



ANALGESIC EFFECT OF THE ETHANOLIC EXTRACT OF *MORINGA OLEIFERA* LINN. PLANT ON INDUCED ALBINO MICE

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ABSTRACT

The ethanolic extract of whole part of *Moringa oleifera* plant in Eddy's Hot plate method shows markedly increase in the pain threshold of mice. The three different Extract Curative doses of 100 mg/kg, 200 mg/kg and 400 mg/kg were compared with standard drug i.e. Aspirin. The increased pain threshold of mice by curative treatment using alcohol extract was significant. *Moringa oleifera* is a deciduous tree of immense medicinal properties. Whole plant specially root, bark, leaves and fruits contain many important phytoconstituents. Literature survey revealed that plant contains flavonoids, glycosides, vitamins, and important inorganic metals that's why used as an important medicine traditionally in many ailments. This laid the basis for selection of whole plant for the anti-inflammatory activity.

KEY WORDS: Ethanolic extract, *Moringa oleifera*, Pain threshold, Aspirin.

INTRODUCTION

Likewise history in India can be traced back to the oldest repository of human knowledge, Charka (1000B.C) and Sushruta (1800B.C), the two eminent physicians in Indian medicines. **Charka Samhita** is the first reported treatise on Ayurveda. It consist of eight section divided into 150 chapters and describes 341 plant used in medicines. The other treatise on ayurveda in **Sushruta Samhita** with special emphasis on surgery. It has six sections covering 186 chapters and describe 395 medicinal plants, 57 drugs of animal origin and 64 mineral and metals as drugs. The next important authority in Ayurveda was Bagabhatta of Sind who practiced during about seventh century. His manuscript entitled "**Astanga Haridaya**" is considered unrivalled for principal and practice of medicine. The manuscript is divided in 6 section covering 120 chapters and contain 7444 verses.^{1,2,3.}

Exhaustive information is available in ayurvedic literature that can be converted into large database giving information of various foods, herbs, medicines and other materials with their taste, actions and utility in different disorders. An innovative method to provide quantitative representations of various ayurvedic concepts, including, Prakruti, Rasa and Guna has been developed by the Indian institute of Chemical Technology, Hyderabad. This patented technology has been registered as Herboprint and essentially gives a three dimensional HPLC fingerprint with ayurvedic property profile. Information about safety, efficacy along with possible indications and contraindications is secured. This is mainly because similar botanicals are used and researched in different parts of the world.^{4, 5}

MATERIAL AND METHODS

Plant material: The fresh whole parts of plant were collected during the month of August 2001, from the Kanpur district (Uttar-Pradesh). The plant material was dried in shade & authenticated by **Dr. Tariq Hussain (Ref. No. 97840)** from National Botanical Research Institute, Lucknow. The dried by plant material converted into moderately coarse powder grinder.

Preparation of extract: The dried plant material subjected to extraction in soxhlet apparatus for 72 hrs. A green colored extract was obtained; the extract was cooled and filtered to

remove the residue. Then solvent was removed firstly by distillation then by rotavapour under reduced pressure and then traces of solvent was removed on water bath. Further extract was dried in dessicator.^{6,7.}

Phytochemical Screening: The ethanolic extract has shown the presence of Carbohydrate, Protein, Amino acid, Steroids, Alkaloids, and Glycosides.

TLC preparation of Ethanolic Extract^{8,9} : The spot of phytochemicals on TLC Plate has shown by using Activated silica gel as adsorbent, Iodine vapour as detecting agent, n-Hexane : Ethyl acetate (80 : 20) as solvent system. Rf value calculated by using following formula-

$$R_f = \text{Distance travelled by spot} / \text{Distance travelled by solvent front}$$

HPTLC & Column chromatography

HPTLC report shown 6 spots of different Rf value which are given below -

0.21, 0.29, 0.35, 0.71, 0.80, 0.87. In column chromatography Rf value of isolated single spot in solvent system Hexane: Ethylacetate (80:20) is 0.87 by using iodine vapour.

EXPERIMENTAL DESIGN

Acute Toxicities

Acute oral toxicity was performed by using OECD guidelines – 423 (Organization of Economic Co-Operation Development) – Fixed Dose Procedure. The purpose of this study is to allow selection of the appropriate starting dose for the main study. All experimental protocols were approved by Institutional Animal Ethical committee of the Institute (approved by CPCSEA Regd.No. BU/PHARM/IAEC/08/033). The limit test for acute toxicity was carried out at 2000 mg/kg oral dose of LEE in group of rats as per OECD 423 guidelines (OECD, 2001). The rats were observed continuously for 24 h for behavioral, neurological, and autonomic profiles and, after a period of 24 and 72 h, for any lethality, morbidity state or death.

