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Evaluation Of Anti-Convulsant Activity Of Methanolic Extract Of Moringa Oleifera Leaves By Electroconvulsive In Wister Albino Rats .

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ABSTRACT:

The native South Asian plant Moringa oleifera has recently garnered significant attention due to its numerous health benefits. This comprehensive review aims to summarize the therapeutic potential of Moringa oleifera leaves, focusing on their pharmacological properties and health advantages. The paper addresses various topics, including the phytochemical composition, as well as the anti-inflammatory, antibacterial, anticancer, and hepatoprotective effects of Moringa oleifera leaves. Additionally, it explores the potential mechanisms of action that may underline these benefits. The review also examines the safety profile and possible adverse effects associated with the consumption of Moringa oleifera leaves. Overall, this analysis provides valuable insights into the therapeutic properties of Moringa oleifera leaves and highlights their potential applications in the diagnosis, treatment, and prevention of various diseases.

Key words: Antioxidant, Anti-inflammatory, Anti-microbial, Hepatoprotective, Anti-epileptic, Anti-fertility, Seizures, Neurotransmitter, Neurological disorder, Epilepsy, Convulsion

INTRODUCTION:

Affecting 65 million people globally, epilepsy ranks as the third most significant contributor to the worldwide burden of neurological disorders.

Moringa oleifera, commonly referred to as 'Sobhanjana,' is an elegant tree characterized by its corky grey bark and brittle branches. It typically reaches a height of 25 to 30 feet and is predominantly found in the Sub-Himalayan region, with significant cultivation in India and Burma. The leaves are generally tripinnate, featuring a slender rachis that is thickened and articulated at the base; the leaflets are either elliptic or obovate, with a rounded tip and inconspicuous nerves. The flowers are white and appear in large, puberulous axillary panicles. Various parts of this plant, including the bark, root, fruit, flowers, leaves, seeds, and gum, have been traditionally utilized for their antispasmodic, stimulant, expectorant, and diuretic properties. Additionally, the plant serves as a tonic for the heart and circulatory system, as well as an antiseptic. In the practices of Ayurveda and Siddha, the leaves and root are employed to treat conditions such as helminthiasis, nausea, dizziness, and tuberculosis.

The main bioassay utilized in the in vivo evaluation of new anticonvulsant agents are convulsions induced by phenytoin and those triggered by maximal electroshock. Several bioactive compounds were recognized in the leaves of M. oleifera such as vitamins, carotenoids, polyphenol, phenolic acids, Flavanoids, alkaloids, glucosinolates, Isothiocyanate, Tannins, Saponins and oxalates and phytates. The leaves are rich in Vitamin A and C. It also contains Niazirin, Niazirinin, three mustard oil glycosides, 4-[(40-O acetyl alpha-L-rhamnosyloxy) benzyl] isothiocyanate, Pyrrole alkaloid (pyrrolemarumine 400-O-a-L- rhamnopyranoside), and 40-hydroxyphe Therefore, this study aims to confirm the efficacy of
M. oleifera Lam. leaves in the treatment of epilepsy and anxiety.

MATERIALAND METHODS :

Drugs and chemicals treatment; Phenytoin, normal saline, and various doses of Moringa oleifera extract were delivered through the intraperitoneal route.

INSTRUMENTS:

1. Soxhlet apparatus was used for preparing the plant extracts.

SOXHLET EXTRACTION PROCESS :

