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Assessment of Inhibitory activities of monoamine oxidase enzyme in Moringa oleifera.

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ABSTRACT: Moringa oleifera, commonly known as the drumstick tree, has gained recognition for its extensive medicinal properties. This study aims to evaluate the inhibitory effects of Moringa oleifera extracts on the monoamine oxidase (MAO) enzyme, which plays a critical role in the breakdown of neurotransmitters like dopamine, norepinephrine, and serotonin. Dysregulation of MAO activity is linked to various neuropsychiatric and neurodegenerative disorders. Through both in vitro and in silico methods, the study found significant inhibition of MAO-A and MAO-B isoforms by Moringa oleifera extracts, with certain phytochemical constituents showing higher efficacy. These results suggest that Moringa oleifera could hold therapeutic potential for conditions associated with MAO dysfunction, such as depression and Parkinson's disease. Further research is needed to isolate specific bioactive compounds and elucidate their mechanisms of action.

KEYWORDS: Neurotransmitters, Monoamine oxidase, neurotransmission, Moringa oleifera, on various physiological processes.

I. INTRODUCTION

The traditional neurotransmitters encompass amino acids and biogenic amines, which play pivotal roles in initiating synaptic transmission within the nervous system. These classic neurotransmitters are synthesized and stored within neurons, being released upon receiving appropriate electrical signals. They are localized in presynaptic terminals and are released into the synaptic cleft in quantal portions, mediating postsynaptic excitatory (EPSP) or inhibitory (IPSP) events. The release of these neurotransmitters occurs selectively upon nerve stimulation in calcium-dependent manner.[1]

Neurotransmitters interact with receptors on postsynaptic or presynaptic sites, with their effects being preventable by specific antagonists and facilitated by specific agonists that mimic their actions. Rapid inactivation of neurotransmitters after release is mediated by specific enzymes or reuptake mechanisms. Experimental application of a neurotransmitter at postsynaptic sites induces effects identical to those of the endogenous substrate.[2]

The structural integrity of neurotransmitters remains conserved, ensuring their biological activity remains stable due to a strict structural-functional relationship. They can either act rapidly by opening ligand-gated ion channels, leading to an immediate current flow, or exert slow-acting effects, inducing long-lasting changes at the postsynaptic site through second-messenger systems. Except for histamine, neurotransmitters are recaptured by highly specific transport systems, which significantly contribute to their rapid inactivation, limiting their temporal and spatial actions. Some neuropeptides exhibit neurotransmitter-like effects, though their adherence to all the criteria mentioned above remains unconfirmed. Such neuropeptides, exhibiting classic neurotransmitter effects, are referred to as putative neurotransmitters or co-transmitters. Additionally, gaseous molecules like nitric oxide (NO) or carbon monoxide (CO) constitute further classes of substances exhibiting neurotransmitter properties.[2]

Neuropsychiatric disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and depression, have become significant social problems attracting worldwide attention. These conditions are complex, multifactorial, and currently lack effective treatments or cures, largely due to an incomplete understanding of their pathogenesis. Among the well-studied etiologies, the abnormal expression of mitochondrial enzyme monoamine oxidases (MAOs) has been recognized as a major cause. Excessive levels of monoamine metabolites produced by the over-expression of MAO can lead to oxidative stress and neuronal damage, contributing to the progression of neurodegenerative diseases. Consequently, MAOs

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present a promising biotarget for the development of MAO inhibitors (MAOIs), which have the potential to offer therapeutic benefits for these neuropsychiatric disorders.[3]

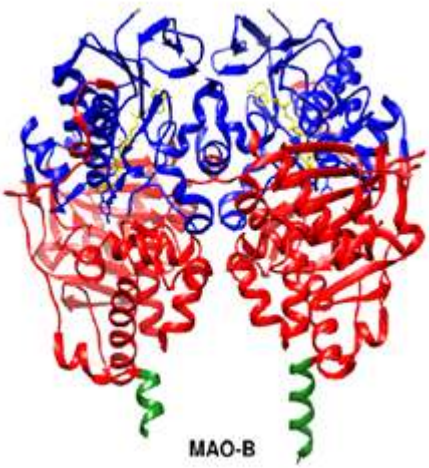
II. MONOAMINE OXIDASE (MAO):