The LD₅₀ value for the ethanolic extract of *M. oleifera* plant was done as per OECD guidelines (Revised draft 423) in adult albino mice were found to be 2000 mg/kg body weight for extracts. The animal not showed any signs of toxicity and

behavioral changes after 24 hrs and 72 hrs below toxic dose.^{10,11.}

Drugs and Chemicals

All the chemicals were analytical grade. Aspirin was collected from Arora Chemicals, New Delhi.

Animals

Healthy albino mice weighing about (20-25gm) of either sex was housed under the uniform laboratory condition fed with commercial diet & water ad-libitum, during the experiment. The animals were procured from Gwalior & permitted for the study under the Institutional Animal Ethical Committee.

Evaluation of Antilithiatic Activity

The animals were divided into five groups of five animals each.

Group 1: served as control & received regular food and drinking water.

Group 2: received MOPEE (100 mg/kg body weight).

Group 3: received MOPEE (200 mg/kg body weight).

Group 4: received MOPEE (400 mg/kg body weight).

Group 5: received standard drug Aspirin (30mg/kg).

This method was first described by the Eddy and Leimback. The instrument is called Eddy's hot plate analgesia meter. The animals are kept in Eddy's hot plate maintained at constant temp. (55±0.5°C) and the reaction of animals such as, paw licking or jump response was taken as the end point.^{12,13.}

The animals were placed individual in Hot plate regulated at temp. (55±0.5°C) before the treatment & its reaction time was determined. After noting the initial reaction time, the treatment should be given to each mouse. Then the each animal placed in the Eddy's hot plate under regulated temp to obtain animal response licking of the forepaws or jump of the Hot plate surface was recorded as the hot-plate latency. Mice with baseline latencies of <5s or >30s were eliminated from the study. The reaction time is noted by stop-watch and then the reaction time were re-determined after 30, 60 & 120 min. after oral administration of standard and test drug.^{14,15.}

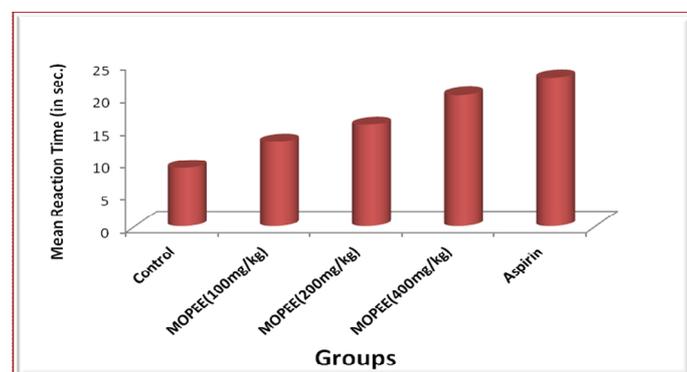
$$\% \text{ protection against thermal stimulus} = \frac{\text{Test mean} - \text{Control mean}}{\text{Control mean}} \times 100$$

RESULT

Table 1: Hot Plate Test in mice

S.No.	Groups	Doses	Mean Reaction Time (in sec)	% Protection
1.	Control	N.S.	9 ± 0.730	-
2.	MOPEE	100	13 ± 1.000*	42.44
3.	MOPEE	200	15.66 ± 1.282**	74.01
4.	MOPEE	400	20.16 ± 0.872**	124.31
5.	Aspirin	30	22.83 ± 0.976**	153.13

All value represented as mean ± SEM and values are overall significant. One - way ANOVA; n = 6 in each group; *P < 0.05; **P < 0.0001; N.S = Normal Saline.



Graph.1. Bar Diagram showing mean reaction time in seconds in Hot Plate Test in mice

DISCUSSION

It is graphical representation of Group-1, Group-2, Group-3, and Group-4 & Group-5. It showed efficacy of *Moringa oleifera* plant as compared with above given standard drug aspirin. When three doses of MOPEE (*Moringa oleifera* plant ethanolic extract) given to male albino mice, it showed markedly increase in the pain threshold.

Results in Table 6.1 demonstrate that the control drug Aspirin 30 mg/kg markedly increased the pain threshold of mice. MOPEE at 100, 200 & 400 mg/kg b.w. significantly (P < 0.05; P < 0.001) increased hot-plate latency in a dose-dependent manner. MOPEE 400 mg/kg exhibited greater activity (124.31%), which is comparable with the standard drug Aspirin (153.13%).

Moringa oleifera is a deciduous tree of immense medicinal properties. Whole plant specially root, bark, leaves and fruits contain many important phytoconstituents. Literature survey revealed that plant contains flavonoids, glycosides, vitamins, & important inorganic metals and used as an important medicine traditionally in many ailments. This laid the basis for selection of whole plant for the analgesic activity.

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