

**ANTIUROLITHIATIC ACTIVITY OF AQUEOUS EXTRACT OF
MORINGA OLEIFERA (Lam.) POD IN RATS**

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Summary

In the present study, aqueous extract of *Moringa oleifera* was evaluated for its antiurolithiatic potential in albino rats of Wistar strain. The vesical calculi were produced by zinc disc insertion into the urinary bladder of rats supplemented with 1%ethylene glycol in drinking water. The drug / extract was administered orally daily in two doses (400mg and 800mg/kg) for four to eight weeks in different groups of animals [Prophylactic and Curative].The reduction in weight of the vesical calculi was used as criteria for assessing the prophylactic or curative antiurolithiatic effect of the pods of this plant. In both the groups the administration of the aqueous extract of *Moringa oleifera* pods [AEMO] has resulted in significant reduction in the weight of calculi as compared to the control group. It can be concluded that pods of *Moringa oleifera* has antiurolithiatic activity.

Keywords: *Moringa oleifera*, antiurolithiatic activity, zinc disc.

Introduction

Moringa oleifera is an important medicinal herb, belonging to the family Moringaceae. It is considered as miracle tree as all the parts of the plant are said to have medicinal properties. It is also known as drum stick tree, horseradish tree, clarifier tree and mother's best friend in different parts of the world, based on their appearance and unique uses. It is mostly cultivated all over the plains of India, in the old world tropics and it is used medicinally in Guinea, Madagascar, Guiana and Burma.^{1, 2, 3}

The various parts of the plant used include flower, pod, leaves, bark and roots. The flowers, which must be cooked, are eaten either mixed with other foods or fried in butter and have been shown to be rich in potassium and calcium⁴. The leaves are eaten as greens, in salads, in vegetable curries, as pickles and for seasoning. The Bark is regarded as an antiscorbid, and it exudes a reddish gum sometimes used for the treatment of diarrhoea. The roots are bitter, act as a tonic to the body and lungs, and have an expectorant activity. They are a mildly diuretic and are used as a stimulant in paralytic afflictions, epilepsy and hysteria. The pods are the most valued and widely used of all the tree parts. The pods are extremely nutritious, containing all the essential amino acids along with many vitamins and other nutrients. The immature pod can be eaten raw or prepared like green peas or green beans, while the mature pods are usually fried and possess a peanut-like flavour. The pods also yield 38 - 40% of non-drying, edible oil known as Ben Oil which is clear, sweet and odourless, and never becomes rancid. Overall, its nutritional value most closely resembles olive oil⁴.

In recent decades, many scientific studies using the extracts of leaves, pods and roots of *Moringa oleifera* are being carried out to confirm many potential uses including wound healing^{5, 6} anti-tumour⁷, anti-hepatotoxic⁸, anti-fertility⁹, hypotensive¹⁰, antiurolithiatic¹¹ acute anti-inflammatory^{6, 12, 13}, and analgesic activity¹⁴.

Even though the root extracts of *Moringa oleifera* have been studied for diuretic activity¹¹, there is no scientific study showing diuretic and antiurolithiatic activity of *Moringa oleifera* pod extract. Therefore this study was conducted in order to evaluate its antiurolithiatic activity in rats.

Materials and Methods

Approval for the project: Approval for the experiment was obtained from the Institutional animal ethics committee (IAEC), Kasturba Medical College, Manipal, vide letter No. IAEC/KMC 07/2008-2009.

Plant material: The pods of *Moringa oleifera* Lam. Available locally were collected between February and May in and around Udipi. The botanical identity has been confirmed by department of botany, Sri Poornapagna College, Udipi. The voucher specimen is preserved in the laboratory for future reference.

Method of extraction: The fresh pods were cut into small pieces, partially crushed and soaked in water overnight which was later subjected to boiling for 6 hours. The resultant extract was then drained and concentrated in a water bath to get the concentrated extract. The yield of the extract was 8.5%. The extract was stored in desiccators and used for further experiments after dissolving it in distilled water.

Animals: Inbred albino rats of Wistar strain, of either sex, aged around 2 to 3 months and weighing 150 – 200g were used. They were housed in standard conditions of temperature (25±2°C), relative humidity of 45-55%, and maintained on 12-hour light: 12-hour dark cycle in animal house of Kasturba Medical College, Manipal. They were fed standard pellet diet (Hindustan Lever rat pellets) and water ad libitum.

Acute toxicity study: The aqueous extract of *Moringa oleifera* pod was administered orally in the escalating dosages, up to 8g/kg to different groups of rats (n=6). The animals were observed for behavioural and physiological variations initially continuously for 4 hours, followed by 4th hourly for 12 hours and there after once daily for 14 days.

