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Research Article

Effect of the Ethanolic Extract of *Moringa oleifera* Linn. Plant on Ethylene Glycol Induced Lithiatic Albino Rats.

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ABSTRACT

The ethanolic extract of whole part of *Moringa oleifera* plant in ethylene glycol induced lithiatic albino rats shows marked increase in renal excretion of calcium and phosphate in two different doses of Curative Regimen of 250 mg/kg and 300 mg/kg as well as Preventive Regimen of 250 mg/kg for 28 days and the effect was compared with standerd drug i.e. Allopurinol. The increased deposition of stone forming constituents in the kidney of calculogenic rats was also significantly lowered by curative and preventive treatement using alcohol extract. *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 antilithiatic activity.

Key Words : Ethanolic extract, *Moringa oleifera*, calculogenic rats, Allopurinol.

INTRODUCTION

Herbal medicines sometimes referred to as Herbals or Botanical medicine, is the use of herb for their therapeutic or medicinal value. A herb is a plant part valued for its medicinal, aromatic or savoury qualities. Herb plants produce & contain a variety of chemical substances which act upon the body. Indian materia medica includes 2500 natural products of therapeutic importance of which 500 are of animal & mineral origin and rest are of vegetable origin. Indian traditional medicine is based on various systems, including Ayurveda, Siddha and Unani. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. These traditional systems of medicine have their uniqueness, no doubt but there is common thread running through these systems in their fundamental principles and practices. Siddha system presents the slogan, "Food is being medicine & medicine is being food" to people. Now-a-days, the trained traditional Siddha practitioners are done well, even with the old highest proficiency processes which were kept by their ancestors and some people make challenges with other systems for healing and curing AIDS like diseases.[1]

The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population presently use herbal medicine for some aspect of primary health care. WHO notes that of 119 plant – derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures.

Herbal medicines can be broadly classified into various basic systems:-

1. Traditional Chinese herbalism, which is a part of Traditional oriental medicine.
2. Ayurvedic herbalism, which is derived from Ayurveda.
3. Western herbalism, which originally came from Greece & Rome to Europe & then spread to North and South America.

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 plant material converted into moderately coarse powder by grinder.

Preparation of extract : The dried plant material subjected to extraction in soxhlet apparatus for 72 hrs. A green coloured 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 waterbath. Further extract was dried in dessicator.

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

TLC preparation of Ethanolic Extract : The spot of phytochemicals on TLC Plate has shown by using

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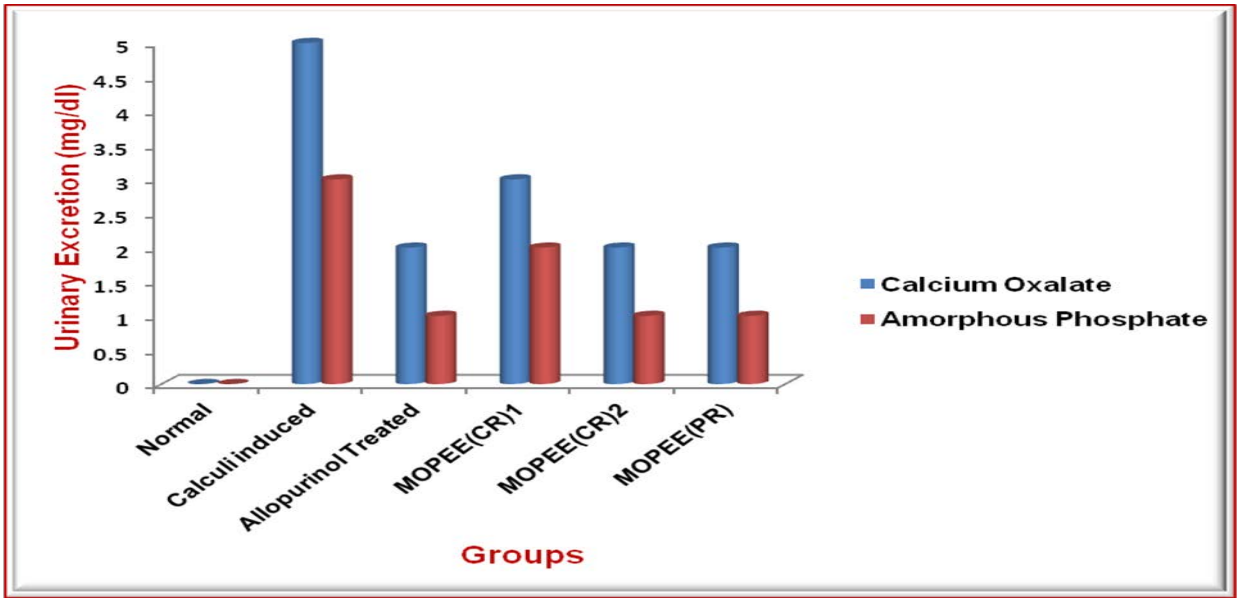
Table 1. Statistical Data Analysis of Urinary Excretion Parameters.