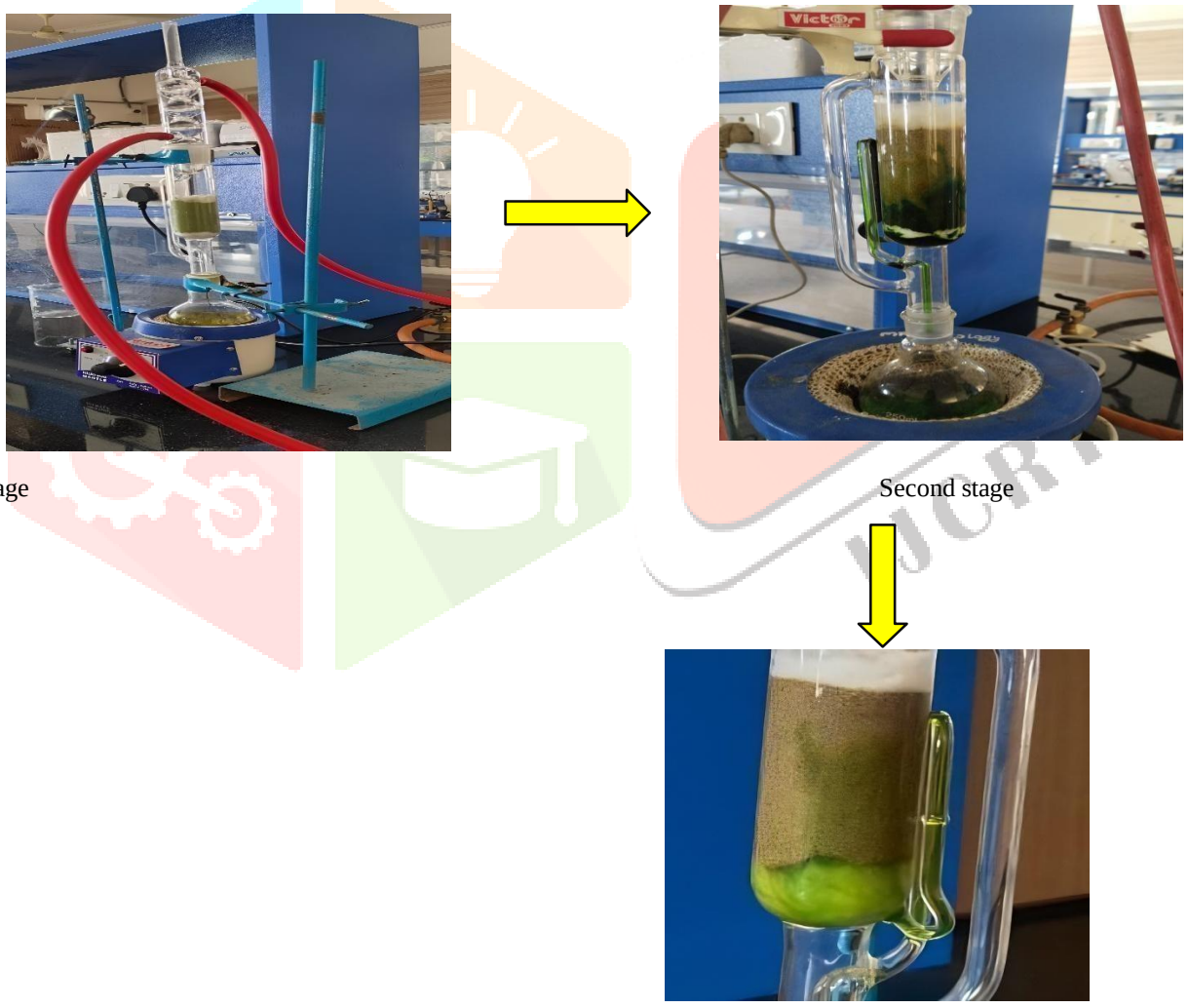


fig1: soxhlet extraction process

Soxhlet extraction is a separation process of compounds using solvents by dissolving the mixture in mother soluble solvent. Soxhlet extraction contain several equipment thimble, round bottom flask, arm, condenser, siphon, stand and a heating mantle. Weight accurately about 50g of dried moringa leaves powder and cotton cloth place in thimble. In a round bottom flask take 150ml of methanol and place in heating mantel and the thimble is placed on the round bottom flask, the condenser is placed on the thimble and connect the water flow The heating is maintained at 70-75 °C When the temperature is increased methanol gets evaporate from the round bottom flask then condensed and fall drop wise in the thimble on the drug. The drug get wet and the extract will fill the cycle and again fall down into the round bottom flask. Like these approximately 5 cycles are fall down. After the extraction Soxhlet apparatus are removed and the extract was taken it into the beaker and kept for heating for evaporator of methanol. After getting the drug take it into the well closed container and kept in a normal temperature and avoid microbials growth.

2. Electro-convulsometer was used for inducing convulsions:

fig-2: electro-convulsometer

PLANT MATERIAL:

The plant material was obtained from the local market in February 2025. The leaves were verified by the Head of the Botany Department at the Botanical Survey of India in Telangana, India. Following authentication, the leaves were shade-dried and ground into a coarse powder.

PREPARATION OF THE PLANT EXTRACT :

MORINGA OLEIFERA METHANOL EXTRACT (MOME)

The plant material was thoroughly cleaned, air-dried, and then ground into a coarse powder using a mortar and pestle. A total of 50 grams of the powdered crude drug was subjected to extraction with 150 liters of 70% methanol using a Soxhlet apparatus, maintained at a temperature of 70-75°C for a duration of 6 to 7 hours. The extraction process continued until the liquid in the side arm of the Soxhlet apparatus became colorless. The extract yielded a percentage of 10%. Subsequently, the methanolic extract was suspended in distilled water.

fig-3: moringa oleifera leaves are shade dried and powdered

EXPERIMENTAL ANIMALS:

Wistar albino mice, both male and female, weighing between 200 and 250 grams, were utilized in this research following the approval of the Institutional Animal Ethics Committee (IAEC). The mice were housed under controlled conditions in the Animal House, residing in polypropylene cages, and were provided with a standard pellet diet and unrestricted access to water.

PROCEDURE:

Dissolve 1000 mg of phenytoin in 10 ml of water by crushing the tablet and mixing it thoroughly. Stir or shake well to ensure the phenytoin is evenly suspended prior to administration. When administering via a feeding tube, it is important to flush the tube with water both before and after the administration. Additionally, avoid mixing phenytoin with enteral feeding as it may decrease absorption.

PROCESS:

Divide the experimental animals into three distinct groups (Vehicle control; Standard drug).

↓

Weigh each experimental animal and label them appropriately.

↓

Administer the drug (both standard and test drug) along with normal saline (vehicle control) 60 minutes before oral administration and 30 minutes before intraperitoneal administration.

↓

Keep the animal secured in the restrainer (to facilitate electrode attachment) or hold it firmly.

↓

Ensure that the contact points for the electrodes (ear electrodes) are clean; apply electrode gel to improve electrical conduction.

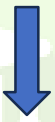
Gently place the electrode (ear electrode) on both ear pinnae.

Set the desired electroshock parameters on the Electroconvulsiometer.

Experimen tal animal	Current (milliamper e mA)	Voltage (V)	Frequency (Hz)	Duration
Mice	50	250	50	0.2

Activate the main switch and press the start button.

Start the stopwatch immediately after pressing the start button and document the time spent in various phases of the MES convulsion.



The Electroconvulsiometer will automatically shut off once the designated time has passed.

Return the animal to its cage after it has recovered from the convulsion.

Repeat the same procedure for the remaining groups.

ACUTE TOXICITY AND AUTOPSY:

An acute toxicity assessment of the extracts was performed using the acute oral toxic class method established by the Organization for Economic Co-operation and Development.