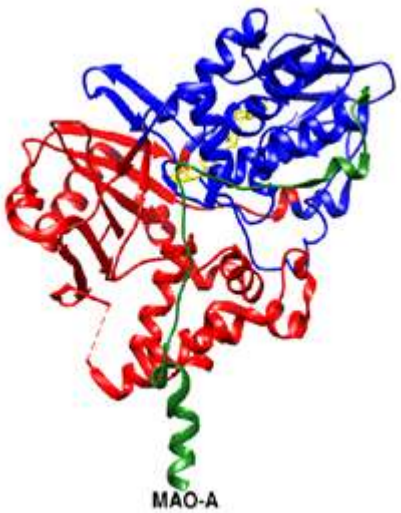
Monoamine oxidase (MAO) represents a category of enzymes present in the body responsible for the breakdown of monoamine neurotransmitters like dopamine, serotonin, and norepinephrine. Two primary forms of MAO exist: MAO-A and MAO-B, each playing distinct roles and serving specific functions within the body. Grasping the disparities between MAO-A and MAO-B is crucial for understanding their impacts on various physiological processes and their associations with health and illness. This comprehensive examination aims to explore the structure, function, regulation, significance, and clinical implications of both MAO-A and MAO-B, encompassing their

involvement in neurotransmission, behavioral patterns, mood regulation, and the pathogenesis of psychiatric conditions.[4]

MAO enzymes play a crucial role in the brain by catalyzing the oxidative deamination of monoamine neurotransmitters and xenobiotic amines. These enzymes exist in two isoforms, MAO-A and MAO-B, which are differentiated by their substrate affinity and inhibitor specificity. MAO-A is primarily found in the presynaptic terminals of catecholaminergic neurons, where it is heavily involved in the breakdown of serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE). On the other hand, MAO-B is present in serotonergic and histaminergic neurons, as well as in astrocytes and ventricular cells. Dopamine (DA) serves as a common substrate for both MAO isoforms. The regulation of these monoamine levels in the brain is vital for maintaining motor, perceptual, and cognitive functions, as well as for modulating mood and emotions.[5]

Abnormal expression or activity of MAO-A has been associated with various neuropsychiatric disorders and behavioral traits, including aggression, panic disorders, antisocial behaviors, major depressive disorder (MDD), bipolar depression (BD), attention-deficit hyperactivity disorder (ADHD), Parkinson's disease (PD), and Alzheimer's disease (AD). The two MAO isoforms exhibit different but overlapping substrate and inhibitor specificities. MAO-A preferentially oxidizes serotonin, norepinephrine, and epinephrine (EN) and can be irreversibly inhibited by low doses of cordyline. Conversely, MAO-B prefers phenylethylamine (PEA) as a substrate and is irreversibly inactivated by low doses of deprenyl (selegiline). Both isoforms also metabolize dopamine (DA) and tyramine.[5]





Understanding the specific roles and regulation of MAO-A and MAO-B is essential for developing targeted therapies for various neuropsychiatric and neurodegenerative disorders. MAO inhibitors (MAOIs) have been used in clinical settings to manage conditions like depression and Parkinson's disease by increasing the levels of monoamine neurotransmitters in the brain. The selective inhibition of MAO-A or MAO-B can lead to distinct therapeutic outcomes and

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side effects, emphasizing the importance of precise modulation of these enzymes in clinical practice. Further research into the molecular mechanisms and regulatory pathways of MAO isoforms will continue to provide insights into their roles in health and disease, paving the way for improved therapeutic use.[6]

