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# A REVIEW ON MULTIPLE SCLEROSIS ACTIVITY OF PLANTS FROM THE ZINGIBEBRACEAE FAMILY

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**Abstract:**

*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 de- struction 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. Subtypes of MS are considered important not only for prognosis but also for treatment decisions and include: relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS (PRMS). Induced symptoms include problems with walking, weakness, stiffness, and trouble with balance. lants have been used as medicines throughout history. Indeed, studies of wild animals show that they also instinctively eat certain plants to treat themselves for certain illnesses. Medicinal plants are widely and successfully used on every continent. In Asia, the practice of herbal medicine is extremely well established and documented. Multiple sclerosis is a chronic inflammatory disease that affects the brain and the spinal cord. Environmental factors such as vitamin D levels, viral infections, obesity, and smoking can contribute to MS development. Genetic factors also have a significant role in MS formation as several genes can increase susceptibility to this disease. The herbal plants showing strong activity on Multiple sclerosis in zingiberace family such as zingiber officinal and curcuma loga.*

**Keywords:** *Multiple sclerosis, Zingiberace family, Astrocytes, Medicine, Animals*

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## INTRODUCTION:

**Drug discovery:**

Drug discovery is the process of identifying chemical entities that have the potential to become therapeutic agents. This process involves the identification of candidates, synthesis, characterization, validation, optimization, screening and assays for therapeutical efficacy. Once a compound has shown its significance in these investigations, it will initiate the process of drug development earlier to clinical trials.

**Preclinical development:**

The preclinical phase of drug development refers to testing of activity and toxicity of drug candidate in invitro in vivo studies before testing in patients can be performed the preclinical development stage begin when a visible candidate for formal preclinical development has been chosen. The main goals of preclinical studies are to determine a starting, safe doses for first in-human study and asses potential toxicity of the product, which typically include new medical devices, prescription drugs, and diagnostics.

**Clinical Development:**

Clinical pharmacology is the study of drugs in humans. It is the science of relationship between drugs and humans, which focuses on drug action, and incorporates pharmacological principles and techniques into the clinical development cycle.

**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 de- struction 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. Subtypes of MS are considered important not only for prognosis but also for treatment decisions and include: relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS (PRMS). 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. 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. 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, mental disorders, and psychological impairment.

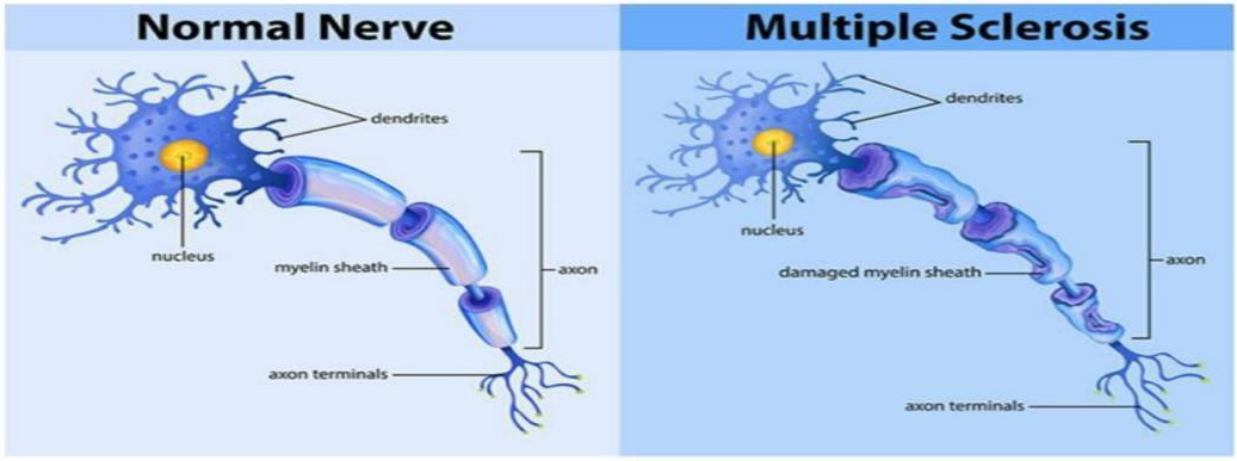


Figure no:1- Difference between normal nerve and nerve with multiple sclerosis

**PATHOPHYSIOLOGY:**

Due to etiological factors

⇩

Activated T cell (which recognise self-age) expressed in CNS and macrophages (B-cell)