Acute toxicity studies involve exposing animals to a single high dose of a substance to assess its immediate effects. These studies help identify potential toxic effects, determine the median lethal dose (LD50), and establish a maximum tolerated dose.

Types of Toxicity Studies

- Acute Toxicity: Single dose exposure to determine immediate effects
- Subacute Toxicity: Repeated dose exposure over 14-90 days to assess effects of repeated administration
- Chronic Toxicity: Long-term exposure (usually 12 months) to evaluate effects of prolonged exposure

Key Components of Acute Toxicity Studies

- Animal Selection: Typically conducted in at least two species, such as rats and non- rodents
- Dose Selection: Single high dose to determine LD50 and maximum tolerated dose
- Observations: Monitoring for signs of toxicity, mortality, and necropsies to identify organ damage
- Histopathology: Examination of tissues to identify potential target organs

Guidelines and Regulations

Acute toxicity studies are guided by organizations like the Organization for Economic Co- operation and Development (OECD) and the Environmental Protection Agency (EPA). These guidelines ensure that studies are conducted in a standardized and reproducible manner.

Importance of Acute Toxicity Studies:

Acute toxicity studies provide essential information for

- Risk Assessment: Identifying potential hazards and risks associated with substance exposure
- Drug Development: Informing safe dosage levels for human clinical trials
- Regulatory Approval: Supporting regulatory decisions for substance approval and use

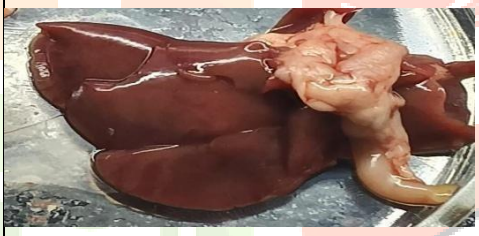
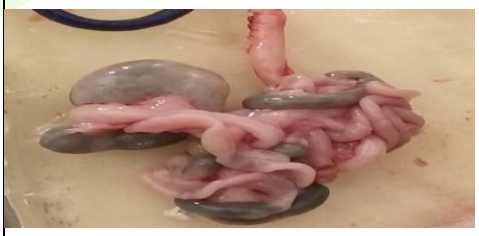
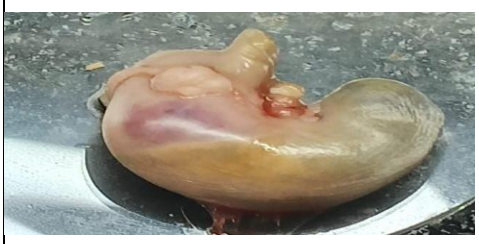
One group served as a control and was administered distilled water, while the other groups received treatments with crude extracts of Moringa oleifera. The extracts were evaluated at a dosage of 2000 mg/kg, with observations for acute symptoms recorded on the first day and monitored over a period of two days.

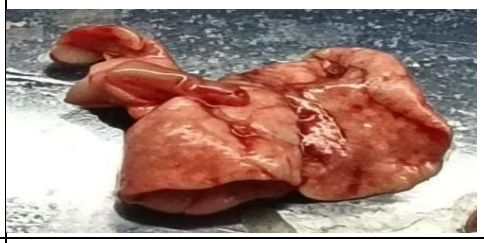
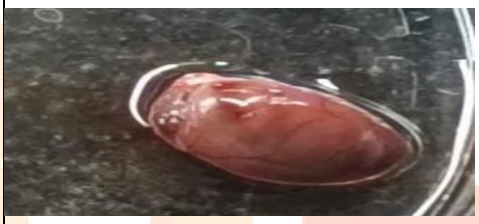
AUTOPSY:

External Examination:

fig-4: albino rat

Internal Examination:

Parts Of Mice	Changes	Organs Images	Organs weight kg/gm
LIVER	No change		5.21
INTESTIN E	No change		11.40
STOMAC H	No change		3.59

KIDNEY	**No change**		**2.94**
LUNGS	**No change**		**1.19**
HEART	**No change**		**0.65**

BLOOD SAMPLE COLLECTION :

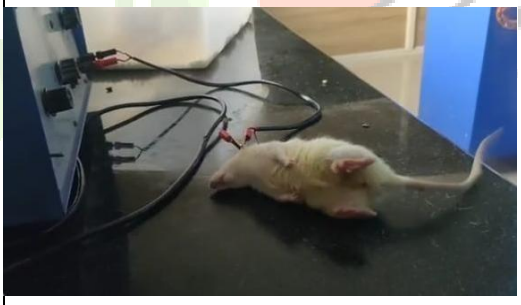
1. BLOOD COLLECTION BY CARDIAC PUNCTURE :

fig6:cardiac puncture

2. HEMATOLOGY STUDY:

3. BIOCHEMISTRY STUDY

EVALUATION OF MAXIMAL ELECTROSHOCK SEIZURE TEST (MEST):

DRUG	DRUG	IMAGES
Phen ytoin	A. Extension:	 No Extension
	B. Flexion	
	C. Stupors	

	D. Clonic	
Mori nga Extra ct	**A. Extension**	 **Less Extension**
	B. Flexion	
	C. Stupors	
	D. Clonic	

fig-6: a maximal electroshock (mes) is a technique used to induce convulsants in experimental models

The method was used with some modifications:

10 mice were fasted and divided into groups

- Control
- Test
- Sample

Animals in each group were stimulated through corneal electrode by a 60 cycle (60 HZ) alternative current unit /MES indicated by hind limb tonic extensor spam was elicted 1-4 with Moringa oleifera Group3- mice were treated with Distilled water (10ml/kg) while Group 6- received phenytoin (30mg/kg i.p).