III. LITERATURE REVIEW OF MORINGA OLEIFERA:

Moringa oleifera, commonly known as Moringa, is a fast-growing tree native to the sub-Himalayan regions of India, Pakistan, Bangladesh, and Afghanistan. Recognized by various names including the drumstick tree, horseradish tree, or ben oil tree, Moringa has been revered for centuries for its nutritional and medicinal properties. Its leaves, pods, seeds, and flowers are all edible and have been integral components of diverse cultural diets and traditional medicinal practices.[7]

One of the most remarkable attributes of Moringa oleifera is its exceptional nutritional profile. The leaves of the Moringa tree are particularly notable for their richness in vitamins, minerals, and protein, making them a valuable dietary supplement, especially in regions experiencing food insecurity. They are densely packed with essential nutrients such as vitamin A, vitamin C, calcium, potassium, iron, and protein, offering potential solutions to malnutrition and dietary deficiencies.[7]

BOTANICAL NAME:

Family: Moringaceae
Genus: Moringa
Species: Moringa oleifera
Common Names: Drumstick tree, Horseradish tree, Ben oil tree

Description: Moringa oleifera is a fast-growing, deciduous tree that can grow over 10 meters tall. It is characterized by a slender, branched trunk and feathery compound leaves. These leaves consist of small leaflets arranged in pairs along the stem. The tree produces clusters of white flowers that develop into long pods containing seeds surrounded by fibrous pulp. The pods transition from green to brown as they mature.[8]

Cultivation: Moringa oleifera is highly adaptable to various climates and soil types. It thrives in hot, arid conditions as well as tropical and subtropical environments. Its ability to endure drought and grow in poor soil conditions makes it suitable for cultivation in regions with limited water resources.[8]

Uses: Throughout history, different parts of the Moringa oleifera tree have been utilized for their nutritional and medicinal benefits. The leaves, pods, seeds, and flowers are all edible and incorporated into a variety of culinary dishes. Moringa oil, extracted from the seeds, is used in cooking, skincare, and various industrial applications. Additionally, Moringa oleifera has a long-standing tradition in traditional medicine, where it is employed to address a wide range of health issues due to its perceived medicinal properties.[9]

Structural overview:

Roots: Moringa oleifera features a taproot system with a primary taproot that penetrates deep into the soil. Lateral roots extend horizontally, providing stability and absorbing water and nutrients from the soil. This intricate root system sustains the tree's resilience and ability to thrive in diverse environments.[10]

Stem: The stem of Moringa oleifera is slender, reaching heights exceeding 10 meters under favorable conditions. It has a fibrous texture and grayish-brown bark with longitudinal fissures. Despite its fragile appearance, the stem serves as a conduit for nutrients and fluids essential for the tree's growth and vitality.[10]

Leaves: Moringa oleifera's compound leaves consist of multiple pairs of small leaflets arranged alternately along a central stem. Each leaf typically has 3 to 9 pairs of leaflets with a single leaflet at the apex. The leaflets are lanceolate or elliptical with serrated margins, varying in size from small to medium depending on the tree's maturity. They play a crucial role in photosynthesis, harnessing solar energy to fuel the tree's metabolic processes.[10]

Flowers: The flowers of Moringa oleifera are small, fragrant, and white or cream-colored. They are pentamerous, consisting of five petals, and form clusters at the ends of branches. Emitting a distinct fragrance, these flowers attract pollinators such as bees and butterflies, facilitating the tree's reproductive cycle.[11]

Fruits: Moringa oleifera produces elongated, cylindrical pods known as drumsticks or pods. These pods can grow 30-60 cm (1-2 feet) long and start green, maturing to a brown color. They contain numerous seeds surrounded by fibrous pulp. These pods are valued for their nutritional content and are a significant food source in many cultures, embodying the tree's abundance and utility.[11]

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Seeds: The seeds of Moringa oleifera are either round or triangular, housed within the pods. They are brown or black and have three papery wings that aid in wind dispersal. Rich in oil and protein, Moringa seeds are utilized in culinary and medicinal applications, reflecting the tree's versatility and nutritional importance.[11]

REPORTED ACTIVITYS:

