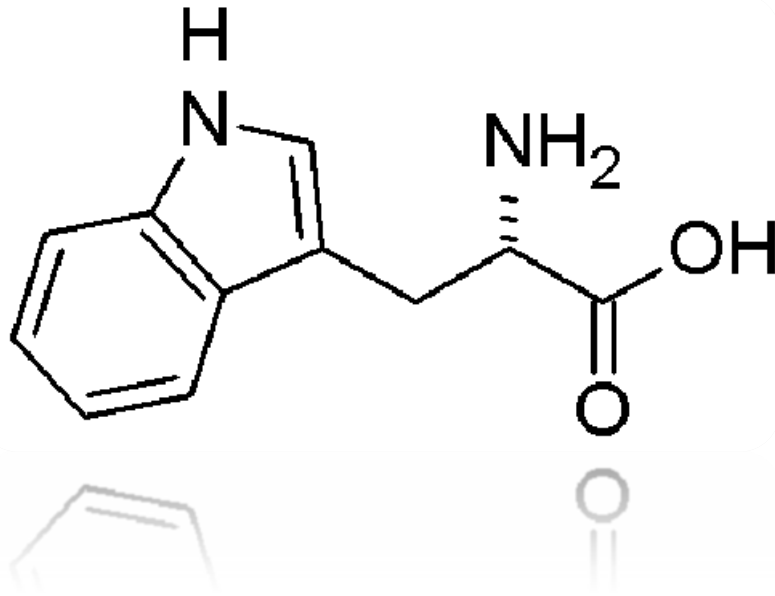
2.2.TRYPTOPHAN

Hopkins and Cole discovered tryptophan in the early 1900s after isolating it from casein protein, and Ellinger and Flamand determined its molecular structure a short time later. *L* tryptophan (i.e. tryptophan) is one of eight essential amino acids (i.e. amino acids that cannot be synthesized in the human body and must be supplied by the diet). For all amino acids, including *L*-tryptophan, only the *L* isomer is used in protein synthesis and can pass across the blood-brain barrier. In humans, tryptophan has relatively low tissue storage and the overall tryptophan concentration in the body is the lowest among all amino acids, although only small amounts are necessary for general healthy nutrition. While typical intake for many individuals is approximately 900 to 1000 mg daily, the recommended daily allowance for adults is estimated to be between 250 mg/day and 425 mg/day, which translates to a dietary intake of 3.5 to 6.0 mg/kg of body weight per day. Some common sources of tryptophan are oats, bananas, dried prunes, milk, tuna fish, cheese, bread, chicken, turkey, peanuts, and chocolate.

Tryptophan was first synthesized in 1949, but by the early 1980's chemical synthesis of tryptophan was replaced with fermentation procedures that greatly increased obtainable yields, making tryptophan supplements more available.

METABOLIC FUNCTIONS OF TRYPTOPHAN

Protien synthesis

The principal role of tryptophan in the human body is as a constituent of protein synthesis. Because tryptophan is found in the lowest concentrations among the amino acids, it is relatively less available and is thought to play a rate-limiting role during protein synthesis.Tryptophan is also the precursor of two important metabolic pathways, kynurenine synthesis and serotonin synthesis.

Kynurenine synthesis

After protein synthesis, the second most prevalent metabolic pathway of tryptophan is for the synthesis of kynurenine, which accounts for approximately 90% of tryptophan catabolism., Kynurenine is a key component in the synthesis of a number of metabolites, but most importantly, it is the precursor of kynurenic and quinolinic acids. Each of these metabolites has the potential to affect other neurotransmitters; specifically kynurenic acidis a glutamate receptor antagonist, while quinolinic acid is a glutamate receptor agonist.

Serotonin synthesis

It is estimated that 95% of mammalian serotonin is found within the gastrointestinal tract, and only 3% of dietary tryptophan is used for serotonin synthesis throughout the body. Nevertheless,serotonin synthesis is one of the most important tryptophan pathways and a topic of intense research[31].

Less than five years after serotonin's (5HT) discovery in the central nervous system by Twarog and Page in 1953, investigators were already studying whether changes in the dietary intake of its amino acid precursor, TRP, could influence brain 5HT levels . There was no clearly stated biochemical rationale at that time as to why one would expect dietary TRP manipulations to influence 5HT levels. This oversight in logic was not remedied until Lovenberg et al. (1968) provided the first set of kinetic constants for tryptophan hydroxylase, which suggested that the enzyme might not be fully saturated at normal brain TRP concentrations. These results provided a biochemical context for interpreting the earlier dietary results, as well as the results of pharmacologic studies showing rapid increases in brain 5HT levels following TRP injection. The observed increases and decreases in brain 5HT presumably paralleled changes in 5HT synthesis rate, which was a direct reflection of the degree of substrate

saturation of tryptophan hydroxylase, the enzyme catalyzing the rate-limiting step in the synthetic pathway. Based on this notion, that TRP hydroxylation rate and 5HT synthesis in brain respond directly to changes in local TRP levels, Tagliamonte A et al. in 1973, said free tryptophan in serum controls brain tryptophan level and serotonin synthesis[32].

Tryptamine synthesis

In addition to tryptophan's three major activities of protein, kynurenine, and serotonin synthesis, tryptamine is another biologically active compound that is derived from tryptophan. The immediate decarboxylation of tryptophan results in the synthesis of trace amounts of tryptamine (i.e. ng/g), which is an important neuromodulator of serotonin.

Melatonin synthesis

Melatonin is a hormone produced in the tryptophan/ serotonin pathway, which regulates diurnal rhythms and influences the reproductive and immune systems,as well as digestive processes and gastrointestinal motility. During periods of darkness, it is actively secreted from the pineal gland to induce neural and endocrine effects that regulate circadian rhythms of behavior,physiology, and sleep patterns.

NAD/NADP synthesis

Tryptophan also plays a role as a substrate for synthesis of the coenzymes nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP).NAD and NADP are coenzymes essential for electron transfer reactions (i.e. redox reactions) in all living cells. These enzymes can be synthesized de novo from ingested tryptophan,or from ingestion of niacin (i.e. vitamin B3).

Niacin synthesis

Interestingly, tryptophan can act as a substrate for niacin synthesis through the kynurenine/quinolinic acid pathway. However, this is a less efficient use of tryptophan since approximately 60 mg of tryptophan are necessary to generate a single milligram of niacin.

Other metabolic functions

Tryptophan also exerts effects on other neurotransmitters and CNS compounds. Dopamine, norepinephrine, and beta-endorphin have been shown to increase following oral dosing of tryptophan.Through serotonin synthesis, tryptophan is also thought to be involved in modulation of the endocrine system and cortisol,as well as prolactin and growth hormone.

In summary, while tryptophan is found in the smallest concentrations of the 20 amino acids in the human body, it has wide-ranging effects and is a critical component of a multitude of essential metabolic functions.

While there are three primary functions of tryptophan (i.e. protein, serotonin, and kynurenine synthesis), it is the tryptophan's role in the synthesis of serotonin in the brain that is responsible for utility of tryptophan for both research and clinical purposes.

Tryptophan once consumed, tryptophan is distributed throughout the human body in the circulatory system. Unlike the other 19 amino acids, approximately 75% to 85% of circulating tryptophan is bound to albumin, with some estimates as high as 95%. It is primarily the non-bound, free tryptophan that is available for transport across the blood-brain barrier.However, since tryptophan has a higher affinity for the blood-brain barrier (BBB) transporter than it does for albumin, albumin bound tryptophan that is in close proximity to the BBB will likely dissociate from the albumin to be taken up into the brain[31].

TRYPTOPHAN AS A THERAPEUTIC AGENT

While dietary intake alone (i.e. ingestion of food) would seldom influence the availability of tryptophan significantly, administration of exogenous tryptophan has been the focus of numerous clinical research and homeopathic applications. One of the earliest examples was an attempt by Lauer and colleagues to augment treatment response for schizophrenia by combining tryptophan administration with iproniazid, a monoamine oxidase inhibitor (MAOI). The success of the combined treatment compared to the MAOI alone changed how researchers thought about the influence of tryptophan treatment on brain function, and began a series of clinical trials testing the

efficacy of treatment for a number of clinical disorders that yielded promising but often inconclusive results[33].

Tryptophan has been used for a broad spectrum of clinical applications, such as treatment of pain, insomnia, depression, seasonal affective disorder, bulimia, premenstrual dysphoric disorder, attention deficit,hyperactivity disorder, and chronic fatigue .

Tryptophan has also been widely used as an over the counter, natural remedy for depression, pain, insomnia, hyperactivity, and eating disorders.[34]

TRYPTOPHAN IN MULTIPLE SCLEROSIS

Suboptimal brain tryptophan concentrations may occur in patients with multiple sclerosis and other degenerative neurological diseases..The tryptophan depletion present in patients with multiple sclerosis and other degenerative diseases might then cause a depression of the synthetic pathways for serotonin and proteins in the brain. Changes in the amount of one amino acid may, in fact,alter the synthesis and degradation of proteins in the brain or, possibly, of a specific population such as myelin proteins [35]. These changes are unlikely to have a primary role in the disease, but might perhaps exacerbate certain symptoms.

Previous studies by Kisser and Harrer in 1974, have demonstrated that administration of tyrosine, L-dopa, and tryptophan effectively counter the negative effect of artificial overheating on the flickerfusion threshold in patients with multiple sclerosis[36]

Markianos M et al. in 2009, evaluated the relationship of CSF neurotransmitter metabolite levels to disease severity and disability in multiple sclerosis.Reduced serotonergic activity has also been connected to psychiatric symptomatology like depression, anxiety, and suicidal ideation, conditions that are often present in MS patients. In this context, it is of interest to study the time when MS patients develop depressive symptoms, as well as their possible differential response to serotonergic activity enhancing drugs according to disease severity. A possible explanation of the specific reductions in serotonin turnover with disability and time may be offered by the data on the influence of inflammation on the metabolism of tryptophan[37].