The duration of electrically induced convulsion was noted for each mouse

The length of the electrically induced convulsion was recorded for each mouse .

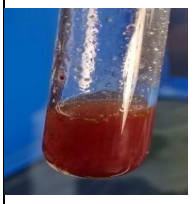
RESULTS :

Prelimanary Phytochemical Screening:

The phytochemical analysis of the methanol extract from Moringa oleifera indicated the presence of carbohydrates, saponins, steroids, triterpenoids, glycosides, and tannins within the extract.

PRELIMINARY TESTS:

TESTS	OBSERVATI ON	IMAGES	INFERENCE
1. Test F **or Alkaloids:** **i. Mayer's Test:** Few ml of filtrate test solution is added to the Mayer's reagent	Yellow color		Presence of Alkaloids
ii. Wagner's Test: Few ml of filtrate is added to 1-2ml of Wagner's reagent and few drops of iodine potassium	Reddish brown/Brow n Color ppt		Presences of Alkaloids
iii. **Hagner's Test**: Few ml of filtrates added to 1-2ml of Hagner's reagent	Orange color		presence of alkaloids

2. Test for flavonoids test: **i. Shinoda Test:** Few ml of filtrate is added to 1-2m1 of Magnesium turnings and concentrated Hcl dropwise.	Pink scarlet or Crimson red		Presence of flavonoids.
ii. Alkaline Test: Few ml of filtrate is added to 1-2ml of NaOH.	Yellow color		Presence of flavonoids.
iii. Zinc Hcl Test: Few ml of filtrate is added to Zinc dust and concentrated Hcl	Red color		Presence of flavonoids.
3. Test for Tannins: **i. Ferric Chloride Test:** Few ml of filtrate is added to the ferric chloride solution.	Blue Color		Presence of Tannins.
4. Test for Protein: i. Million's Test: To 3ml of extract add 5ml of million's reagent	White ppt is formed and warm the ppt dissolves giving red color soln		Presence of proteins.
5. Test for Amino acids: **i. Ninhydrin Test:** Heat 3ml of extract and 3drops of 5% Ninhydrin solution in boiling water bath for 10 min	Purple or bluish color appears		Presence of Amino acids

OBSERVAYION TABLE:

Observation: Control group:

S. No	Body Weight(g)	Treatment	Time(sec) in various phase of convulsion				
			Flexion	Extensor	Clonus	Stupor	Recovery / Death
1	150	saline	4	10	5	60	Recovery
2	160	Saline	7	13	4	120	Recovery
3	170	saline	5	11	4	90	Recovery

Observation of Test group:

S. No	Body Weight(g)	Treatment	Time(sec) in various phase of convulsion				
			Flexion	Extensor	Clonus	Stupor	Recovery / Death
1	155	Phenytoin	2	0	2	40	Recovery
2	160	Phenytoin	5	0	3	90	Recovery
3	170	Phenytoin	3	0	2	50	Recovery

Observation of sample (Extract) Moringa:

S. No	Body Weight(g)	Treatment	Time(sec) in various phase of convulsion				
			Flexion	Extensor	Clonus	Stupor	Recovery / Death
1	170	Moringa oleifera Extract	2.6	5.0	2.5	45	Recovery
2	180	Moringa oleifera Extract	3	3.2	3	100	Recovery
3	150	Moringa oleifera Extract	2.5	4	3	70	Recovery
4	200	Moringa oleifera Extract	2	3	2.7	120	Recovery

