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

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ABSTRACT

Epilepsy may be defined as an intermittent derangement of the nervous system due presumably due to a sudden, excessive, disorderly discharge of cerebral neurons. *Moringa Oleifera* is a small, graceful, deciduous tree with sparse foliage, often resembling a leguminous species at a distance, especially when in flower, but immediately recognized when in fruit. The tree grows to 8 m high and 60 cm dbh. Bole crooked, often forked from near the base. *Moringa oleifera* Lam (Moringaceae) is a member of the genus, *Moringa*, which has 14 other species. , *M. oleifera* is the only acceptable name for this plant; and no synonyms have been reported for it.

Moringa Oleifera was collected in bulk quantity and exposed to shade drying. After drying Crude drug were exposed to size reduction and passed through the sieve of 40 mesh size the supernatant were collected and exposed to successive extraction by hot percolation by soxhlet apparatus with petether and ethanol and cold maceration with water. All the extracts made free from solvent and stored in wide mouth bottle and yield was calculated. After extraction all the extracts were exposed to biological evaluation by different models of anticonvulsion activity like MES nad PTZ induced convulsion mmethods. After that all the extracts were exposed to immunomodulatory activity by different models like NBT, Neutrophil locomotion and

chemotaxis and Candicidal assay etc. Among all the extracts alcoholic extracts of both the drugs showed significant anticonvulsant and immunomodulatory activity, so alcoholic extracts of both the drugs have been taken for isolation, characterization and identification of lead compound. After literature surbey and extensive TLC of alcoholic extract of both the plant a perfect mobile has been discovered and lead compounds of flavonoid category has been isolated by preparative TLC technique from both plant extracts, the compounds were further exposed to HPTLC technique with same mobile phase as in case of TLC, the results of HPTLC again indicates towards the flavonoids category. The types of flavonoids and the molecular and structural formula were confermed by the interpretation of data obtained by I.R. and N.M.R. technique.

The Present Investigation was aimed to study an anticonvulsant activity and immunomodulatory activity of Moringa oleifera. Animals models of anticonvulsant namely the pentylenetetrazole (PTZ) and maximal electroshock induced convulsion (MES) and immunomodulatory activity namely the nitrobluetetrazolium quantitative test (NBT). The extract of Moringa Oleifera possesses anticonvulsant activity and immunomodulatory activity.

KEY WORDS: *Moringa Oleifera* , Anticonvulsant, Immunomodulatory.

Introduction

Epilepsy may be defined as an intermittent derangement of the nervous system due presumably due to a sudden, excessive, disorderly discharge of cerebral neurons. Epilepsy is one of the most common neurological disorders characterized by sudden, transient alterations of brain function usually with motor, sensory autonomic or psychic symptoms often accompanied by loss of, or altered consciousness. Coincidental pronounced alteration in the electro encephalogram (EEG) might be detected during these episodes.

Immunomodulation is any procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reaction is named as immunostimulation and primarily implies stimulation of non-specific system i.e. stimulation of the function and efficiency of granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may be because of environmental or chemotherapeutic factors.

Moringa Oleifera is a small, graceful, deciduous tree with sparse foliage, often resembling a leguminous species at a distance, especially when in flower, but immediately recognized when in fruit. The tree grows to 8 m high and 60 cm dbh. Bole crooked, often forked from near the base. *Moringa oleifera* Lam (Moringaceae) is a member of the genus, *Moringa*, which has 14 other species. , *M. oleifera* is the only acceptable name for this plant; and no synonyms have been reported for it.

M. oleifera is also known as drumstick tree or horseradish tree, dandalonbin, Mulangay, Mlonge, Benzolive, Sajna, Kelor, Punjabese, Sujna, Marango and Saijihan.[5] It is native to the Himalayas of north-western India and currently, it is widely distributed in India, the Philippines, Ceylon,

Thailand, Malaysia, Myanmar, Pakistan, Singapore, the West Indies, Cuba, Jamaica and Nigeria, and still spreading to other areas.