Moringa oleifera has been studied extensively for its various reported activities, including its medicinal, nutritional, and therapeutic properties. Here are some of the reported activities of Moringa oleifera backed by scientific research:

Antioxidant Activity:

Moringa oleifera is rich in antioxidants like vitamins C and E, flavonoids, and phenolic compounds. These antioxidants neutralize harmful free radicals, reducing oxidative stress and protecting cells from damage. Antioxidant activity is crucial for maintaining overall health and may help prevent chronic diseases like cancer, cardiovascular diseases, and neurodegenerative disorders.[12]

Anti-inflammatory Activity:

Extracts from Moringa oleifera possess anti-inflammatory properties due to their ability to inhibit inflammatory mediators and pathways. By reducing inflammation, Moringa oleifera may alleviate symptoms associated with inflammatory conditions such as arthritis, asthma, and inflammatory bowel disease. Its anti-inflammatory effects contribute to its therapeutic potential in managing chronic inflammatory diseases.[13]

Antimicrobial Activity:

Moringa oleifera extracts exhibit antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and viruses. These antimicrobial properties make Moringa oleifera a potential candidate for developing natural antimicrobial agents or adjunctive therapies for combating infections. Further research is needed to explore its efficacy and safety in clinical settings and its potential to address antibiotic resistance.[14]

Anticancer Activity:

Studies suggest that Moringa oleifera extracts possess anticancer properties by inhibiting cancer cell growth and inducing apoptosis (programmed cell death). Its bioactive compounds may target various cancer pathways, making it a promising candidate for cancer prevention and treatment. However, more extensive research, including preclinical and clinical studies, is necessary to elucidate its mechanisms and evaluate its efficacy against different types of cancer.[15]

Hypoglycemic Activity:

Research indicates that Moringa oleifera may help regulate blood sugar levels by enhancing insulin secretion and sensitivity. Its hypoglycemic effects make it potentially beneficial for individuals with diabetes or those at risk of developing the condition. Further clinical trials are needed to determine the optimal dosage, safety profile, and long-term effects of Moringa oleifera supplementation in managing diabetes.[16]

Hypolipidemic Activity:

Moringa oleifera has been shown to reduce lipid levels in the blood, including cholesterol and triglycerides. Lowering lipid levels contributes to cardiovascular health by reducing the risk of atherosclerosis and cardiovascular diseases.[17]

Its hypolipidemic effects highlight its potential as a natural adjunctive therapy for managing dyslipidemia and preventing cardiovascular complications.

Hepatoprotective Activity:

Studies suggest that Moringa oleifera extracts have hepatoprotective effects by protecting the liver from damage caused by toxins, drugs, and oxidative stress. Its hepatoprotective properties may help prevent liver diseases such as fatty liver disease, hepatitis, and cirrhosis. Further research is warranted to elucidate the underlying mechanisms and evaluate its efficacy in clinical settings.[18]

Neuroprotective Activity:

Moringa oleifera has been reported to exhibit neuroprotective properties, potentially protecting neurons from damage and degeneration. Its neuroprotective effects hold promise for the prevention and treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. However, more studies are needed to understand its mechanisms of action and assess its neuroprotective efficacy in relevant disease models and clinical trials.[19]

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Wound Healing Activity:

Moringa oleifera extracts have shown potential in promoting wound healing by accelerating the closure of wounds and enhancing tissue regeneration. Its wound healing properties may be attributed to its anti-inflammatory, antimicrobial, and antioxidant effects. Further research is necessary to determine the optimal formulations and applications for utilizing Moringa oleifera in wound care products and clinical practice.[20]

Immunomodulatory Activity:

Some studies suggest that Moringa oleifera may modulate the immune system by enhancing immune function and boosting the body's defenses against infections and diseases. Its immunomodulatory effects may be mediated by its bioactive compounds, which regulate immune cell activity and cytokine production. Further investigations are warranted to elucidate its immunomodulatory mechanisms and evaluate its potential therapeutic applications in immune-related disorders.[21]