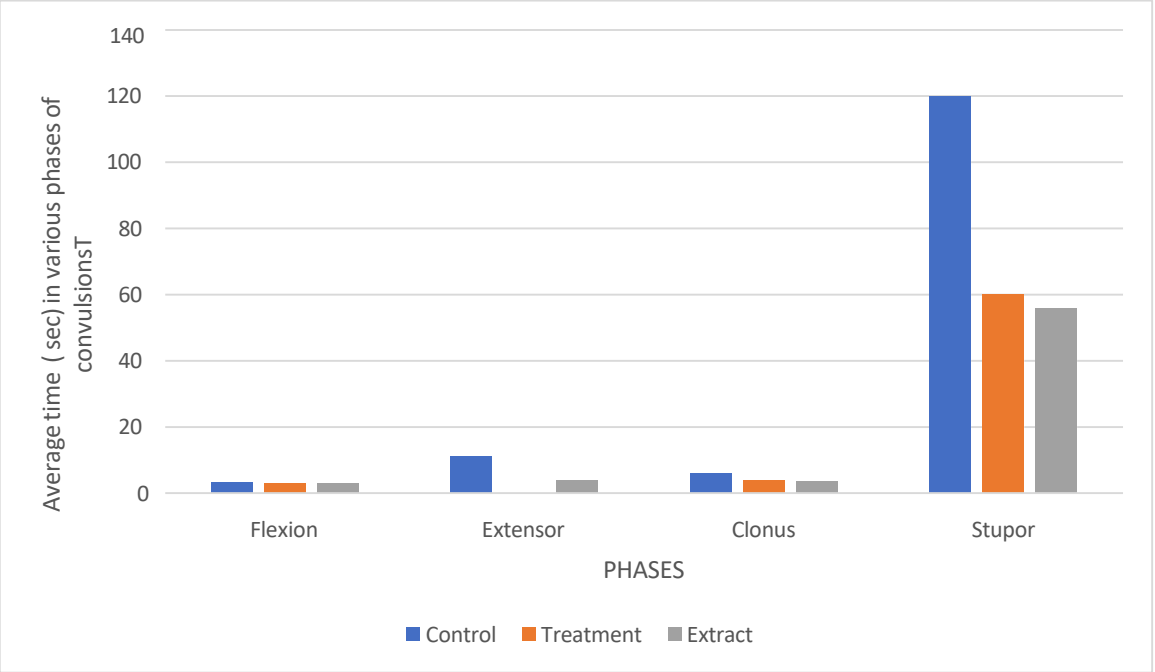
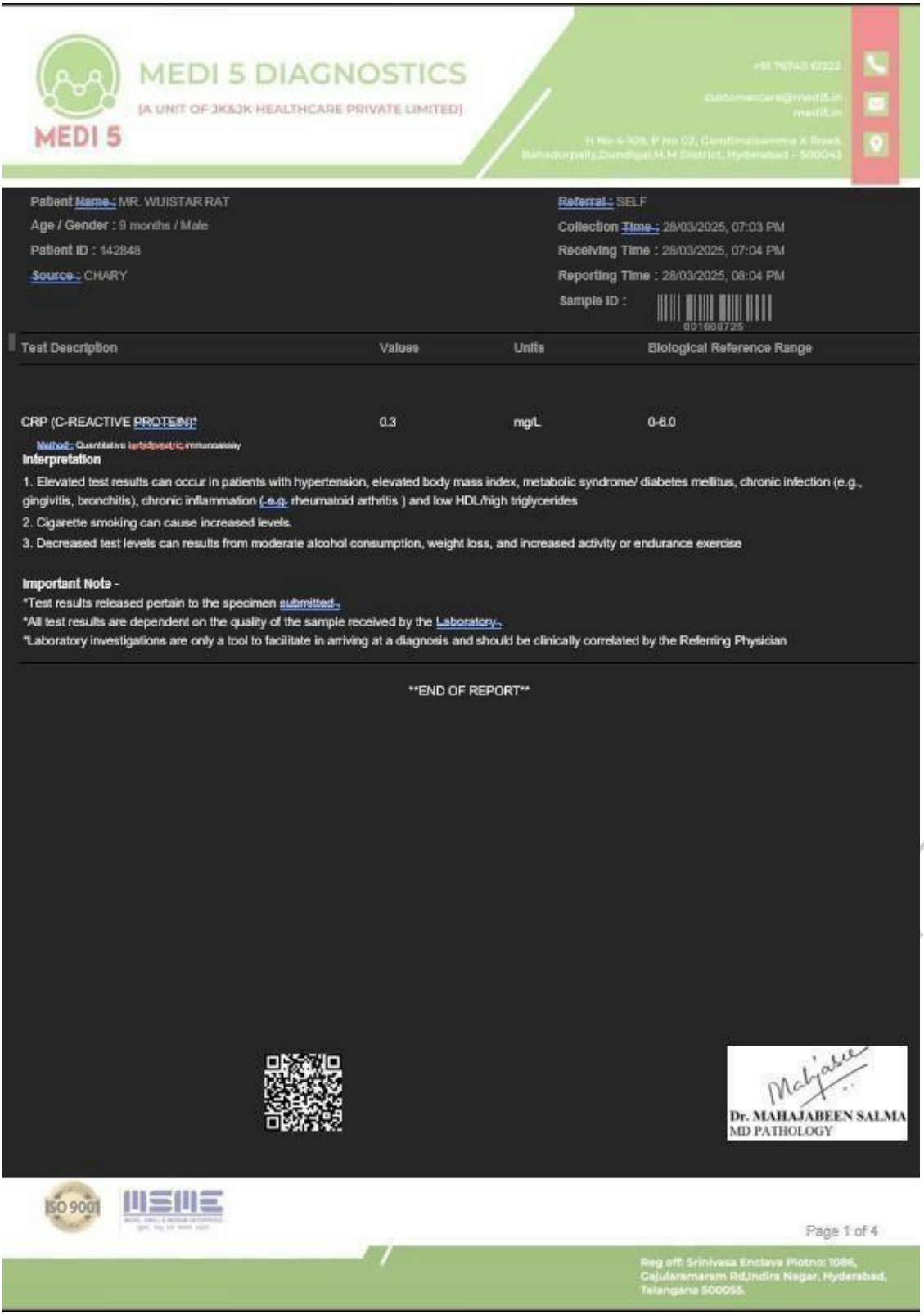


fig-7:

1. **effect of moringa oleifera phenytoin induced seizures maximal electroshock seizure in mice.**
2. **effect of moringa oleifera must induced seizure.**

BLOOD SAMPLE REPORTs:



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Patient Name :- MR. WUISTAR RAT
Age / Gender : 9 months / Male
Patient ID : 142848
Source :- CHARY

Referral :- SELF
Collection Time :- 28/03/2025, 07:03 PM
Receiving Time : 28/03/2025, 07:04 PM
Reporting Time : 28/03/2025, 08:04 PM
Sample ID : 001608725

Test Description	Values	Units	Biological Reference Range
CRP (C-REACTIVE PROTEIN)*	0.3	mg/L	0-6.0

Method :- Quantitative turbidimetric immunoassay

Interpretation

1. Elevated test results can occur in patients with hypertension, elevated body mass index, metabolic syndrome/ diabetes mellitus, chronic infection (e.g., gingivitis, bronchitis), chronic inflammation (-e.g. rheumatoid arthritis) and low HDL/high triglycerides
2. Cigarette smoking can cause increased levels.
3. Decreased test levels can results from moderate alcohol consumption, weight loss, and increased activity or endurance exercise

Important Note -

*Test results released pertain to the specimen submitted-.
*All test results are dependent on the quality of the sample received by the Laboratory-.
*Laboratory investigations are only a tool to facilitate in arriving at a diagnosis and should be clinically correlated by the Referring Physician