Opitz et al. 2007 suggested that tryptophan degradation is altered in autoimmune diseases.Pro inflammatory cytokines shift tryptophan metabolism towards kynurenine and quinolinic acid by activation of the tryptophan degradation enzymes tryptophan-2,3-oxygenase and indoleamine-2,3-dioxygenase, reducing tryptophan availability for transport to CNS and metabolism to serotonin[38]. This has been confirmed by Heyes et al. 1992 .He found increased concentrations of kynurenine and quinolinic acid and reduced tryptophan have in CSF and postmortem tissue of patients with inflammatory diseases[39].

It is known, that in MS, the decreased amount of 5-HT synthesis in the brain may lead to the local 5-HT-deficit. A significant role in this deficit may play 5-HT metabolites, N-acetylserotonin (NAS) and melatonin. The levels of these metabolites are dependent on availability of 5-HT. Wen.J et.al in 2016 demonstrated that NAS and melatonin exhibit antioxidant and anti-inflammatory properties. It also acts as immune signaling agents . NAS exerts similar as a brain-derived neurotrophic factor (BDNF), activating the brain-derived neurotrophic factor (BDNF) receptor. However, melatonin decreases the number of Th1 and Th17 cell populations and the cytokines synthetized . It also exerts a positive effect on mitochondrial function and reduces oxidative stress. It has been shown that NAS and melatonin in experimental autoimmune encephalomyelitis (EAE) in mice reduce clinical scores and the loss of mature oligodendrocytes, demyelination, and axon injury[40].

The ability to change the rates of serotonin synthesis in the brain by manipulating concentrations of serum tryptophan is of immense importance. As the sole precursor of serotonin, experimental research has shown that L-tryptophan's role in brain serotonin synthesis is an important factor involved in mood, behavior, and cognition. Furthermore, clinical trials have provided some initial evidence of L tryptophan's efficacy for treatment of psychiatric disorders, particularly when used in combination with other therapeutic agents[31].

SCOPE OF WORK

3. SCOPE OF WORK

Multiple sclerosis (MS) is an autoimmune disease characterized by neuronal demyelination, astrogliosis, and microglial activation. It is generally believed that MS is an inflammatory disease controlled by T cell–induced autoimmune reaction against the myelin sheet which principally affects the white matter. FDA approved agents include four preparations of interferon-beta , glatiramer acetate, mitoxantrone, and natalizumab and the recently approved first oral medication fingolimod.

The most commonly used animal model of MS is the cuprizone (CPZ) model, a toxin-induced model of MS. Characteristic histomorphological and pathological manifestation of the CPZ-induced demyelination typically mimics type III MS lesions.

`Taurine and tryptophan being aminoacids are vital for normal growth and development. Numerous studies have been conducted in which ability of taurine to mitigate oxidative stress is investigated. Taurine in this regard, plays beneficial role in a number of diseases of varied pathology. Similarly all the research on tryptophan revolves around the serotonergic pathway through which it has proved to improve mood,behaviour and cognition. This can even offer a symptomatic relief to patients suffering from depression and cognitive disability in multiple sclerosis.

The scope of the work is to bring the treatment regimen for the people being affected by multiple sclerosis, since only symptomatic treatments are present that are aimed at maintaining the function and improving the quality of life. The present work is to evaluate the effect of TAURINE and TRYPTOPHAN in multiple sclerosis and to find out the better and safe treatments. The study is evaluated by the various parameters like locomotor activity, sensation, learning and memory along with in -vitro parameters like anti oxidant estimations.

PLAN OF WORK

4. PLAN OF WORK

CUPRIZONE MICE MODEL OF MULTIPLE SCLEROSIS

TAURINE	TRYPTOPHAN

IN VIVO STUDIES

EX VIVO STUDIES

DATA COLLECTION

STATISTICAL ANALYSIS

RESULTS

```
                    ┌──────────────────┐              ┌──────────────────────┐
                    │    LOCOMOTOR     │─────────────▶│   ACTOPHOTOMETER     │
                    │     ACTIVITY     │              │                      │
                    └──────────────────┘              └──────────────────────┘


                    ┌──────────────────┐              ┌──────────────────────┐
                    │  MOTOR ACTIVITY  │─────────────▶│    ROTAROD TEST      │
                    └──────────────────┘              └──────────────────────┘

┌──────────────┐                                      ┌──────────────────────┐
│   IN VIVO    │                                      │    MORRIS WATER      │
│   STUDIES    │                                      │     MAZE TEST        │
│              │                                      └──────────────────────┘
└──────────────┘            ┌──────────────────┐      ┌──────────────────────┐
                            │    LEARNING      │─────▶│      PASSIVE         │
                            │  AND MEMORY      │      │   AVOIDANCE TEST     │
                            └──────────────────┘      └──────────────────────┘

                                                      ┌──────────────────────┐
                                                      │    Y -MAZE TEST      │
                                                      └──────────────────────┘
```

```
                  ┌─────────────────────────────────────────────┐
                  │  ASSAY OF SUPEROXIDE DISMUTASE(SOD)         │
                  └─────────────────────────────────────────────┘
                             ▲
                             │
                                    ┌──────────────────────────┐
                                    │   ASSAY OF GLUTATHIONE    │
                                    │        REDUCTASE          │
                                    └──────────────────────────┘
                                         ▲
                                         │
   ┌────────────────────────┐
   │     EX VIVO TESTS      │──────────┐
   └────────────────────────┘          │     ┌──────────────────────────┐
                                        ├────▶│   ASSAY OF GLUTATHIONE    │
                                        │     │       PEROXIDASE          │
                                        │     └──────────────────────────┘
                                        │
                                        │        ┌──────────────────────────┐
                                        ├───────▶│   ASSAY OF CATALASE       │
                                        │        └──────────────────────────┘
                                        │
                                        │   ┌────────────────────────────────┐
                                        └──▶│  ESTIMATION OF INTERLEUKIN -6  │
                                            └────────────────────────────────┘
```

ASSAY OF SUPEROXIDE DISMUTASE(SOD)

ASSAY OF GLUTATHIONE REDUCTASE

EX VIVO TESTS

ASSAY OF GLUTATHIONE PEROXIDASE

ASSAY OF CATALASE

ESTIMATION OF INTERLEUKIN -6

MATERIALS AND METHODS

5.1. EXPERIMENTAL ANIMALS

The Swiss Albino mice weighing 20-50g were used for the study.The inbred animals were procured from animal house of C. L. Baid Metha College Of Pharmacy,Thoraipakkam,Chennai-97.They were housed six per cage under standard laboratory conditions at a temperature 22±2°c wth 12:12 hrs light and dark cycle. The animals were provided with standard pellet feed (Hindustan Lever Limited ,Bangalore) and drinking water was provided *ad libitum* throughout the experimentation period. The animals were acclimatized to laboratory conditions one week prior to initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The study was approved by Institutional Animal Ethical Committee (IAEC),approval no:16/321/PO/Re/S/01/CPSCEA valid upto 14/03/21

5.2.EXPERIMENTAL DESIGN

On the first day of the experiment the animals were divided randomly into six groups of six animals each. Multiple sclerosis was induced by feeding 0.2% cuprizone diet to the II,III,IV,V and VI groups and continued upto 5 weeks. Contol (Group I) animals were given distilled water orally.To add cuprizone to standard mouse chow, the chow must be ground into a powder and mixed with the cuprizone.

Taurine Administration:

Taurine is orally administered by oral gavage at dose level of 250 and 500 mg/kg/day from day 1 till cuprizone feeding is stopped .

Oral tryptophan administration

Tryptophan is weighed with the aid of a precision analytical balance and diluted with a magnetic stirrer to produce solutions containing 50 mg/kg and 100mg/kg of tryptophan and given by oral gavage from day 1.

5.3.CHEMICALS LIST

All the chemicals used in the study were of analytical grade.Cuprizone,Taurine and Tryptophan were bought from Sigma Alrich.

5.4. METHODS AND ASSESSMENT

Grouping of animals

Male Swiss Albino mice weighing 18-25 g were used for the study.They were divide into six groups of six animals each and maintained at an ambient temperature and relative humidity with 12 hrs light/dark cycle.

Group – I: Control (untreated)
Group – II: 0.2% of Cuprizone (model group)
Group – III: Cuprizone + Taurine 250 mg/kg
Group – IV: Cuprizone + Taurine 500 mg/kg
Group – V: Cuprizone + Tryptophan 50mg/kg
Group – VI: Cuprizone + Tryptophan 100mg/kg

5.5. IN-VIVO PHARMACOLOGICAL STUDIES

5.5.1.ASSESSMENT OF LEARNING AND MEMORY

Morris Water Maze[41]

The Morris water maze method is performed to evaluate spatial working and reference memory. The experimental apparatus consists of a circular tank (120 cm in diameter, 45 cm in height). An invisible platform (15 cm in diameter, 35 cm height) was placed 1.5 cm below the surface of the water. Water was kept opaque by dissolving small quantity of milk at a temperature of 21-23⁰c. The pool was located in a test room and many cues external to the maze were visible from the pool, which could be used by the mice for spatial orientation. The position of the cues was kept constant through the task. The training trials were carried out. The platform is located in a constant position throughout the test period in the middle of one quadrant, equidistant from the center and edge of the pool. In each training session, the latency to escape to the hidden platform was recorded.