Material and Methods:

Selection of crude drugs

The crude drug, steam of *Moringa Oleifera* and rhizomes of selected for the study was collected from Himachal region were authenticated.

Standardization

Powdered materials was subjected to standardization by adopting various physical and chemical parameters described in the literature.

Extraction and phytochemical investigation

Powdered crude drugs was extracted in successive manner by soxhlet method using solvents like pet-ether (40-60^{0}C), Ethanol by hot percolation method and with water by cold maceration with chloroform water I.P. in the increasing polarity order.

Extracts obtained were subjected to qualitative chemical tests adopting standard procedures.

Pharmacological activities of selected extracts

The extracts was selected for the screening of anticanvulsant and immunomodulatory activities by using models like MES induced, PTZ induced convulsions and particle ingestion and phagocytosis, candidacidal assay, neutrophilic locomotion and chemotaxis respectively.

Characterization

Extracts exhibiting significant activity was selected for the characterization and the isolated compound was subjected for the spectral studies.

Results and Discussion:-

ACUTE TOXICITY STUDIES:

Moringa Oleifera are common plants and used for food purposes from ancient time, so the toxicity is very less.

The acute toxicity study was done as per the OECD guidelines. All the extracts (petether, alcohol and aqueous) of *Moringa Oleifera* were administered orally in different dosed, where 24 hr toxicity was recorded to identify the toxic dose. The doses of the text compounds were then fixed on the basis of their acute toxicity study as 200 mg/kg for evaluation.

Pharmacological activities

Animal division and dose preparation:

For ***Moringa Oleifera*****:**

animals were first weighed and were selected for the experiment depending on the weight. Rats of either sex were used. The rats were then divided into five groups of six rats each.

Group 1 received saline; group 2 received 5mg/kg b.w. of Diazepam; group 3, 4 and 5 received 200 mg/kg b.w. Pet ether extract; Alcoholic and Aqueous Extracts respectively.

A. Maximal Electroshock (MES) – Induced Convulsions in Rats:

The anticonvulsant property of the drug in this model was assessed by its ability to protect against MES induced convulsions. The method used was as described by Dandiya & Sakina, 1990.

Maximal electroshock (Inco Electroconvulsiometer model # 100-3) of 150 mA current for 0.2 Sec was administered through ear electrodes to induce convulsions in the control and drug treated animals

.

The drugs and chemicals were prepared fresh; the concentration, dose and the duration before induction of convulsion were as follows:

Anticonvulsant activity by Maximal Electroshock Method (*Moringa Oleifera*)

Treatments	Duration in Various phases (Time in Sec.) (Mean±SEM)				Recovery or death	percentage inhibition
	Flexure	Extensor	Clonic	Stupor		
Control	1.30±0.15	20.15±0.25	0	0	Death	0
Pet ether extract (200 mg/kg b.w.)	2.05±0.15	14.28±0.58*	7.26±0.32	36.46±0.52	Recovery after 60.05sec.	29.14%
Aqueous extract (200 mg/kg b.w.)	2.16±0.25	13.58±0.22*	9.21±0.64	101.38±0.66	Recovery after 126.33sec.	33.45%
Alcoholic extract (200 mg/kg b.w.)	1.58±0.25	9.24±0.45**	5.50±0.35	28.33±0.45	Recovery after 44.83sec	54.13%
Diazepam (5mg/kg b.w.)	1.12±0.64	6.63±0.75***	4.35±0.48	20.20±0.15	Recovery after 32.30sec.	67.12%

Effect of various crude extracts on Pentylenetetrazole induced Kindling in Rats:

The anti-convulsant activity in this model was assessed by its ability to protect against pentylenetetrazole induced kindling seizures. The method used was described by Gupta & Malhotra, 1997 and Gee Hollinges, 1981.

Male Wistar rats were first weighed and were selected for the experiment depending on the weight.