END OF REPORT

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Sample ID : 001808725

Test Description	Values	Units	Biological Reference Range
COMPLETE BLOOD COUNT (CBC)			
Hemoglobin (Hb)	9.5	gm/dL	11.1 - 14.1
Method: SLS, Sample: WB EDTA			
Erythrocyte (RBC) Count	3.17	mil/cumm	3.9 - 5.1
Method: Hydrodynamically focused DC detection, Sample: WB EDTA			
Packed Cell Volume (PCV)	28.50	%	30 - 38
Method: Calculated, Sample: WB EDTA			
Mean Cell Volume (MCV)	89.91	fL	72 - 84
Method: Calculated, Sample: WB EDTA			
Mean Cell Haemoglobin (MCH)	29.97	pg	25 - 29
Method: Calculated, Sample: WB EDTA			
Mean Corpuscular Hb Concn. (MCHC)	33.33	gm/dL	32.0 - 36.0
Method: sample: WB EDTA, Method: Calculated			
Red Cell Distribution Width (RDW)	13.5	%	11.5 - 14.0
Method: Electrical Impedance, Sample: WB EDTA			
R D W (SD)	24.8	fl	35 - 56
Method: Method:calculated,Sample Type : WB EDTA			
Total Leucocytes (WBC) Count	16.7	10^3/µl	6.0-16.0
Method: Flow cytometry, Sample: WB EDTA			
Neutrophils	38	%	15 - 46
Method: Flow cytometry, Sample: WB EDTA			
Lymphocytes	52	%	45 - 76
Method: sample: WB EDTA, Method: Flow cytometry			
Monocytes	08	%	3 - 6
Method: Flow cytometry, Sample: WB EDTA			
Eosinophils	02	%	0 - 3
Method: Flow cytometry, Sample: WB EDTA			

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Sample ID : 001608725

Test Description	Values	Units	Biological Reference Range
Basophils Method:- Flow cytometry, Sample: WB EDTA	00	%	0 - 1
Absolute Neutrophil Count Method:- calculated	6.35	* 10^9/L	2.0 - 7.0
Absolute Lymphocyte Count Method:- Calculated	3.68	* 10^9/L	1.0 - 4.0
Absolute Monocyte Count Method:- Calculated	1.34	* 10^9/L	0 - 1.0
Absolute Eosinophil Count Method:- Calculated	0.33	* 10^9/L	0 - 0.5
Absolute Basophils Count Method:- Calculated	0.00	* 10^9/L	0 - 0.2
Platelet Count Method:- Hydrodynamically focused DC detection, Sample: WB EDTA	108	10^3/µl	200 - 550
Mean Platelet Volume (MPV) Method:- Electrical Impedance, Sample: WB EDTA	7.7	fL	7.2 - 11.7
PCT Method:- sample: WB EDTA, Calculated	0.8	%	0.2 - 0.5
PDW Method:- Calculated, Sample: WB EDTA	16.8	%	9.0 - 17.0

Note:
Tests done on Automated Six Part Cell Counter. (WBC, RBC,Platelet count by impedance method, colorimetric method for Hemoglobin, WBC differential by flow cytometry using laser technology other parameters are calculated). All Abnormal Haemograms are reviewed confirmed microscopically.

Important Note -
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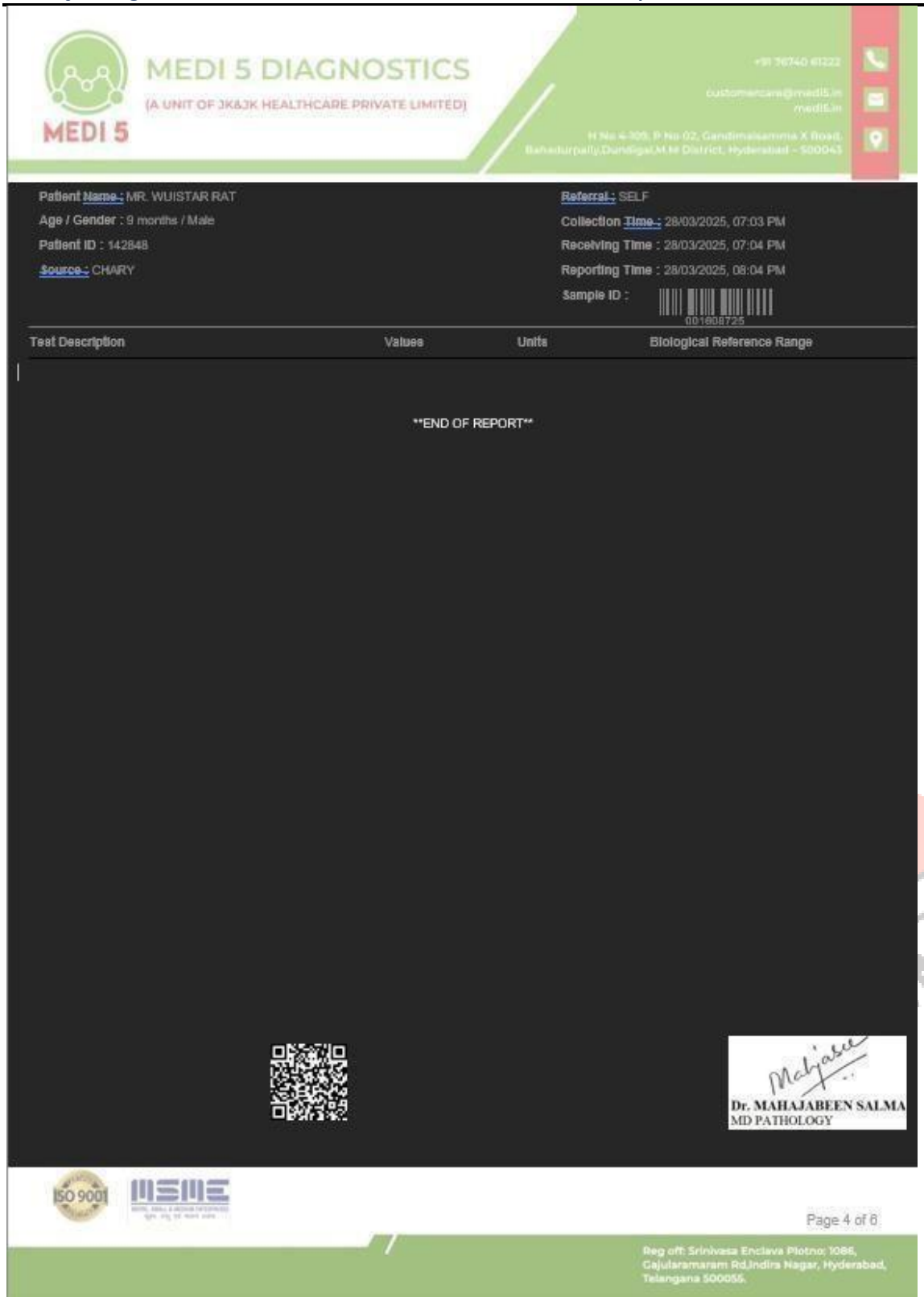