On the day of experiment, the platform was removed and the animals were tested for its memory where the time spent by each animal in target quadrant searching for the hidden platform is noted as an index of retrieval
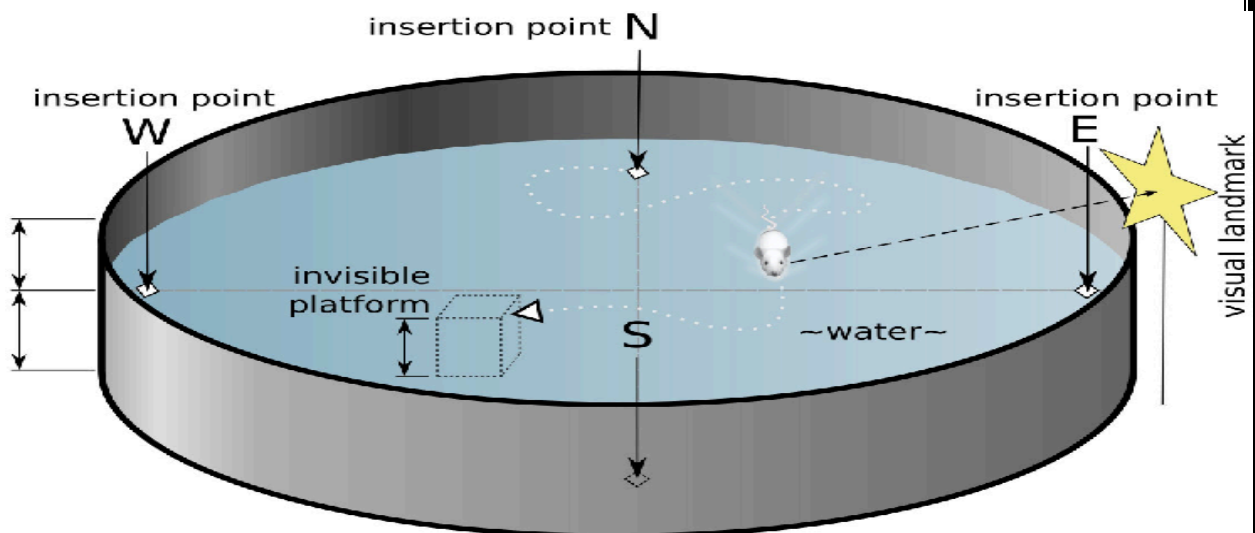

Figure.8.Diagrammatic representation of Morris water maze

Passive Avoidance Test[42]

The behavioural activity was assessed using the active avoidance paradigm. The apparatus consists of a sound proof experimental chamber with a grid floor which could be electrified and with a provision for a buzzer tone. The enclosure had a clear transparent front sliding door, through which the animal would be introduced into the chamber. A flat wooden surface, placed on the inner central surface of the chamber acted as the shock free zone. The stimulus provided was a foot shock of 6 mA given for a period of 15s from the electrified grid floor. Mice were initially trained to escape the foot shock by climbing/steeping up on the wooden surface, i.e., the shock free zone. The initial trail was carried out by using three trail sessions interspersed with an interval of 15s. During each of the initial trails, the mice were allowed to explore the apparatus for 15s. This was followed by the foot shock for 15s. Only those mice which were sensitive to the foot shock and could climb/step up on to the flat wooden surface were included in the study

Y-Maze Test[43]

This working is based on spontaneous exploloration and alteration between arms – neither water nor food restrictions are required. Three identical arms are mounted symmetrically on an equilateral traiangular centre. Y-shaped maze with three opaque arm spaced 120° apart. Large Y-maze: three 40×8×15cm arms and small Y-maze: two 15.24×7.62×12.7cm arms, one 20.32×7.62×12.7cm arm. The number of entries into and the time spent, in each of the two types of arm, were counted during a 5 minutes test period. The open-arm entries and open arm time and close arm entry and close arm time were recorded. Scoring consists of recording each arm entry (defined as all four paws entering arm).

5.5.2.ASSESSMENT OF LOCOMOTOR ACTIVITY

Actophotometer[44]

Locomotor activity (horizontal activity) was measured using Actimeter. Actophotometer which operates on photoelectric cells connected with a counter. When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally. Each mice was placed individually in the activity cage floor for 10 min and the locomotion count was observed from the digital reading displayed in the actimeter. Actimeter is the combination of hole board and actophotometer in which both rats and mice can be placed. Impulsive behaviour was assessed on day 10 and 20. The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using one-way ANOVA followed by Dunnet's "t" test to assess the significance difference if any among the groups. $P < 0.05$ was considered as significant.

Figure 9. Digital actophotometer

5.5.3.ASSESSMENT OF MOTOR CO ORDINATION

Rotarod Activity[46]

Motor coordination and balance was assessed using a rotarod apparatus. In total, four training trials per day with an interval trial time of one hour were performed. Mice falling off during a training trial were put back on the rotating rod. Following the training days, a one day test of three trials was performed using two speed levels (10 & 20 R.P.M) mode of the apparatus over 5-min. Mice are placed individually in separate lanes on rod rotating at 5rpm such that animals may walk forward to keep balance. The rotating rod is allowed to rotate at 10 rpm and mice from each group are placed individually on the rod and the fall off time was recorded for each mice. Similar procedure is followed by increasing the speed to 20 rpm. Total 6 groups were involved and their average hanging duration was concluded using MEAN±SEM.

Figure 10. Rotarod apparatus

5.6. IN –VITRO PHARMACOLOGICAL STUDIES

5.6.1. ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)[47]

Reagents:

Carbonate buffer (100mM, pH 10.2)
Epinephrine (3mM)

Procedure:

The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100µl of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480nm in spectrophotometer for 2min at an interval of 15sec. Parallel blank and standard were run for determination of SOD activity.

One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation.

Reagents	Uninhibited (Standard)	Inhibited (Sample)	Blank
Carbonate buffer	0.900ml	0.800ml	1.0ml
Supernatant	-	0.1ml	-
Epinephrine	0.1ml	0.1ml	-

The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer

5.6.2.ESTIMATION OF GLUTATHIONE REDUCTASE (GRD) [48]

Glutathione reductase was assayed by the method by Stahl *et al*.

Reagents:

1. Phosphate buffer (0.3M; pH 6.5)
2. EDTA (0.25M)
3. Glutathione oxidized, GSSG (0.012M)
4. NADPH (0.03M); Nicotinamide Adenine Dinucleotide Phosphate reduced tetra sodium salt, NADPH.Na$_4$ (Mw.833.35)

Procedure:

The reaction mixture containing 1ml phosphate buffer, 0.5ml EDTA, 0.5ml GSSG and 0.2ml of NADPH was made up to 3ml with distilled water. After the addition of 0.1ml of tissue homogenate, the change in optical density at 340nm was monitored for 2min at 30sec interval.

One unit of the enzyme activity was expressed as nmoles of NADPH oxidized/min/mg protein

Reagents	Sample	Blank
Phosphate buffer	1.0ml	1.5ml
EDTA	0.5ml	0.5ml
GSSG	0.5ml	-
NADPH	0.2ml	0.2ml
Supernatant	0.1ml	0.1ml
DDW	0.8ml	0.8ml

5.6.3. ESTIMATION OF CATALASE[49]

Reagents

1. **Phosphate buffer solution (50mM)**

   i. Dissolve 6.81g of KH2PO4 in 1000ml distilled water

   ii. Dissolve 6.9g of Na2HPO4 in 1000ml distilled water

390ml from solution (A) are mixed with 610ml from solution (B), the pH is adjusted to 7

2. **Hydrogen peroxide (H2O2) 30mM**

   0.34ml of 30% H2O2 is diluted with phosphate buffer to 100ml

Procedure

Catalase activity was measured by the method of Aebi. 0.1ml of supernatant was added to cuvette containing 1.9ml of 50mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0ml of freshly prepared 30mM H2O2. The rate of decomposition of H2O2 was measured spectrophotometrically from changes in absorbance at 240nm. Activity of catalase was expressed as units/mg protein. A unit is defined as the velocity constant per seconds.

The reaction occurs immediately after the addition of H2O2. Solutions are mixed well and the first absorbance (A1) is read after 15sec (t1) and the second absorbance (A1) after 30sec (t1). The absorbance is read at wavelength 240nm.

5.6.4. ESTIMATION OF GLUTATHIONE PEROXIDASE[50]

Reagents

      1. Phosphate buffer, pH 7.0 (75mM)

      2. Glutathione reductase (60mM)

      3. Sodium azide (0.12M)

      4. Disodium EDTA (0.15mM)

      5. NADPH (3mM)

      6. H2O2 (7.5mM)

Procedure

3ml cuvette containing 2.0ml of phosphate buffer (75mmol/L, pH 7.0), 50µl of glutathione reductase (60mmol/L), 50µl of NaN3 (0.12mol/L), 0.1ml of Na2EDTA (0.15mM/L), 100µl of NADPH (3.0mmol/L) and tissue supernatant were added. Water was added to make a total volume of 2.9ml. The reaction was started by the addition of 100µl of (7.5mmol/L) H2O2, and the conversion of NADPH to NADP was monitored by a continuous recording of the change of absorbance at 340nm at 1min interval for 5min. Enzyme activity of GSHPx was expressed in terms of mg of protein.

5.6.5.ESTIMATION OF INTERLEUKIN-6 (IL-6)[51]

Reagents:

1. RIPA buffer (50Mm Tris-HCL,150Mm NACL Ph-7.4)
2. 1mM EDTA
3. 1mM PMS
4. Protease inhibitor cocktail.

Procedure:

One half of the brain tissue was homogenized using disposable rotor homogenizer in 10 volumes of RIPA (radio-immune precipitation assay) buffer (50mM Tris-HCL, 150mM Nacl, Ph 7.4) supplemented with 1mM EDTA, 1mM PMSF(phenyl methyl sulfonyl fluoride) and protease cocktail was separated to sub cellular fraction. The homogenate centrifuged at 29000g for 20min. The pellet was resuspended with RIPA buffer containing 1mM EDTA and protease cocktail, whilst the supernatant was centrifuged at 2900 for 45min the supernatants was used for the determination of IL-6 by specific quantitative sandwich ELISA kits according to the manufacturers instruction.

5.7.METHODS FOR HISTOPATHOLOGICAL STUDY

The mice from each group are anesthetised using chloroform. The brain was carefully removed without any injury after opening the skull and dissected carefully without any damage to the tissues, the collected organs were washed with ice cold normal saline and fixed in10% buffered neutral formalin.The tissues were processed for routine paraffin embedding and 5 micron sections were stained with Mayer's Haematoxilin Eosin stain.

5.8.STATISTICAL ANALYSIS

The statistical analysis was carried by one way ANOVA followed by Dunnet's 't' test P values <0.05 (95% confidence limit) was considered statistically significant, using Software Graph pad Prism 8.