IV. EXPERIMENTATION

Materials Needed:

- Moringa oleifera plant material (leaves)
- Solvent (ethanol, methanol, water, or a mixture)
- Blender or grinder
- Weighing scale
- Soxhlet extractor (optional)
- Rotary evaporator
- Filtration apparatus (filter paper, funnel)
- Distilled water
- Glassware (beakers, flasks, etc.)
- Drying oven or lyophilizer

` The preparation of plant material is a critical step in ensuring the integrity and potency of bioactive compounds found in Moringa oleifera. The following detailed guide outlines the process of collecting, cleaning, drying, and grinding Moringa plant material, which is essential for various applications including nutritional supplements, medicinal formulations, and research purposes.

1. Collection and Cleaning

Collection:

- Selection: Choose healthy, fresh Moringa oleifera leaves, seeds, or roots. Ensure that the plant material is free from visible signs of disease or pest infestation.
- Timing: Harvest the plant material during the early morning hours when the bioactive compounds are at their peak concentration. For leaves, the optimal time is before flowering, while seeds and roots can be collected when they are mature.[22]

Cleaning:

- Initial Rinse: Place the collected plant material in a large container and rinse thoroughly with tap water to remove soil, debris, and any foreign particles.
- Distilled Water Wash: Follow up by washing the plant material with distilled water to eliminate any remaining contaminants. This step ensures the removal of potential chemical residues or microorganisms that might affect the quality of the final product.
- Inspection: After washing, inspect the plant material carefully to ensure it is clean. Remove any remaining impurities or damaged parts.[23]

2. Drying

Air-Drying:

- Setup: Arrange the cleaned plant material on a clean, dry surface or a drying rack. Ensure the material is spread out in a single layer to allow even drying.
- Environment: Place the drying setup in a shaded, well-ventilated area. Direct sunlight should be avoided as it can degrade sensitive bioactive compounds.
- Duration: Allow the plant material to air-dry for several days until it is completely devoid of moisture. The drying time will depend on the ambient humidity and temperature.[24]

Oven-Drying:

- Preparation: Preheat a drying oven to a low temperature, ideally below 40°C (104°F). This low temperature helps in preserving the bioactive compounds which might be sensitive to heat.
- Arrangement: Place the plant material on baking sheets or trays, ensuring they are spread out in a single layer.
- Drying Process: Insert the trays into the oven and allow the material to dry slowly. Periodically check the plant material to prevent overdrying or burning.
- Completion: The drying process is complete when the plant material is crisp and brittle to touch, indicating the removal of moisture.[24]

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3. Grinding

Preparation:

Cool Down: If using an oven, allow the dried plant material to cool down to room temperature before proceeding to the grinding stage. This prevents any heat-induced degradation during grinding. Clean Equipment: Ensure that the blender or grinder used for grinding is clean and dry to prevent contamination.[25]

Grinding Process:

Blending: Place the dried plant material into the blender or grinder in small batches. Grinding in smaller amounts ensures a more consistent and fine powder. Consistency: Blend until the material reaches a fine, uniform powder. The texture should be smooth without large, unground particles.

Sifting (Optional): For an extra finepowder, sift the ground material through a fine mesh sieve. This step is optional but helps in obtaining a consistent particle size, especially useful for certain applications.[25]

4. Storage

Container: Store the fine Moringa powder in airtight containers to prevent exposure to air and moisture which can degrade the bioactive compounds.[26]

5. Extraction:

Weighing: Weigh a specific amount of the powdered plant material (500 grams) and place it in a suitable container.

Solvent Addition: Add an appropriate solvent (e.g., ethanol, methanol, water, or a mixture) in a ratio of 1:10 (plant material to solvent). For example, add 500 ml of solvent for 50 grams of plant material.[27]

Maceration:

Process: Allow the mixture to macerate (soak) at room temperature for 24-48 hours, shaking occasionally to ensure thorough mixing and extraction of compounds.