⇩

Enters the brain from peripherral circulation

⇩

Production of inflammatory cytokines and reactive O2 species

⇩

Inflammation

⇩

Then activated T- cell and B-cell cause

⇩

Demyelination and destruction of oligodendrocytes

⇩

Formation of plaque

⇩

Causes scarring and destruction of sheath

⇩

Compensatory system starts causing subsidation of edema and inflammation

⇩

After that some remyelination process occurs which is often incomplete

⇩

Multiple sclerosis

Common symptoms in multiple sclerosis
- Sexual Problems
- Bladder problems
- Bowel problems
- Fatigue
- Vision problems

**MANAGEMENT AND TREATEMENT:**

There is currently no cure for MS. Treatment focuses on managing symptoms, reducing relapses and slowing the disease's progression. Your comprehensive treatment plan may include:

**Disease-modifying therapies (DMTs):** Several medications have FDA approval for long-term MS treatment. These drugs help reduce relapses (also called flare-ups or attacks). They slow down the disease's progression. And they can prevent new lesions from forming on the brain and spinal cord.

**Relapse management medications**:

If you have a severe attack, your neurologist may recommend a high dose of corticosteroids. The medication can quickly reduce inflammation. They

slow damage to the myelin sheath surrounding your nerve cells.

**Physical rehabilitation**:
Multiple sclerosis can affect your physical function. Staying physically fit and strong will help you maintain your mobility.

## PHARMACOLOGICAL SCREENING METHODS USED IN MULTIPLE SCLEROSIS:

**In vitro methods:**
Astrocytes In the healthy CNS, astrocytes maintain homeostasis including preserving the integrity of the blood-brain barrier. In the damaged brain, they often appear hypertrophic and form the scar tissue typical of chronic MS lesions. When damaged themselves, astrocytes may lose the ability to maintain the blood-brain barrier, thus contributing to further damage. In contrast, astrocytes also help the repair process by secreting growth factors, thus promoting regeneration. To examine these aspects, several human and animal cell lines are available, as well as primary cultures. Primary astrocyte cultures are relatively slow growing, yet have the advantage of not only being suitable for repeated passage, but also amenable to cryopreservation. Astrocyte cell lines have been derived from mice and rats using transfection systems, or derived from astrocytomas in respond differently as compared to primary astrocytes from post mortem foetal or adult tissues, as well as from biopsies from neurosurgery patients.

**Neurons:**
Both primary and neuronal cell lines are widely available, including from mice, rats and humans. Many of these are derived from embryos, although more recent technology has produced neuronal cultures from pluripotent stem cells from rats. In general, primary human neurons are difficult to maintain, probably since most tissues are obtained post-mortem. However, some cells have been generated during surgery. Neuronal cell lines are available and do overcome some of the problems of low cell numbers and difficulties in culturing primary neurons.

**Blood Brain Barrier (BBB) :**
The blood-brain barrier (BBB) is considered an important subject in MS research since it is widely considered that the BBB, and indeed the blood spinal cord barrier and blood-CSF barrier, are defective in people with MS. While this dysfunction could be the primary trigger of the disease, allowing access of potentially pathogenic T cells, it could also be the consequence of inflammation in the CNS. Whatever the initial cause, the idea that the BBB is compromised has led to the development of several immunotherapeutic approaches to prevent cells from entering the CNS. The role of the BBB, mechanisms of BBB damage, and how immune cells can gain access to the CNS across the BBB, has been studied using in vitro BBB model transendothelial electrical resistance can be measured, while other models take into account the dynamics and physiological role of the BBB.

**In vivo methods:**
**Behavioral tests:**
The behavioral studies on animals give the visible manifestation of activity of the central nervous system. The behaviour of animals can be correlated with measurement of brain electric or chemical activity to evaluate the disease condition. In the present study the disease progression and its statuses were evaluated with various behavioral tests of animals such as maze apparatus testing, rota rod tests, Actophotometer etc.