TABLES AND GRAPHS

Table 1:Effect of Taurine and Tryptophan in Morris water maze test

Sl No	GROUPS	ESCAPE LATENCY (secs)		
		DAY 7	DAY 14	DAY 21
1	CONTROL	15.60±.5	17.80±.2	18.60±.2
2	CPZ group	43.32±.5	47.26±.37	49.52±.37 *
3	CPZ+TAURINE LOW DOSE	32.36±.4	37.91±.32	35.86±.37 **
4	CPZ+TAURINE HIGH DOSE	29.56±.50	28.12±.37	22.67±.5 ***
5	CPZ+TRYPTOPHAN LOW DOSE	29.87±.4	33.35±.32	36.72±.3 ****
6	CPZ+TRYPTOPHAN HIGH DOSE	25.98±.37	20.67±.50	21.59±.30

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

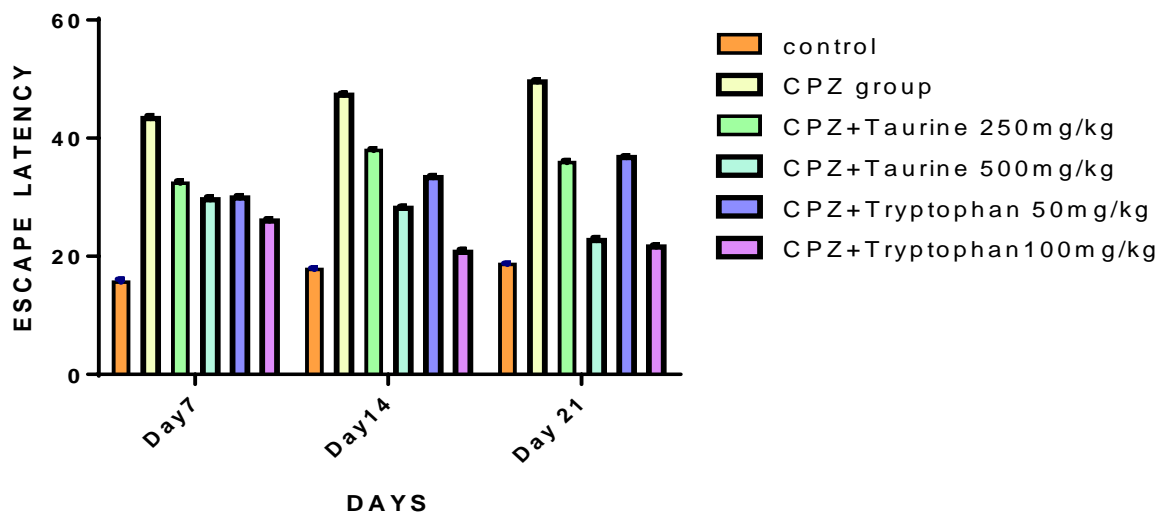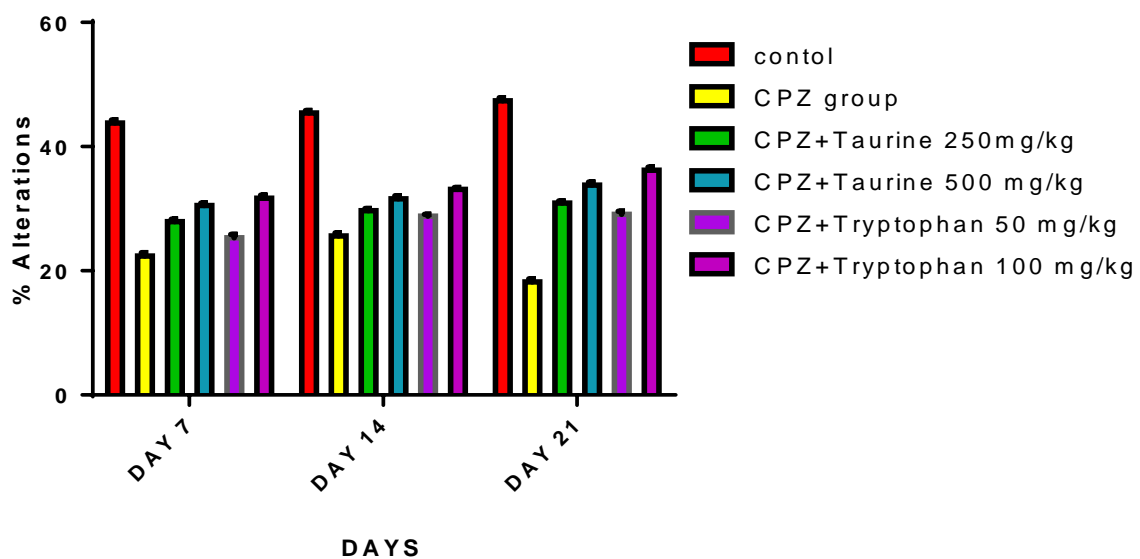**Graph 1:Effect of Taurine and Tryptophan in Morris water maze test**

Table 2: Effect of Taurine and Tryptophan in Y maze

Sl No	GROUPS	Percentage Correct Alternations		
		DAY 7	DAY 14	DAY 21
1	CONTROL	43.80±.50	45.40±.40	47.50±.45
2	CPZ group	22.40±.5	25.60±.5	18.2±.5
3	CPZ+TAURINE LOW DOSE	27.90±.4	29.70±.32	30.90±.37 ***
4	CPZ+TAURINE HIGH DOSE	30.50±.4	31.60±.5	33.80±.5 **
5	CPZ+TRYPTOPHAN LOW DOSE	25.30±.58	28.80±.3	29.10±.5 **
6	CPZ+TRYPTOPHAN HIGH DOSE	31.70±.5	33.10±.3	36.20±.5

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$.
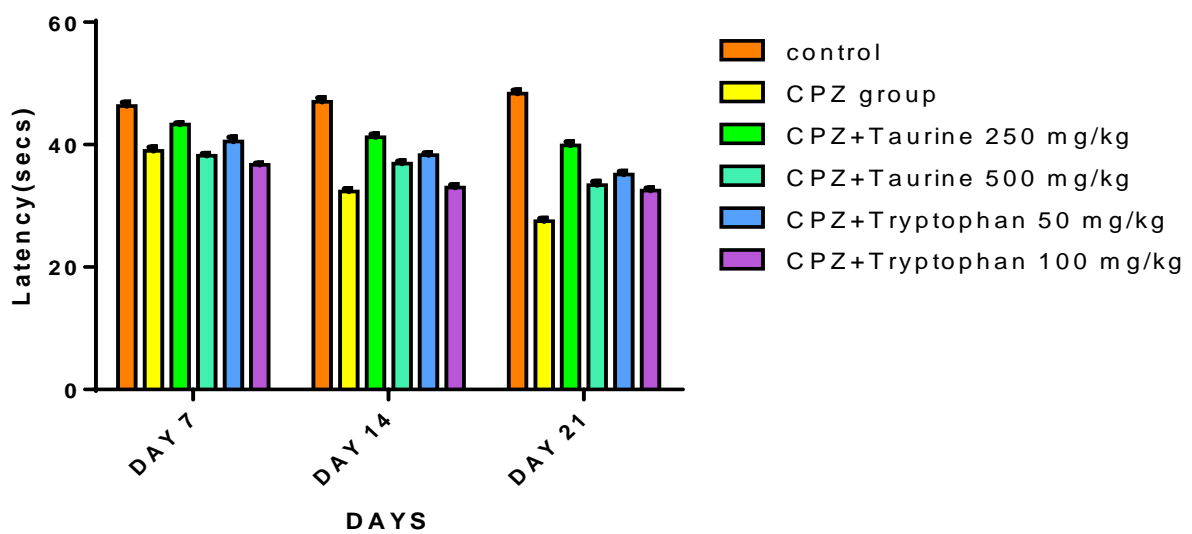
Graph 2:Effect of Taurine and Tryptophan in Y maze Test

Table 2: Effect of Taurine and Tryptophan in Passive Avoidance Test

Sl No	GROUPS	Latency		
		DAY 7	DAY 14	DAY 21
1	CONTROL	46.33±.56	47.00±.63	48.33±.58
2	CPZ group	39.00±.58	32.33±.49	27.50±.43
3	CPZ+TAURINE LOW DOSE	43.3±.3	41.2±.5	39.9±.6 ***
4	CPZ+TAURINE HIGH DOSE	38.2±.3	36.9±.4	33.4±.6 **
5	CPZ+TRYPTOPHAN LOW DOSE	40.5±.7	38.3±.3	35.1±.5 **
6	CPZ+TRYPTOPHAN HIGH DOSE	36.7±.3	33.0±.4	32.5±.4 ***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet′s 't' test, ns non-significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Graph:3.Effect of Taurine and Tryptophan in Passive Avoidance Test