Alternative (Soxhlet Extraction): For a more efficient extraction, use a Soxhlet extractor. Place the plant material in the extraction chamber, add the solvent to the flask, and heat to allow the solvent to evaporate, condense, and wash through the plant material repeatedly.[28]

Filtration: After the extraction period, filter the mixture through filter paper using a funnel to remove solid plant residues. Collect the filtrate (liquid extract).[28]

V. OBESERVATIONS FROM THE TESTS CONDUCTED :

The Monoamine Oxidase (MAO) inhibition assay is a useful and efficient test for

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evaluating the inhibition of the MAO enzyme, which is implicated in the pathogenesis of psychiatric diseases. In this assay, a MO (Moringa oleifera) extract was found to inhibit both MAO-A and MAO-B enzymes, with the IC50 values of MO extract(100mg) are 4.4 µM and 1.2 µM, and for MO extract(150mg) is 4.8 µM and 1.4 µM respectively. This suggests that the MO extract is a more potent inhibitor of MAO-B compared to MAO-A. Among the tests, samples 100mg & 150mg demonstrated significant inhibitory activity against MAO enzymes. This suggests that these particular samples contain the bioactive compounds responsible for the MAO inhibition observed in the methanol extract.

Graphical Representation:

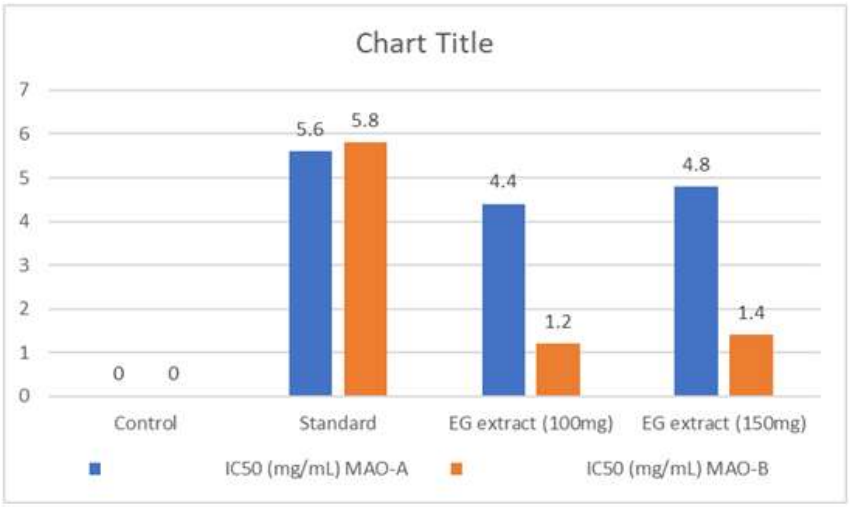

Table. Effects of the MeOH extract of Moringa oleifera on the MAO inhibition assay.

	IC50 (mg/mL)	
MATERIAL	**MAO-A**	**MAO-B**
Control	**<10**	**>10**
Standard	**5.6**	**5.8**
MO extract (100mg)	**4.4**	**1.2**
MO extract (150mg)	**4.8**	**1.4**

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VI. CONCLUSION:

The assessment of inhibitory activities of monoamine oxidase (MAO) enzymes in Moringa oleifera reveals significant potential for the plant's application in neurological and psychological health management. Monoamine oxidases, including MAO-A and MAO-B, are critical enzymes involved in the metabolism of neurotransmitters such as serotonin, dopamine, and norepinephrine. Inhibiting these enzymes can be beneficial in treating various neuropsychiatric and neurodegenerative disorders, including depression, anxiety, and Parkinson's disease.

Moringa oleifera has demonstrated notable MAO inhibitory activities in several studies, primarily attributed to its rich composition of bioactive compounds, including flavonoids, phenolic acids, and alkaloids. These compounds not only exhibit MAO inhibitory properties but also possess antioxidant and anti-inflammatory effects, which may contribute synergistically to neuroprotection and the amelioration of neurological symptoms.

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