**T-Maze**
The T- Maze is the apparatus used in the study of fear and anxiety. The test is based on the natural aversion of rats for open and elevated areas. The number of entries into the open arms and the time spent in the open arms are used as indices of open-space induced anxiety in rat. T- Maze is also used for estimation of special memory. The apparatus can be used for the measurement of short time memory, general locomotor activity and stereotypic behaviour.


***Fig no:2 T Maze***

**Procedure**
Rats were placed in the central square of EPM facing an open arm and were then allowed to explore the apparatus for 5 minutes. The behaviour of the animals was recorded. Rearing, head –dips, grooming, stretchattends and line crosses were scored and the measures of arm entries and time in each arm are recorded. The maze apparatuses were cleaned with 5% ethanol before each trial.

**Acto photometer:**
**Principle:**
The test used for the estimation of locomotor activity of experimental animals. The test is designed to study the spontaneous or induced or locomotors activity of experimental animals. The optical sensors and emitters used will record the horizontal movements of the animals on a six digital counter display. Based on the reading in the digital counter display, the locomotor activity of the animal was assessed in the Actophotometer.


***Fig no: 3 Actophotometer***

**Procedure**
Before kept the animal in the apparatus, turned the equipment on and checked that all the photocells are working for accurate recording. Then the experimental animals were placed in the activity box for 10 minutes. The basal activity score was also recorded.

**Rota rod:**
**Principle:**
Rota rod test is carried out for the assessment of coordination and balance and it also provide the measurement of locomotor activity. The rota rod consists of the rotating cylinder upon which the animal is placed. On the rotation of the cylinder the animal must foreword to keep from falling off the cylinder. The animal with deficits affecting balance or coordination wills.


***Fig no: 4 Rota Rods***

**Procedure**
The apparatus was turned on and the animals were placed on the rotating cylinder. The fall of different groups of animals from the cylinder was recorded and correlated with neurological functioning all from the apparatus more quickly than the animals with normal motor function.

**Experimental pharmacology**
**Care and maintained of laboratory animals:**
To be defined as laboratory "The species must be bred and raised under idea condition and kept in a rigorously controlled environment under constant monitoring so that all microbiologic and genetic factors are known. Most frequently used animal are mouse, mice, rat, hamster, guinea pig, pig, dog, cat, monkey.

**Housing of experimental animals:**
Proper housing and good management of animal facilities are essential for the animal wellbeing, the quality of the research data, and the health and safety of personnel. At a minimum, animal must have sufficient space to turn around and to express normal postural adjustments, it must have ready access to food and water, and it must have enough clean bedded or unobstructed area in which to move and rest.

**Maintenance:**
The person in charge of the establishment must ensure regular inspection and maintenance of the animal. Wherever it is possible species should be housed in a separate room. The factors which should maintained for laboratory animals are food, drinking water, bedding, sanitation. The factors required in micro environment are temperature, humidity, ventilation.

**Iaec Approval:**
With procedures specified for the purpose by the Committee IAEC accords quality and Institutional Animals Ethics Committee (IEAC) means a body comprising of a group of persons recognised and registered by the committee for the purpose of control and supervision of experiments on Animals (CPCSEA) in a establishment which us constituted and operated in accordance consistency in review of research proposals and to prevent infliction of unnecessary pain & sufferings before, during and after experiments on animal to follow the CPCSEA guidelines.

**Function of IAEC**
AEC should provide independent, competent and timely review of the ethics of a proposed study before

the commencement of the same and regularly monitor the ongoing studies

**Toxicity studies:**
Toxicology is the study of how chemical substances interact with living systems and affect normal processes, and the use of this information to predict safe exposure levels.Toxicology studies are crucial to the discovery and development of safe products such as new drugs, cleaning products, plastic food containers, flame retardant infant clothing and food additives, to name a few. Toxicologists perform research in whole animals to ensure the short- term and long-term safety of such products before they are brought to market. If research on a new substance predicts significant risks to human or animal health, or to the environment, then that substance may never reach +market place.