Table 3:Effect of Taurine and Tryptophan on Locomotor activity

Sl No	GROUPS	Latency		
		DAY 7	DAY 14	DAY 21
1	CONTROL	296.17±.8	295.12±.17	295.45±.57
2	CPZ group	180.33±.7	150.16±.60	126.33±.33
3	CPZ+TAURINE LOW DOSE	193.4±.1	205.8±.6	220.7±.6 ***
4	CPZ+TAURINE HIGH DOSE	199.9±.9	228.8±.4	230.6±.6 ***
5	CPZ+TRYPTOPHAN LOW DOSE	195.6±.6	204.8±.6	220.5±.6 ***
6	CPZ+TRYPTOPHAN HIGH DOSE	200.70±.26	219.60±.70	240.12±.6 ***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$
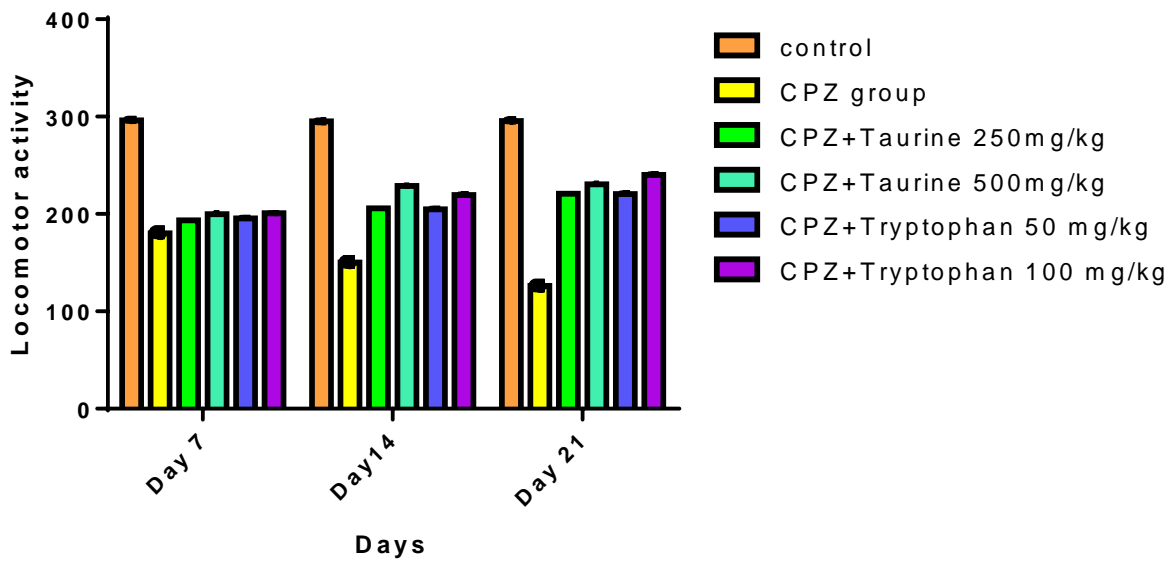
Graph 4: Effect of Taurine and Tryptpohan on locomotor activity

Table 4:Effect of Taurine and Tryptophan in Rotarod test

Sl No	GROUPS	Fall off time (secs)		
		DAY 7	DAY 14	DAY 21
1	CONTROL	40.2±.3	42.6±.5	44.6±.5
2	CPZ group	14.82±.3	11.60±.5	7.20±.3
3	CPZ+TAURINE LOW DOSE	18.21±.3	20.60±.3	22.45±.3 ***
4	CPZ+TAURINE HIGH DOSE	23.2±.5	24.5±.5	26.2±.4 ***
5	CPZ+TRYPTOPHAN LOW DOSE	17.8±.3	20.9±.4	23.5±.5 ***
6	CPZ+TRYPTOPHAN HIGH DOSE	24.6±.3	25.9±.3	29.7±.4 ***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$.

Graph 5: Effect of Taurine and Tryptophan in Rotarod test
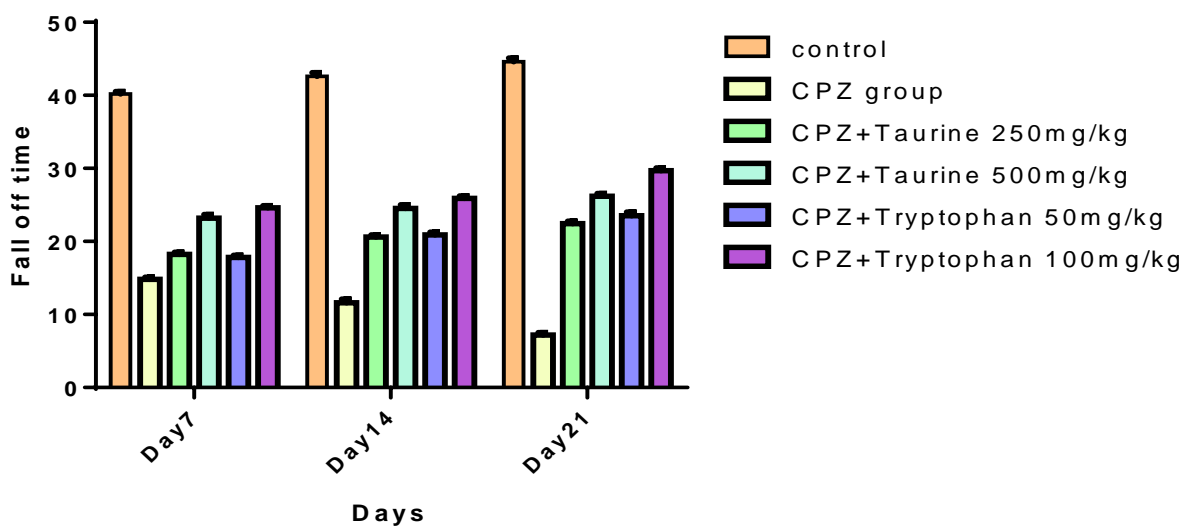
Table 5:Effect of Taurine and Tryptophan on SOD

SI no.	GROUPS	Units/mg wet tissue
1	CONTROL	13.01±.1
2	CPZ GROUP	8.08±.05
3	CPZ+TAURINE LOW DOSE	9.92±.1 ***
4	CPZ+TAURINE HIGH DOSE	10.52±.1 ***
5	CPZ+TRYPTOPHAN LOW DOSE	9.9±.1 ***
6	CPZ+TRYPTOPHAN HIGH DOSE	10.52±.05 ***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
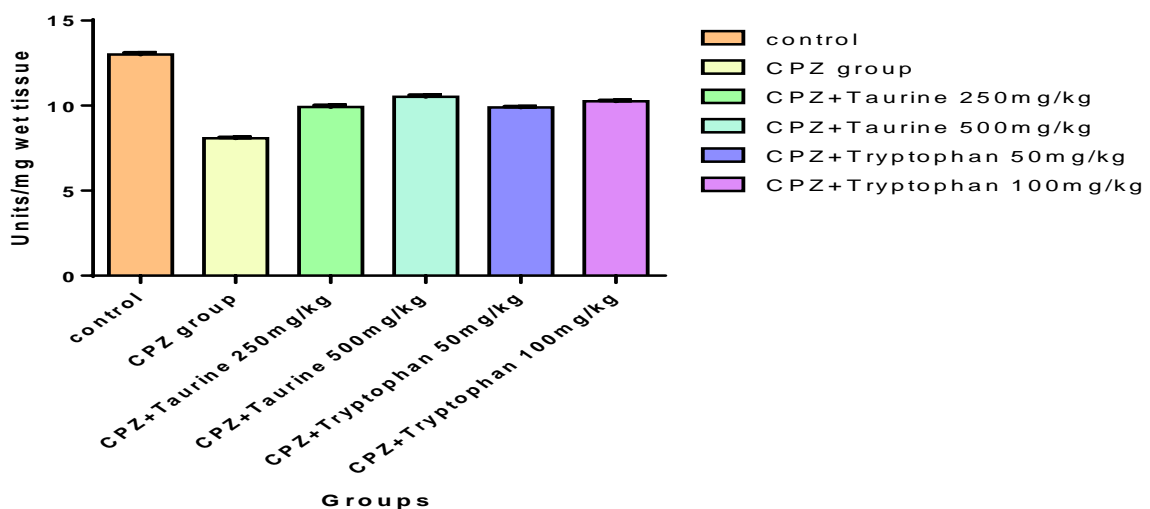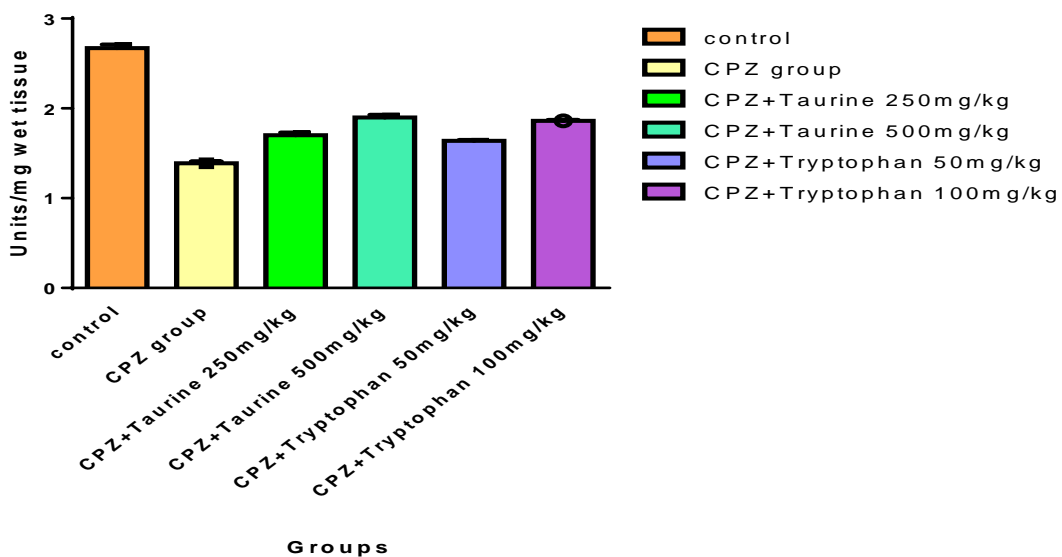
Graph 6: Effect of Taurine and Tryptophan on SOD

Table 5:Effect of Taurine and Tryptophan on Catalase

Sl no.	GROUPS	Units/mg wet tissue
1	CONTROL	2.67±.04
2	CPZ GROUP	1.39±.02
3	CPZ+TAURINE LOW DOSE	1.7±.03 **
4	CPZ+TAURINE HIGH DOSE	1.9±.03 ***
5	CPZ+TRYPTOPHAN LOW DOSE	1.64±.01 ****
6	CPZ+TRYPTOPHAN HIGH DOSE	1.86±.01 ****

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$.