Establishment of PTZ Kindled Seizures in Rats:

'Pentylenetetrazole' was dissolved in saline. Kindled seizures were induced by intraperitoneal injection of subconvulsant doses of PTZ i.e., 30 mg/kg, in rats, on alternate days, three times a week for nearly 10 weeks. The rats were observed for a period of 30min after subconvulsant PTZ manually and seizure activity scored using scoring system ranges from 0 to 5 (Table No.5).

Animals showing five stage 5 seizures were considered to be kindled after which, the PTZ treatment was stopped. To ascertain whether the increased sensitivity to PTZ is persistent,

the rats were rechallenged with subconvulsant PTZ (30mg/kg, ip), on 3rd and 10th day after PTZ treatment had ended. Only rats which had a stage 5 seizures on both the days were used for experiments with different drugs. The selected rats were then divided into 5 groups of 6rats each.

For *Moringa Oleifera*:

Group 1 received saline; group 2 received 5mg/kg b.w. of Diazepam; group 3, 4 and 5 received 200 mg/kg b.w. Pet ether extract; Alcoholic and Aqueous Extracts respectively.

Effects of various extracts of *Moringa Oleifera* on PTZ induced convulsions

Groups	Treatment	Onset of clonic convulsions (sec/min)	Onset of tonic convulsions (sec/min)	Animal status after 30 min.		Animal status after one day	
				No. of live animals	% Protection	No. of live animals	% Protection
1.	Control	46.88±0.599	650.33±0.999	0	0	0	0
2.	Diazepam (5mg/kg b.w.)	No Convulsion	No Convulsion	All	100	All	100
3.	Pet ether Extract (200 mg/kg body wt.)	221.88±0.888	485.00±2.501	3	50	2	34
4.	Alcoholic Extract (200 mg/kg body wt.)	235.49±0.548	564.99±2.548	5	84	5	84
5.	Aqueous Extract (200 mg/kg body wt.)	143.99±1.115	30.98±0.398	2	33.33	2	34

Values expressed are mean SEM from 6 rats. $p < 0.0001$*** as compared to control group.

Results:

Preliminary phytochemical investigation:

As per qualitative chemical tests, sterols, steroids, triterpenoids, triterpenoid glycosides, and tannins were major chemical constituents of both the powders. .

Acute toxicity studies:

All the extracts (petroleum ether, alcoholic and aqueous) of *Moringa Oleifera* and were exposed to acute toxicity studies on mice according to OECD guidelines and a dose of 200mg/kg body wt was decided for study.

Anticonvulsant activity of *Moringa Oleifera*

(i) MES induced:

The alcoholic extract among all exhibited a significant ($^{**}P<0.01$) reduction in various phases of epileptic seizure on comparison with the reference standard Diazepam 5 mg/kg, i.p. There was also a significant reduction in the time required for the righting reflex (recovery) in the extract treated group.

(ii) PTZ induced:

200 mg/kg alcoholic extract exhibited a significant anticonvulsant effect by increasing latency, onset of clonic convulsion and decreases onset of tonic seizures.

After 30 min of interval, 84 % of animals survived. In case of pet ether, after 30 min 50 % animals survived at a dose of 200 mg/kg, but 100 % survival in case of standard drug all the results are shown.

Conclusion:-

Moringa Oleifera are well known plants and has dietry values. The plant was taken with the aim to prove scientifically their anticonvulsant and immunomodulatory potential reported.

Moringa Oleifera was collected in bulk quantity and exposed to shade drying. After drying Crude drug were exposed to size reduction and passed through the sieve of 40 mesh size the supernatant were collected and exposed to successive extraction by hot percolation by soxhlet apparatus with petether and ethanol and cold maceration with water. All the extracts made free from solvent and stored in wide mouth bottle and yield was calculated. After extraction all the extracts were exposed to biological evaluation by different models of anticonvulsion activity like MES nad PTZ induced convulsion mmethods. After that all the extracts were exposed to immunomodulatory activity by different models like NBT, Neutrophil locomotion and chemotaxis and Candicidal assay etc. Among all the extracts alcoholic extracts of both the drugs

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