**Acute Toxicity:**
This test is divided into stages, and the result from each stage determines whether to terminate or proceed to the next stage.
**Stage 1:** This stage requires four animals (consisting of two different strains). These animals are divided into four groups of one animal each. Animals in Groups 1 and 3 are of the same strain, while those in Groups 2 and 4 are also of the same strain. The animals are kept under intense observation for an hour after administration and then 10 min at 2 h interval for 24 h.
**Stage 2:** This stage involves three animals (consisting of two different strains). The animals are divided into three groups of one animal each. Animals in Groups 1 and 3 are of the same strain while that in Group 2 of a different strain. Higher doses of the test substance are administered to the animals and then carefully observed for 1 h after administration and periodically for 24 h.
**Stage 3:** This stage involves three animals (consisting of two different strains) which are assigned to three different groups of one animal each. Animals in Groups 1 and 3 are of the same strain while that in Group 2 of a different strain. Careful observation is performed for 1 h of post administration and then 10 min at 2 h.

**Dose calculation:**
The dose of a drug is the quantitative amount administered or taken by a patient for the intended medicinal effect. In preclinical research, the experimental animals are dosed according to their body weight. The selection of doses is usually decided based on acute oral toxicity studies.

**Pharmacological screening of plants from zingeberacea:**
A few very influential ones and a majority of non-influential ones. A screening method is a that exerts extracts, isolates, and identifies a or group of components in a sample with the minimum number of steps and the least manipulation of the sample. Screening method is the identification of a few parameters that have largest influence on the model outputs. This method aims to provide adequate information about the sensitivity of the to its input, while decreasing the computation cost.

**TEM examination :**
A piece of CC was isolated and postfixed in the aforementioned fixative solution at +4 °C overnight. After the preparation of the blocks, samples were sectioned using an ultramicrotome to about 90 nm thick. Sections were rinsed with phosphate buffer saline (0.12 M; pH 7.4) and postfixed with osmium tetroxide solution (1%) in PBS for 60 min and dehydrated. Uranyl acetate (2%) and Reynold's lead citrate were utilized to contrast the sections. Photographs of the CC axons cut in cross-section were taken, and quantification of the myelinated axons was performed on 10 images (×3000) per specimen. The myelin thickness was measured using at least 50 axons per sample.

**CPZ - induced demyelination**
An MS model was induced in all experimental groups, except for the control group. For this purpose, 0.6% CPZ dissolved in corn oil was administered daily by oral gavage for four weeks.

**Basket test**
Before sampling and sacrificing the animals, the basket test was performed twice a day to determine the motor function of the animals. This test is useful in assessing motor coordination and sensorimotor deficits in rodent models of CNS disorders. A rat was placed in the center of a mesh-like basket and then the basket was inverted quickly and carefully. The rat's performance was evaluated by recording the latency to fall within 180 s.

**In vitro method of curcumin:**
Natarajan and Bright found that myelin basic protein-stimulated immune spleen cells from 6-week-old female SJL/J mice treated with 20 µg/ml curcumin showed lower proliferation of neural Ag-specific Th1 cells and a reduction in IFN-γ production. Splenic macrophages and microglia from SJL/J mice stimulated with 50 ng/ml IFN-γ and 1 µg/ml lipopolysaccharide (LPS) or anti-CD40 antibody showed a reduction in production of IL-12 during

treatment of curcumin in a dose-dependent fashion. Consequently, the curcumin's effect on activated T cells, where IL-12 is induced, was a significant dose dependent decrease in proliferation and differentiation through blocking of tyrosine phosphorylation in the STAT3 and STAT4 signalling pathway via the upstream JAK2 and TYK2. When BV2 cells (immortalized murine microglial cells) were pretreated with curcumin before addition of LPS, a significant reduction in release and accumulation of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, via suppression of NF- κB P65 nuclear protein was observed.

**In Vivo Methods:**

Curcumin's effect on the reduction in inflammation and demyelination in the CNS was studied in an induced EAE model. By analysing spinal cord sections from 6-week female SJL/J mice, treated with either 50 or 100 μg curcumin for 25 days, a significant decrease in inflammation and CNS demyelination in a dose-dependent fashion was demonstrated. The same study also investigated the efficacy of curcumin on the reduction of intensity and duration of paralysis induced in the EAE model. Injection of 50 μg curcumin decreased the period of paralysis from 16 down to 10 days (37.5% reduction) and by doubling the injection dose, this duration was reduced to 8 days. In chronic and relapsing EAE induced in 6-week-old female C57BL/6 and SJL/J mice, treatment with 100 μg 15d-PGJ2 or curcumin resulted in paralytic disease with only minor symptoms and intensity. In another study, investigating curcumin's role on manifestations of EAE, the mean clinical score in 6 to 8-week-old female C57BL/6 mice treated with 100 μg curcumin was 0.6 in comparison to 1.95 in the control group (69% reduction), indicating curcumin's efficacy in attenuating EAE. Treatment of adult female Lewis rats with 12.5 mg/kg PNC for 18 days in an induced EAE model of MS resulted in lowering of 20 the peak EAE score compared to both the control group and a curcumintreatment with no polymerized curcumin dissolved in PBS group.