Graph 7:Effect of Taurine and Tryptophan on Catalase

Table 9: Effect of Taurine and Tryptophan on Glutathione peroxidase

SI no.	GROUPS	Units/mg wet tissue
1	CONTROL	7.51±.04
2	CPZ GROUP	4.11±.02
3	CPZ+TAURINE LOW DOSE	4.91±01 ****
4	CPZ+TAURINE HIGH DOSE	5.98±.02 ****
5	CPZ+TRYPTOPHAN LOW DOSE	5.32±.01 ****
6	CPZ+TRYPTOPHAN HIGH DOSE	6.43±.03 ****

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

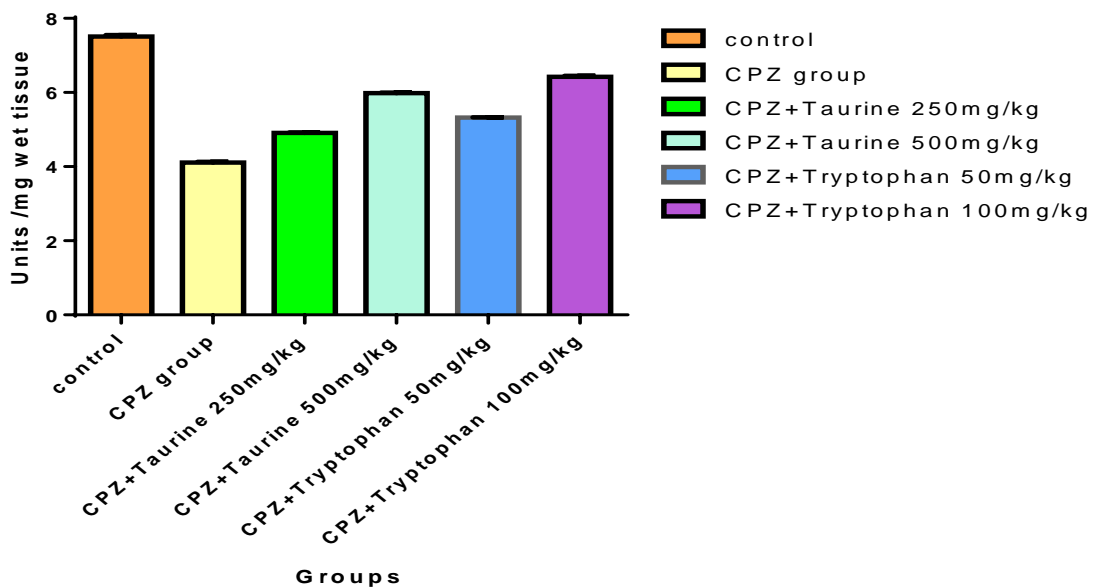**Graph 9:Effect of Taurine and Tryptophan on Glutathione peroxidase**

Table 10:Effect of Taurine and Tryptophan on Glutathione reductase

SI no.	GROUPS	Units/mg wet tissue
1	CONTROL	6.03±0.05
2	CPZ GROUP	3.15±.01
3	CPZ+TAURINE LOW DOSE	3.95±.01 ***
4	CPZ+TAURINE HIGH DOSE	5.11±.01 ***
5	CPZ+TRYPTOPHAN LOW DOSE	3.98±.02 ***
6	CPZ+TRYPTOPHAN HIGH DOSE	4.98±0.06 **

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

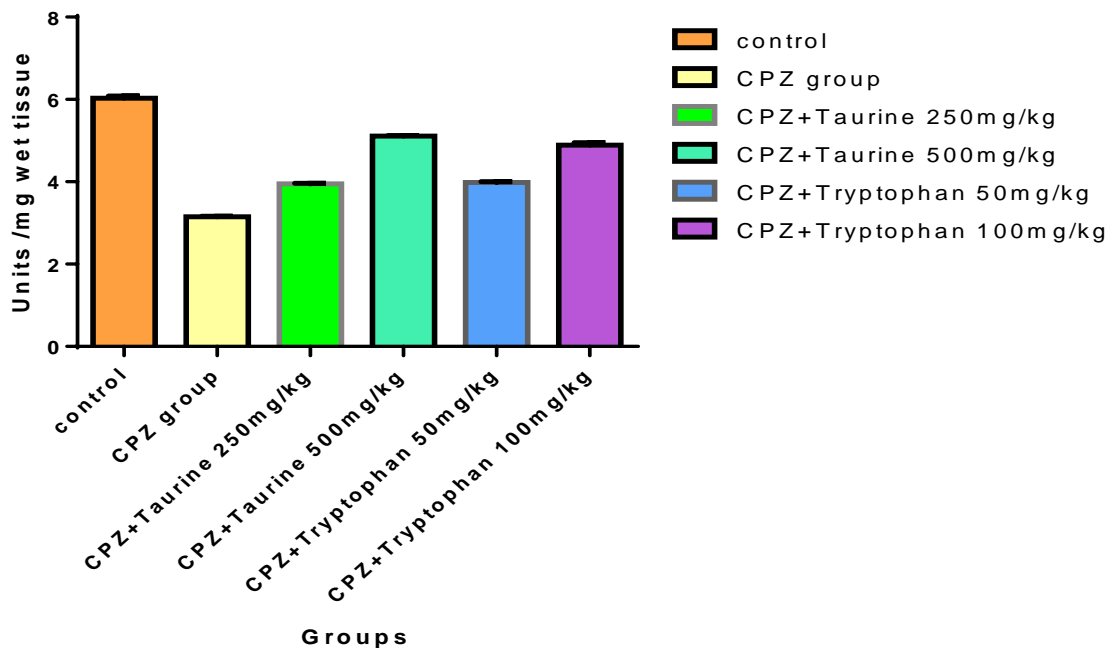**Graph 8: Effect of Taurine and Tryptophan on Glutathione reductase**

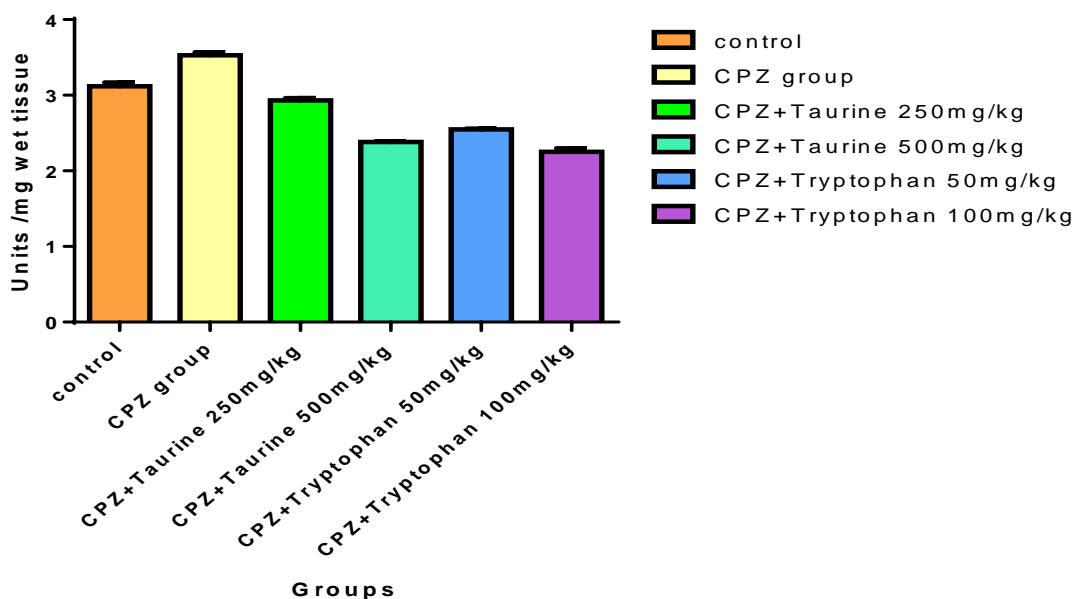Table 11: Effect of Taurine and Tryptophan on Interleukin – 6

SI no.	GROUPS	Interleukin -6
1	CONTROL	3.12±.05
2	CPZ GROUP	3.53±.04
3	CPZ+TAURINE LOW DOSE	2.93±0.03[**]
4	CPZ+TAURINE HIGH DOSE	2.38±0.01[****]
5	CPZ+TRYPTOPHAN LOW DOSE	2.55±0.01[***]
6	CPZ+TRYPTOPHAN HIGH DOSE	2.25±0.05[***]

Values are represented in Mean ± SEM, n=6

Comparison: a- Group II vs Group III and Group IV and Group V and Group VI

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test, ns non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Graph 10: Effect of Taurine and Tryptophan on Interleukin-6

RESULTS

7.1.ASSESSMENT OF LEARNING AND MEMORY

7.1.1. Effect of Taurine and Tryptophan in Morris water maze

For the groups III, IV, V and VI , there is significant decrease in the escape latency on to the hidden platform as compared with group II animals. The results are given in table 5 and plotted in graph 1.

7.1.2.Effect of Taurne and Tryptophan in Y-maze

For the group III, IV,V and VI ,there is significant increase in the percentage of alteration as compared to group II animals. The results are given in table 6 and plotted in graph 2.

7.1.3.Effect of Taurine and Tryptophan in Passive avoidance test

The group III, IV,V and VI animals showed marked decrease in the step down latency when compared to Group II animals. The results are given in table 7 and plotted in graph 3.

7.2. ASSESSMENT OF LOCOMOTOR ACTIVITY

7.2.1. Effect of Taurine and Tryptophan in Actophotometer

The group III, IV, V and VI showed an increase in the locomotor activity when compared to the group II animals. The results are given in table 8and plotted in graph 4.

7.3. ASSESSMENT OF MOTOR CORDINATION

7.3.1. Effect of Taurine and Tryptophan in Rotarod

The group III, IV, V and VI showed significant increase in fall off time when compared with group II animals.The results are given in table 9 and plotted in graph 5.

7.4. ESTIMATION OF ANTI OXIDANTS ENZYME

7.4.1. Effect of Taurine and Tryptophan in SOD

The group III, IV,V and VI animals showed significant increase in SOD level compared to group II animals. The results are given in table 10 and plotted in graph 6.

7.4.2. Effect of Taurine and Tryptophan in catalase

The group III, IV,V and VI animals showed significant increase in Catalase level when compared with group II animals. The results are given in table 11 and plotted in graph 7.