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Patient Name : MR. WUISTAR RAT

Age / Gender : 9 months / Male

Patient ID : 142848

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Referral : SELF

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Reporting Time : 28/03/2025, 08:04 PM

Sample ID : 001808725

Test Description	Values	Units	Biological Reference Range

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Sample ID : 001808725

Test Description	Values	Units	Biological Reference Range
LIVER FUNCTION TEST			
Bilirubin - Total Method:- Diazo method	1.4	mg/dL	0.3 - 1.2
Bilirubin - Direct Method:- Diazo method,Walter& Gerarde method	0.5	mg/dL	Adults and Children: 0 - 0.4
Bilirubin - Indirect Method:- Serum, Calculated	0.90	mg/dL	0.1 - 1.0
SGOT Method:- IFCC without PDP	28	U/L	< 35
SGPT Method:- IFCC without PDP	53	U/L	< 45
SGOT/SGPT Method:- calculated	0.53	ratio	0.7 - 1.4
GGT-Gamma Glutamyl Transpeptidase Method:- Serum, UV - Assay according to Szasz	52.3	U/L	11.0 - 81.0
Alkaline Phosphatase-ALPI Method:- PNPP-DEA method	124	IU/L	90-180
Total Protein Method:- Biuret	10.0	g/dL	6.4 - 8.3
Albumin Method:- Bromo Cresol Green(BCG)	4.2	g/dL	Adults: 3.5 - 5.2
Globulin Method:- Calculated	5.80	g/dL	1.8 - 3.6
A/G Ratio Method:- Calculated	0.72	ratio	1.2 - 2.2

Important Note -

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Reg off: Srinivasa Enclava Plotno: 1086, Cajularamaram Rd,Indira Nagar, Hyderabad, Telangana 500055.

MEDI 5 DIAGNOSTICS

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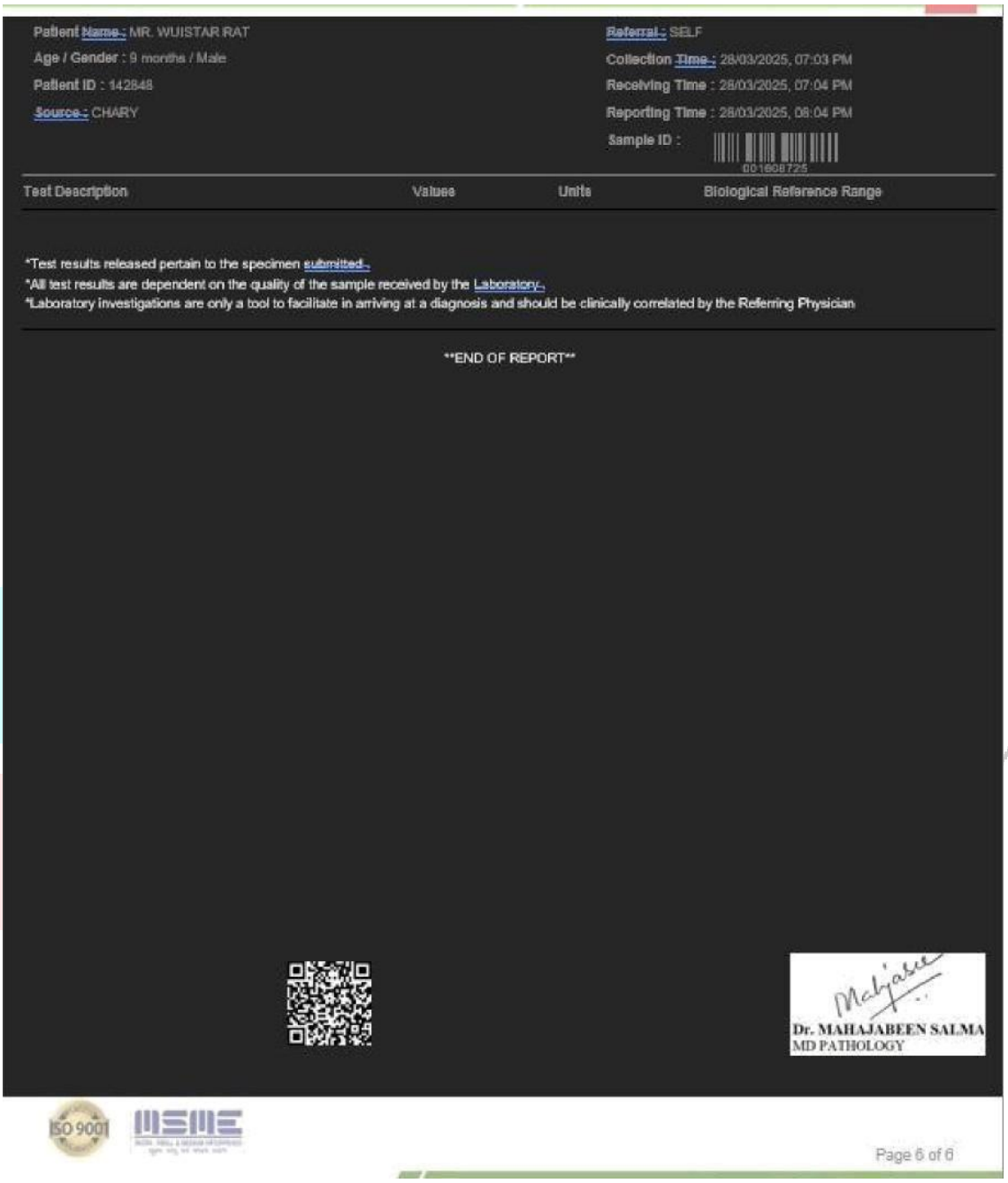
MEDI 5