**Multiple Sclerosis Activity Of Plants From The ZingieBaracea Family Medicinal Herbes From Zingiberacea :**

Medicinal Plants have been used as medicines throughout history. Indeed, studies of wild animals show that they also instinctively eat certain plants to treat themselves for certain illnesses. Medicinal plants are widely and successfully used on every continent. In Asia, the practice of herbal medicine is extremely well established and documented. As a result, most of the medicinal plants that have international recognition come from this region, particularly from China and India.

**MULTIPLE SCLEROSIS ACTIVITY IN *ZINGIEBARACEA* FAMILY**

***Zingiberace* family**

Family *Zingiberaceace* or the ginger family is a family of flowering plants made up of more than 1300 species of aromatic perennial herbs with creeping horizontal or tuberous rhizomes. Its members are divided into about 52 genera and distributed throughout tropical Africa, Asia, and the Americas.

***Zingiber officinale:***

Ginger (*Zingiber officinale)* is a flowering plant whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine. The inflorescences bear flowers having pale yellow petals with purple edges, and arise directly from the rhizome on separate shoots. Ginger is in the family *Zingiberaceace*, Ginger originated in Maritime Southeast Asia and was likely domesticated first by the Austronesian peoples.

**Fig no:5 *Zingiber officinale***

**Taxonomical classification**

Scientific name: *Zingiber officinale*
Family: *Zingiberaceae*
kingdom: Plantae
Order: Zingiberace; Griseb.
Vernacular name: Ichi, Ginger, Alla

**MATERIALS AND METHODS:**

Animals All experiments on rat models were conducted at the Central Laboratory of Isfahan University of Medical Sciences.

(1) The normal group that was not subjected to any intervention (Cont.)

(2) The M.S group including CPZ-induced MS rats that did not receive any treatment (Cup)

(3) Sham group consisting of rats with MS that received NaCMC as the fingolimod and ginger solvent

(4) Standard treatment group: M.S + fingolimod treatment (0.5 mg/kg/day)

**Preparation of ethanolic extract of ginger**

The rhizomes of ginger were purchased from an herbal medicine market in Isfahan (Iran). The ginger was identified, authenticated, and its extract was prepared under the supervision of Dr. Ghanadian, an academic staff of the Department of Pharmaceutical Chemistry and Pharmacognosy. Initially, 20 kg of fresh ginger rhizome was air-dried in shade and turned into powder mechanically (2.45 kg), and then its extract was prepared by maceration using 70% ethanol as a solvent for four days, repeated three times. The collected extracts were combined and concentrated using a rotary evaporator at 45 °C and low pressure (50 mbar) to obtain a gummy ethanol-free extract. The gummy extract was lyophilized by a freeze-dryer at -50 °C and 0.250 mbar for 48 h to obtain the dried powder (355 g). The extract yield was 14.5% of the dried weight of plant material.

**Invivo Method:**

Extract standardization by the determination of total polyphenols Ginger extract was standardized by the determination of total phenolic content through the colorimetric method and Folin-Ciocalteau according to the mg of GAEs. A standard calibration curve was prepared against 10 different concentrations of gallic acid as the standard. Subsequently, 20 µL of each concentration of the standard solution was taken, mixed with 1.58 mL of water and 100 µL of Folin–Ciocalteau reagent and shaken for 10 min. Also, 300 µL of the saturated NaHCO3 solution was added to the mixture, and after 2 h, the OD absorbance was measured at 765 nm by an ultraviolet/visible spectrophotometer.