7.4.3. Effect of Taurine and Tryptophan in glutathione reductase

The group III, IV,V and VI animals showed significant increase in Glutathione reductase level compared with group II animals. The results are given in table 12 and plotted in graph 8.

7.4.4. Effect of Taurine and Tryptophan in glutathione peroxidase

The group III, IV,V and VI animals showed significant increase in Glutathione peroxidase level when compared with group II animals. The results are given in table 13 and plotted in graph 9.

7.5. ESTIMATION OF NEURO INFLAMMATION

7.5.1. Effect of Taurine and Tryptophan in IL-6

The group III, IV,V and VI animals showed significant decrease in the level of IL-6 in comparison with Group II animals. The results are given in table 14 and plotted in graph 10.
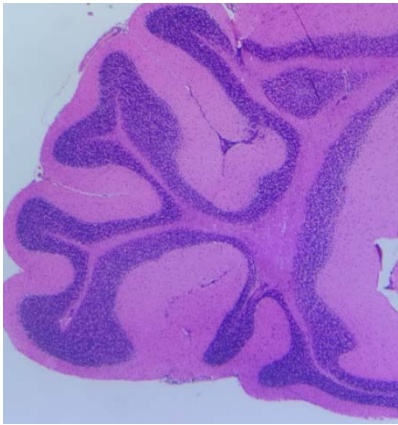
8. ASSESSMENT OF HISTOPATHOLOGICAL CHANGES
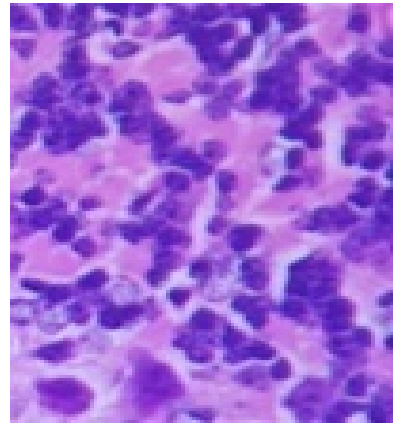

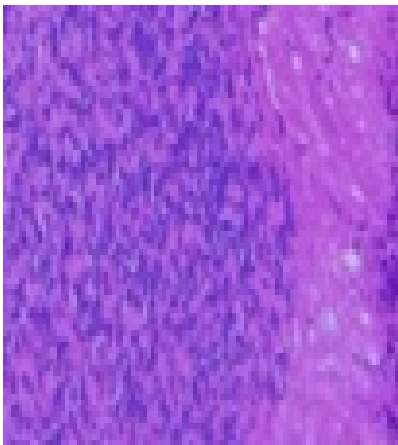Figure 11. CONTROL

Figure 12. CPZ GROUP
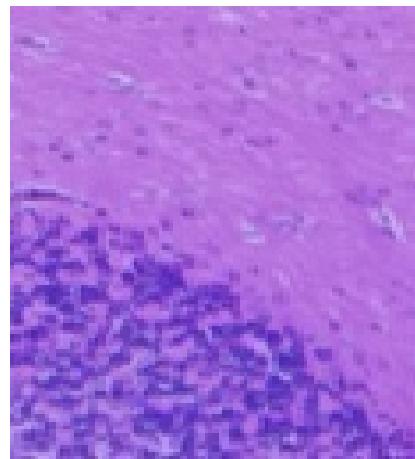

Figure13. CPZ+ TAURINE HIGH DOSE

Figure14. CPZ+TAURINE L D
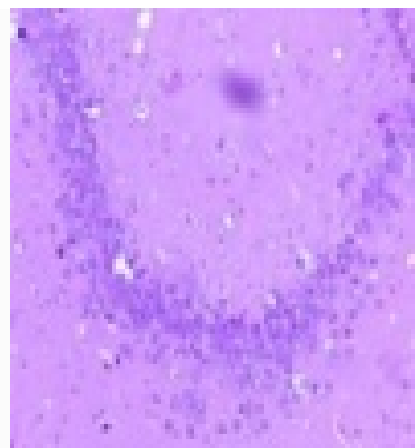

Figure15. CPZ+TRP HIGH DOSE

Figure16. CPZ+TRP LOW DOSE

Figure 11: Control animal brain shows clear healthy neuronal arrangement of neurons in cerebellum.

Figure 12: Cuprizone treated brain shows total derangment and degeneration of neurons and inflammation is clearly seen.

Figure 13: Animal brain treated with Taurine at high dose shows full recovery of the neuronal cells and neuronal arrangement

Figure 14: Animal brain treated with Taurine at low dose shows partial recovery of neuronal cells and neuronal derangement and slightly degenerated cells are seen.

Figure 15: Tryptophan high dose treated brain shows full regeneration of the neuronal cells and cellular arrangement is similar to control group.

Figure 16: Tryptophan low dose treated brain shows partial damaged neuronal cells with inflammation.

DISCUSSION

8. DISCUSSION

Multiple sclerosis (MS) is the most prevalent inflammatory demyelinating disease of the central nervous system. Besides other pathophysiological mechanisms, mitochondrial injury and oxidative stress are crucially involved in the development and progression of this disease. Cuprizone is a neurotoxin with copper-chelating ability used in animal model of multiple sclerosis. This study aimed at evaluating the ameliorative capability of **Taurine** and **Tryptophan** in cuprizone-induced behavioral and histopathological alterations in Swiss albino mice.

In this study, cuprizone treatment adequately induced oxidative stress and mitochondrial injury in the experimental animals, which can be manifested in the brain histomorphology and neurobehavioral outcomes of the animals.

From our findings oral treatment of Taurine and Tryptophan reduced the cuprizone-induced demyelination, restored the antioxidant levels and neuronal integrity .

The CPZ induced oxidative stress caused a reduction in the activities of SOD and CAT in mice treated with cuprizone. This points out that there is a superfluous generation of ROS by the actions of CPZ that cannot be controlled by the intrinsic antioxidant system in the brain. SOD and CAT are important enzymes critical in the antioxidant system. Exacerbating the level of superoxide and hydrogen peroxide will eventually downplay the activities of SOD and CAT, respectively, thereby resulting into oxidative stress. Taurine and Tryptophan treatment normalized the level of SOD, CAT, Glutathione reductase and Glutathione peroxidase .The proposed mechanism is that taurine acts as an antioxidant in its own capacity, thereby helping the intrinsic antioxidant system to mop up the excessively generated reactive species while tryptophan take part in synthesis of melatonin and NAS which are proved to have antioxidant effects.

Assessment of neuronal inflammation by interleukin -6 levels which is a proinflammatory cytokine revealed that cuprizone is able to produce neuronal inflammation. Taurine and Tryptophan reduced the level of interleukin-6 dose

dependently, compared to CPZ group which shows the anti neuroinflammatory activity of these compounds.

Our findings from the neurobehavioral test in the present study revealed that CPZ treatment significantly depleted memory, motor co ordination and locomotor activity. As a measure of learning and memory, animals were subjected to Morris water maze, Y maze and Passive avoidance test. Animals maintained on CPZ diets for 5 weeks had an increased escape latency period which indicates depletion of long term memory. Likewise, in the spontaneous alternation test which was used to assess short-term memory, these animals presented with decreased percentage correct alternation. This observed depletion in the memory of experimental animals suggests the possible negative effect CPZ has on the cortico-hippocampal region responsible for memory formation and long-term potentiation. Taurine and Tryptophan treatment restored the long and short-term memory in experimental animals which is evident from their decreased escape latency and increased percentage alternations. This is consistent with the report that Taurine is able to improve the performance of animals in memory tests and increase the exploration activity whie Tryptophan is be to improve cognition through its serotonergic pathway.

Histochemical findings in this study support previous reports that CPZ extensively damages neurons in corpus callosum and other areas of brain. Taurine and Tryptophan restored neuronal integrity and and prevented neuronal degeneration by their neuroprotective action which is already established.

SUMMARY

AND CONCLUSION

9. SUMMARY

Multiple sclerosis (MS) is a demyelinating neurodegenerative disease, representing a major cause of neurological disability in young adults. Taurine and Tryptophan are two amino acids known to pass blood brain barrier and exhibit antioxidant, anti-inflammatory, and neuroprotective effects in several brain pathologies. Cuprizone model of MS is particularly beneficial in studying demyelination/remyelination. Our study examined the potential neuroprotective effects of Taurine and Tryptophan in cuprizone-intoxicated Swiss Albino mice. Mice were fed with chow containing 0.2 % cuprizone for 4 weeks Taurine (250 mg/kg/day and 500 mg/kg/day , p.o.) and Tryptophan (50 mg/kg/day and 100 mg/kg/day , p.o.) were given for 3 weeks starting from the second week. At the end of the experiment, animals were tested on morris water maze, Y maze, rotarod, passive avoidance paradigm and actophotometer to evaluate changes in learning, memory and motor coordination. Mice were then sacrificed to measure the brain content of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase . The activitiy of pro inflammatory cytokine interleukin 6 were also assessed. stained brain sections were compared to assess the myelin status. Taurine and Tryptophan effectively enhanced learning memory and motor coordination, reduced cuprizone-induced demyelination, improved mitochondrial function, and alleviated oxidative stress, The present study indicate a pro-remyelinative effect for Taurine and Tryptophan which might represent a potential additive benefit in treating MS.

10. CONCLUSION

The present study was aimed to evaluate the efficacy of Taurine and Trypyophan in Cuprizone model of multiple sclerosis. It was hypothised that Taurine and Tryptophan would be able to ameliorate the neuronal degeneration and neuroinflammation present in Multiple Sclerosis. The study was evaluated by assessment of various in vivo parameters like of learning and memory, locomotor activity and motor co ordination.The in vitro parameters like SOD, Catalase ,Glutathione reductase, Glutathione peroxidase and proinflammtoiry cytokine InterleuikZin-6 were also assessed. This study identified cuprizone as a neurotoxin capable of inducing oxidative stress. It also revealed the protective effect of Taurine and Tryptophan against CPZ-induced behavioral and neuropathological deficits in Swiss Albino mice.