S.N.	Groups	Dose (mg/kg)	Urine Parameters (mg / dl)	
			Calcium Oxalate	Amorphous Phosphate
1.	Normal	vehicle	0 ± 0.0	0 ± 0.0
2.	Calculi induced	vehicle	5 ± 0.05	3 ± 0.01
3.	Allopurinol Treated	50	2 ± 0.08	1 ± 0.02
4.	MOPEE (CR)1	250	3 ± 0.01	2 ± 0.04
5.	MOPEE (CR)2	300	2 ± 0.05	1 ± 0.06
6.	MOPEE (PR)	250	2 ± 0.08	1 ± 0.01



Graph. 1. Bar Diagram representation of Oxalate, Calcium & Phosphate Concentration in Urine

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-

Rf = Distance travelled by spot / Distance travelled by solvent front

HPTLC & Column chromatography : HPTLC report shown 6 spots of different Rf value which are given -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:Ethyacetate (80:20) is 0.87 by using iodine vapour.

Experimental Design: Acute Toxicities : Acute oral toxicity was performed by using OECD guidelines – 423 (Organisation 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 Animals 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 behavioural, neurological, and autonomic profiles and, after a period of 24 and 72 h, for any lethality, morbidity state or death.

The LD_{50} 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 behavioural changes after 24 hrs and 72 hrs below toxic dose.

Animal selection : Adult Albino male Wistar rats weighing 140-200 gm were used for assessment of antilithiatic activity. Animals were procured from DRDE, Gwalior and maintained at Central Animal Facility of the Institute. They were maintained standard environmental condition (R.H.-55-65%, room temperature 25±2ºC and 12 hr light / dark cycle) and were fed with standard pellet diet and water *ad libitum*. Each experiment group constituted of five animals, housed in separate cages. All experimental protocols were approved by Institutional Animals Ethical committee of the Institute (approved by CPCSEA Regd. No.716/02/a/CPCSEA).

Drugs and Chemicals : Allopurinol tablet (Zyloric tablet, Glaxo Smith Kline Pharmaceuticals Limited, Dr. Annie Besant Road, Mumbai, Bangalore, India; Batch no. M425; Mfg. Date : Jan-2010; Exp. Date : Feb-2013) were used as standard drug. Ethylene

glycol was obtained from Merck Laboratories, Mumbai, India.

Evaluation of Antilithiatic Activity: The animals were divided into six groups of six animals each.

Group 1: served as control & received regular food and drinking water. Group 2: served as induced & received 0.75% Ethylene Glycol in distilled water as vehicle till 28 days. Group 3: received standard drug allopurinol (750 mg/kg body weight) from 15th day till 28th day. Group 4: received EtE (250 mg/kg body weight) from 15th day till 28th day and served as curative regimen (CR). Group 5: received EtE (300 mg/kg body weight) from 15th day till 28th day. Group 6: received EtE (250 mg/kg body weight) from 1st day till 28th day and served as preventive regimen (PR).

Collection and Analysis of Urine: All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40ºC. Urine was centrifuged and the crystals of urine were analysed under optical microscope at 10 x to 40 x resolution. Urine was analysed for calcium oxalate and phosphate content. [2], [3], [4], [5].

STATISTICAL ANALYSIS

Data were expressed as the mean ± standard error of means (SEM). The mean and SEM was calculated by Graph Pad Instat 3.0. The statistical analysis was carried out employing analysis of variance (ANOVA) by using the software PRISM (Graph Pad) 5.0. All the values were compared to the saline control. The differences were determined statistically using Dunnett's t-test. Differences were considered statistically significant at $p < 0.05$. [6], [7].

RESULT AND DISCUSSION

Where, n = 6, number of animals. Data analyzed by one-way ANOVA followed by Dunnett's test. All groups compared with control group. The result of *in-vivo* model indicates that the MOPEE not only cure but also prevented the growth of urinary stones, which significantly reduced the level of lithiasis causing factors such as calcium, oxalate and phosphate. This plant based therapy used as adjunct therapy particularly in urolithiasis as there are no satisfactory drugs in modern medicine which can dissolve the stone and the physicians remain to be depend on alternative systems of medicine for better relief. Currently known herbal drugs exert their antilithogenic effect by altering the ionic composition of urine, e.g., decreasing the calcium and oxalate ion concentration or increasing magnesium and citrate excretion.

It is graphical representation of amorphous phosphate & calcium oxalate crystals obtained from pathological report. It showed efficacy of *Moringa oleifera* plant as compared with above given standerd drug allopurinol. When two doses of MOPEE (Moringa oleifera plant ethanolic extract) given to male wistar albino rat, it showed presence of 3 ± 0.01 calcium & 2 ± 0.04 phosphate.

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