**Statistical analysis**

Statistical analysis was carried out using the software SPSS (IBM, SPSS Statistics Version 24). The results of the experiments were expressed as mean ± standard error of the mean (SEM). The analysis was performed using the one-way analysis of variance (ANOVA), followed by the LSD post-hoc test. Statistical significance was defined at $p < 0.05$.

***Curcuma longa:***

Curcuma longa, a member of the ginger family (*Zingiberaceace*), has rhizomes below the ground. a yellow rhizome commonly known as turmeric. The medicinal use of this plant is part of traditional Chinese medicine and traditional medicine of India (Ayurveda), used to treat different conditions of inflammatory origin such as joint pain and skin inflammation, and is also used for diarrheal conditions and fever Curcumin has been shown to possess a variety of pharmacological activities, including antioxidant, anti-inflammatory, cancer chemo preventive, and neuroprotective activities.

**Fig no: 6 *Curcuma longa***

**Taxonomical classification**

Scientific name: *Curcuma longa*
Family*: Zingiberaceace*
Kingdom: Plantae
Order: Zingiber ales
Venicular name: Turmeric, Indian saffron

**In Vitro And In Vivo Methods Of *Curcumina Longa:***

As MS is a disabling disease, it impacts all aspect of a patient's life, and considering the current high cost and side effects of existing therapeutic agents, it is important to provide an effective, low-cost, and safe treatment.

**In vitro method**

Natarajan and Bright found that myelin basic protein-stimulated immune spleen cells from 6- week-old female SJL/J mice treated with 20 µg/ml curcumin showed lower proliferation of neural Ag-specific Th1 cells and a reduction in IFN-γ production. Splenic macrophages and microglia from SJL/J mice stimulated with 50 ng/ml IFN-γ and 1 µg/ml lipopolysaccharide (LPS) or anti-CD40 antibody showed a reduction in production of IL-12 during treatment of curcumin in a dose-dependent fashion. Moreover, human astrocyte cells, the most numerous brain glial cell population, play a dual neuroprotection and neurodegeneration role in autoimmune diseases. Pretreatment of LPS-induced astrocytes with the highest dose of curcumin (5 µg) reduced MMP-9 and IL-6, however, at the same time, had no effect on neurotrophin-3 and insulin-like growth factor-1 production, which are factors that play a crucial role in myelinogenesis. using the lumbar spinal cord of EAE induced female Lewis rats

treated in culture with h 12.5 mg/kg PNC for 18 days in an induced EAE model of MS resulted in lowering of the peakEAE score compared to both the control group and a curcumintreatment with no polymerized curcumin dissolved in PBS group. MS are demyelination and axonal damage. Demyelinated lesions can occur anywhere within the brain and spinal cord, leading to disease complexity and heterogeneity of clinical signs and symptoms. Based on the main findings detailed above, curcumin will lead to a promising treatment for MS. The clinically studied chemical properties of curcumin and its various effects on MS shows the possibility to do further research and develop better drugs based on curcumin for treating Multiple sclerosis.12.5 mg/kg polymerized nanocurcumin (PNC) once daily from Day 12–29, found a reduction in expression of proinflammatory genes, including IL-1, IL-17, TNF-αr1, MCP-1, and NF-κb. Further, they found enhancement of expression of other anti-inflammatory genes such as IL-4 and a Foxp3-regulatory factor of IL-10 production.

## DISCUSSION:

The present study showed that the treatment of multiple sclerosis with zingiberaceace family, zingiber officinal inhibited demyelination and alleviated remyelination of corpus callosum in rats.it could serve as a therapeutic agent in Multiple sclerosis. curcuma loga studies showed that this plant could improve Body Mass Index, glycemia, lipids, adiponectin, C reactive proteins, and cytokines levels. No severe adverse effects were referenced in the included studies. We conclude that the use of Curcuma longa can help to control risk factors in patients with Multiple sclerosis.

## CONCLUSION:

Multiple sclerosis is a chronic inflammatory disease that affects the brain and the spinal cord. Environmental factors such as vitamin D levels, viral infections, obesity, and smoking can contribute to MS development. Genetic factors also have a significant role in MS formation as several genes can increase susceptibility to this disease. The herbal plants showing strong activity on Multiple sclerosis in zingiberace family such as zingiber officinal and curcuma loga. In this review of multiple sclerosis activity on zingiberace family show that the remyelination occur in the neurons.

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