REFERENCES

REFERENCES

1. Nazem Ghasemi, Shahnaz Razavi, Elham Nikzad..Multiple sclerosis :pathogenesis, symptoms,diagnosis and cell based therapy .Cell Journal. 2017; 19(1): 1-10

2. Hauser SL, Goodwin DS. Multiple sclerosis and other demyelinating diseases. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, eds. Harrison's Principles of Internal Medicine, vol. II, 17th ed. New York: McGraw-Hill Medical; 2008:2611–2621.

3. Hammer A, Nilsagård Y, Forsberg A, Pepa H, Skargren E, Oberg B. Evaluation of therapeutic riding (Sweden)/hippotherapy (United States). A single-subject experimental design study replicated in eleven patients with multiple sclerosis. Physiother Theory Pract 2005; 21(1)

4. Savarin C, Bergmann CC, Gaignage M, Stohlman SA. Selfreactive CD4+ T cells activated during viral-induced demyelination do not prevent clinical recovery. J Neuroinflamm 2015; 12: 207.

5. Salverti M, Ristori G, Buttinelli C, Fiori P. Falcone M, Britton W,Adams E, Paone G, Grasso MG, Pozzilli C. The immune response to mycobacterial 70-kDa heat shock proteins frequently involves autoreactive T cells and is quantitatively disregulated in multiple sclerosis. J Neuroimmunol 65:143-153, 1996.

6. Shimoji Y, Ng V. Matsumura K, Fischerti VA, Rambukkana A. A 21-kDa surface protein of tviycohacterium leprue binds peripheral nerve laminin-2 and mediates Schwann cell invasion. Proc Nail Acad Sci U S A 9617:9857-9862, 1999.

7. J. D. Lutton, R. Winsron, T. C. Rodman. Multiple Sclerosis: Etiological Mechanisms and Future Directions. Exp Biol Med 229:12-20, 2004.

8. Marrie RA. Environmental risk factors in multiple sclerosis aetiology. Lancet Neurol 2004; 3(12): 709-18

9. Confavreux C, Vukusic S: Natural history of multiple sclerosis: a unifying concept. Brain 2006; 129: 606–616

10. Pugliatti M, Rosati G, Carton H, Riise T, Drulovic J, Vécsei L, Milanov I: The epidemiology of multiple sclerosis in Europe (review). Eur J Neurol 2006;   13: 700–722.

11. Alonso A, Hernán MA: Temporal trends in the incidence of multiple sclerosis: a systematic review (review). Neurology 2008;   71:   129– 135

12. Grytten Torkildsen N, Lie SA, Aarseth JH, Nyland H, Myhr KM: Survival and cause of death in multiple sclerosis: results from a 50year follow-up in Western Norway. Mult Scler 2008;   14:   1191–1198

13. Multiple Sclerosis: Perspectives in Treatment and Pathogenesis. Ian S. Zagon and Patricia J. McLaughlin (Editors), Codon Publications, Brisbane, Australia. ISBN: 978-0-9944381-3-3;140

14. Ingrid Loma and Rock Heyman: Multiple Sclerosis: Pathogenesis and Treatment. Current Neuropharmacology, 2011; 9: 409-416

15. Multiple Sclerosis: Perspectives in Treatment and Pathogenesis. Ian S. Zagon and Patricia J. McLaughlin (Editors), Codon Publications, Brisbane, Australia. ISBN: 978-0-9944381-3-3;157-158

16. Markus Kipp, Tim Clarner , Jon Dang , Sjef Copray, Cordian Beyer. The cuprizone animal model: new insights into an old story. Acta Neuropathol .2009; 118:723–736.

17. Harris Ripps,Wen Shen**.** Review: Taurine: A "very essential" amino acid. Molecular   Vision. 2012; 18:2673-2686.

18. Ta mai, I., Senmaru, M., Terasaki, T. & Tsuji, A. Na + - and Cl − -Dependent transport of taurine at the blood-brain barrier.Biochemical pharmacology.1995; 50: 1783–1793 .

19. Shao, A. & Hathcock, J. N. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Regulatory toxicology and pharmacology: RTP. 2008;50:376–399.

20. Rahmeier, F. L. et al. The effect of taurine and enriched environment on behaviour, memory and hippocampus of diabetic rats.Neuroscience letters. 2016;630: 84–92.

21. Altamura, C., Maes, M., Dai, J. & Meltzer, H. Y. Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology.1995;5: 71–75 .

22. Gao-Feng Wu, Shuang Ren, Ri-Yi Tang, Chang Xu, Jia-Qi Zhou, Shu-Mei Lin, Ying Feng,Qun-Hui Yang, Jian-Min Hu & Jian-Cheng Yang. Antidepressant effect of taurine in chronic unpredictable mild stress induced depressive rats. Scientific Reports.2017; 7: 4989

23. Wu JY, Tang XW, Schloss JV, Faiman MD. Regulation of taurine biosynthesis and its physiological significance in the brain. Adv Exp Med Biol 1998; 442:339-45.

24. Y. Su, W. Fan, Z. Ma, X. Wen, W. Wang, Q. Wu, H. Huang. Taurine improves functional and histological outcomes and reduces inflammation in traumatic brain injury, Neuroscience. 2014; 266:56–65.

25. Li, X. W., Gao, H. Y. & Liu, J. The role of taurine in improving neural stem cells proliferation and differentiation. Nutr Neurosci. 2016;18:287–301 .

26. Hernández-Benítez R, Pasantes-Morales H, Saldaña IT, Ramos-Mandujano G. Taurine stimulates proliferation of mice embryonic cultured neural progenitor cells. J Neurosci Res 2010; 88:1673-81.

27. Chen G, Nan C, Tian J, Jean-Charles P, Li Y, Weissbach H, Huang XP. Protective effects of taurine against oxidative stress in the heart of MsrA knockout mice. J Cell Biochem. 2012; 113:3559-66. [PMID: 22740506].

28. Das J, Sil PC. Taurine ameliorates alloxan-induced diabetic renal injury, oxidative stress-related signalling pathways and apoptosis in rats. Amino Acids. 2012; 43(4):1509-23.

29. Marcinkiewicz J, Kontny E. Taurine and inflammatory diseases. Amino Acids. 2014; 46, 7–20

30. Schaffer SW, Azuma J, Mozaffari M. Role of antioxidant activity of taurine in diabetes. Can J Physiol Pharmacol 2009; 87:91-9. [PMID: 19234572].

31. Jong CJ, Azuma J, Schaffer S. Mechanism underlying the antioxidant activity of taurine: prevention of mitochondrial oxidant production. Amino Acids 2012; 42:2223-32

32. Dawn M Richard, Michael A Dawes, Charles W Mathias, Ashley Acheson, Nathalie Hill-Kapturczak1 and Donald M Dougherty. L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. International Journal of Tryptophan Research 2009:2:45–60

33. Alessandro Tagliamonte, Giovanni Biggio, LidiaVargiu, Gian Luigi Gessa. Free tryptophan in serum controls brain tryptophan level and serotonin synthesis. Life Sciences. 1973;12(6):277-287

34. Lauer JW, Inskip WM, Bemsohn J, et al. Observations on schizophrenic patients after iproniazid and tryptophan. *AMA Arch Neurol Psychiatry*. 1958;80:122–30.

35. Young SN. Behavioral effects of dietary neurotransmitter precursors:Basic and clinical aspects. *Neurosci Biobehav Rev*. 1996;20:313–23.

36. F. Monaco, s. Fumero, a. Mondino, and r. Mutani. Plasma and cerebrospinal fluid tryptophan in multiple sclerosis and degenerative diseases. Journal of Neurology, Neurosurgery, and Psychiatry.1979; 42: 640-641

37. Kisser, W., and Harrer, G. (1974). Amino acid determinations in serum of patients with multiple sclerosis.Wiener Klinische Wochenschrift, 86, 80-82.

38. Manolis Markianos et.al.Relationship of CSF neurotransmitter metabolite levels to disease severity and disability in multiple sclerosis. Journal of Neurochemistry . 2009;108 :158–164

39. Opitz C. A., Wick W., Steinman L. and Platten M. Tryptophan degradation in autoimmune diseases. Cell. Mol. Life Sci.2007; 64:2542– 2563.

40. Heyes M. P., Saito K., Crowley J. S. et al. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. Brain.1992; 115:1249–1273

41. Wen J, Ariyannur PS, Ribeiro R, Tanaka M, Moffett JR, Kirmani BF, Namboodiri AM,Zhang Y.Efficacy of N-acetylserotonin and melatonin in the EAE model of multiple sclerosis. Journal of NeuroImmune Pharmacology. 2016;**11**:763-773.

42. Morris R, Development of a water maze procedure for studying spatial learning in the rat. J Neuroscience Methods, 1984, 11:47-60.

43. Chowdary SG. Neuropharmacological Screening of ethanolic extract of Nelumbo nucifera Gaertner seeds. Indian Journal of Research in Pharmacy and Biotechnology. 2013 Sep 1;1(5):635.

44. Kulkarni,S.K. and Dandiya,P.C.,Indian J. Med.Res . 63: 468 , 1975.

45. Standard Operating Procedure. Standard Behavioural Functional Neuroscience Laboratory. Behavioural Assay.

46. Yang M, Crawley JN. Simple behavioral assessment of mouse olfaction. Current protocols in neuroscience. 2009 Jul 1;48(1):8-24.

47. Deacon RM. Measuring motor coordination in mice. Journal of visualized experiments: JoVE. 2013(75).

48. Misra H.P, Fridovich I. Biochemistry, 1967, 15:681.

49. L. A. Goth, L. A. Almasheddy,Simple method for determination ofserum catalase activity and revision of reference range.Clin.Chim.Acta,196(1996),143-152.

50. Beutler E, Duron O, Kelly B.M. Improved method for the determination of blood glutathione. J Lab Clin Med, 1963; 61:882-8.

51. Boring, L., J. Gosling. Molecular cloning and functional expression of murine JE (monocyte chemoattractant protein 1) and murine macrophage inflammatory protein 1alpha receptors: evidence for two closely linked C-C chemokine receptors on chromosome . J Biol Chem 1996; 271(13): 7551-8.