Antiuro lithiatic study : Method of induction of urolithiasis by insertion of zinc disc¹⁵⁻¹⁹. Rats were anaesthetized with intraperitoneal ketamine(50mg/kg). A suprapubic incision was made and the abdomen was opened. The urinary bladder was then carefully exposed and the urine in the bladder was aspirated with a sterile syringe. A small nick was made at the apex end of urinary bladder and the sterile zinc disc (previously weighed) was carefully inserted into the bladder. Then the bladder was closed in a single stitch using chromic catgut (4-0). The abdomen was then closed in layers with chromic catgut and skin was closed with silk thread. The rats were allowed to recover from anaesthesia. Food and 1% ethylene glycol in water was given ad libitum. The stone was allowed to form and grow inside the bladder during the study period. After the study period the rats were sacrificed and zinc disc implants/stones were removed from the bladder and dried. Stones taken out were weighed. The difference between initial and final weights indicated the amount of stone formed.

Grouping of animals for different treatments and procedure of the study: Adult albino rats of Wistar strain, weighing between 150-200 g, were selected for the study. In this study the rats were divided into 6 groups with 6 animals in each group receiving different treatments. Group I- Prophylactic control(1%ethylene glycol for 4 weeks),Group II-Prophylactic treatment (1%ethylene glycol+AEMO 400mg/kg orally for 4 weeks), Group III -Prophylactic treatment (1%ethylene glycol+AEMO 800mg/kg orally for 4 weeks), Group IV-Curative control (1%ethylene glycol for 4 weeks followed by water for 4 weeks),Group V-Curative treatment (1%ethylene glycol for 4 weeks followed by AEMO 400mg/kg for 4 weeks), Group VI-Curative treatment (1%ethylene glycol for 4 weeks followed by AEMO800mg/kg for 4 weeks). Prophylactic activity against urolithiasis was tested in Groups I to III in this study and, after 4 weeks, animals were sacrificed and vesical calculi were collected, weighed and statistically evaluated. Curative property was tested in Groups IV to VI in the study and, at the end of eight weeks, animals were sacrificed and vesical calculi were collected, weighed and statistically evaluated.

Weight of calculi: The difference between the weight of the implanted zinc discs at the time of implantation and final weight of the dried calculi taken out from the bladder at the end of the 4th and 8th week period indicated the weight of deposited stone.

Statistical analysis: The data obtained from the study was statistically evaluated using a parametric test ANOVA (Analysis of Variance) and Turkey as post hoc test. This was done with SPSS (Statistical package for social science) software

Results

The results of different groups studied using aqueous extract of pods of *Moringa oleifera* 400mg/kg & 800mg/kg are shown in table 1. In this study, the extracts from pods of the plant showed significant decrease in the weight of stones compared to control after the study period.

Table 1.

Weight of deposits on zinc discs in various experimental groups of rats

Groups [n=6 in each group]	Weight of calculi (mg) (Mean± SEM)
Group I - Prophylactic control	273.5 ± .10
Group II - Prophylactic treatment (AEMO,400mg/kg)	139.5 ± 6.1*
Group II - Prophylactic treatment (AEMO,800mg/kg)	128.1 ± 11.5*
Group IV - Curative control	256.1 ± 42.69
Group V - Curative treatment (AEMO,400mg/kg)	129 ± 10.4*
Group VI - Curative treatment (AEMO,800mg/kg)	160 ± 21.81*

*p<0.05

Discussion

In the present study, aqueous extract of pods of *Moringa oleifera* 400mg/kg & 800mg/ were evaluated for the antiurolithiatic potential in albino rats of Wistar strains. The method used for induction of stones in this study was zinc disc foreign body insertion technique into the bladder of rats supplemented with 1% ethylene glycol in drinking water, which is commonly used in these type of studies.

In this study, the weight of the stones was used as criteria for assessing the preventive or curative effect of the pods of *Moringa oleifera*. Two doses of extract for prophylactic and curative groups were used. In both groups the administration of the extract have resulted in significant reduction in the weight of stones compared to the control group.

The decrease in the bladder stone formation was inconsistent with the increase in the dose of the extract which could be due to variability in the response due to physiological variation. The stone formation in the control group itself was variable to a certain extent ranging from 240 mg to 296 mg in prophylactic control group and 236 mg to 296 mg in curative control group, which is indicative of normal physiological variation. Among the different strains of rats used for preclinical studies, Wistar rats are much less susceptible to persistent bladder infection and struvite stone formation in comparison to other strains. In a study where female Fischer 344 (F344), Lewis (LEW), Sprague-Dawley (SD), and Wistar (WIS) rats were inoculated with a host-adapted strain of

Ureaplasma parvum, and necropsied at 2 weeks post inoculation; 100% of F344, 42% of SD, 10% of LEW, and 10% of WIS rats remained infected. Severe bladder lesions and struvite calculi were seen in 64% of F344 rats; in other rat strains, bladder lesions were mild or absent²⁰. In this study there was no signs of infection and the weight of stones formed in the bladder were significantly reduced after the administration of aqueous extract of *Moringa oleifera* in both preventive and curative groups of animals. The extract is more effective in preventing the stone formation in bladder of rats in our experiments.

Conclusion

In conclusion we can confirm the possibility of antiurolithiatic activity of pods of *Moringa oleifera* as there was reduction in size of the stones. Further studies are needed to prove the stone dissolving property of aqueous extract of pods of *Moringa oleifera* (400mg/kg & 800mg/kg) in other animal models and also further studies are required for isolation of active ingredients /compounds in the extract.

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