Patient Name : MR. WUISTAR RAT
Age / Gender : 9 months / Male
Patient ID : 142848
Source : CHARY

Referral : SELF
Collection Time : 28/03/2025, 07:03 PM
Receiving Time : 28/03/2025, 07:04 PM
Reporting Time : 28/03/2025, 08:04 PM
Sample ID :

Test Description	Values	Units	Biological Reference Range

*Test results released pertain to the specimen submitted.
*All test results are dependent on the quality of the sample received by the Laboratory.
*Laboratory investigations are only a tool to facilitate in arriving at a diagnosis and should be clinically correlated by the Referring Physician

END OF REPORT

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DISCUSSION:

Since ancient times, medicinal plants have been recognized as accessible, cost-effective, and potent sources of treatment. Individuals often turn to traditional and various forms of complementary and alternative medicine for chronic ailments that do not respond effectively to conventional therapies. This includes neurological conditions such as epilepsy, anxiety, and pain, which can benefit from these alternative approaches. Consequently, there is an urgent need for the pharmacological assessment and standardization of these medicinal plants. Moringa oleifera has been traditionally noted for its potential in treating epilepsy and anxiety. Given the demand for medicinal plants as alternatives to modern medicine and the traditional uses of M. oleifera, this study aims to investigate the antiepileptic and anxiolytic properties of Moringa oleifera leaf extract (MOME) through various behavioral animal models. The initial critical step in identifying a potential antiepileptic drug (AED) is the classical maximal electroshock (MES) test conducted on mice. The MES test is widely utilized in AED research due to its straightforward seizure induction process and a high success rate

in identifying clinically effective AEDs. Established AEDs like phenytoin, carbamazepine, and valproic acid are known to prevent hind limb tonic extension induced by maximal electroshock, thereby demonstrating their efficacy, effective for managing generalized tonic-clonic and partial seizures

In the current study, mice administered with MEMO demonstrated protection against seizures induced by MES. This finding indicates that MEMO exhibits considerable antiepileptic properties and could be beneficial in the management of both grand mal and petit mal epilepsy. This research presents scientific evidence regarding the therapeutic benefits of Moringa oleifera leaves, which have been traditionally utilized for the treatment of epilepsy in Ethiopia. The findings offer a scientific basis for the application of Moringa oleifera leaf extract in alleviating epilepsy, as recognized in Ethiopian traditional medicine.

Moringa oleifera extract was found to reduce the duration of maximal electroshock seizures. At a dosage of 400 mg/kg, it provided complete protection against mortality, comparable to that of Phenytoin, a well-established anticonvulsant. In contrast, normal saline and a lower dose of 50 mg/kg of the extract did not offer any protection to the mice from convulsions or death. The extract demonstrated a protective effect ranging from 50% to 100% against seizures induced by maximal electroshock (MES) at 150 mA for 0.2 seconds. This protective effect suggests that the extract may interact with sodium-ergic neurotransmission. The MES test is considered a reliable method for identifying anticonvulsant drugs that are effective against myoclonic and absence seizures. Moringa oleifera significantly reduced the incidence of electrically induced seizures in mice, which are characterized by the tonic extension of the hind limbs, with the cessation of this activity being indicative of successful intervention.

The capacity of M. oleifera to reduce the duration of tonic-clonic seizures in the MEST test indicates its effectiveness against generalized tonic-clonic seizures. M. oleifera has shown significant efficacy against MEST seizures, suggesting that it is likely to be effective against both absence seizures and generalized tonic-clonic seizures. The MEST test is designed to detect substances that act against generalized tonic-clonic seizures, while the PTZ test is used to identify compounds that are effective against generalized absence and myoclonic seizures.

CONCLUSION:

In conclusion, the findings of this study suggest that the methanolic extract of Moringa oleifera leaves possesses bioactive compounds that could be advantageous in treating epilepsy. This supports the traditional medicinal use of the plant for managing epilepsy. Additional research is recommended to isolate and characterize the specific bioactive compounds that contribute to its anticonvulsant effects.

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8. PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL STUDY OF MORINGA OLEIFERA PLANT OF INDIAN ORIGIN

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