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Research Article

EFFECT OF ETHANOLIC EXTRACT OF *NYMPHAEA ALBA* LINN ON UROLITHIATIC RATS

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ABSTRACT

Mechanisms of pathogenesis involved in lith/crystal formation, including recent developments in our understanding of related changes in human kidney tissue and of underlying genetic causes, in addition to current therapeutics. Changes in dietary practices may be a key driving force behind the crystal formation. The urolithiasis was induced by inserting zinc disc (a foreign body) in the urinary bladder. This was also followed by supplementing 1% ethylene glycol in drinking water. The reduction in weight of the stones was used as criteria for assessing the preventive or curative regimen. In the present study, ethanolic extract of dried leaves of *Nymphaea alba* Linn was administered orally. This was evaluated for its antiurolithiatic potential in albino rats of Wistar strains. It was studied by administrating two different doses of the plant for prophylactic and curative groups. Oral administration of the *Nymphaea alba* Linn has resulted in significant reduction in the weight of bladder stones compared to the control group.

Keywords: *Nymphaea alba* Linn, Zinc discs, Ethylene glycol, Antiurolithiatic activity.

INTRODUCTION

Urolithiasis is defined as the presence of one or more calculi in any location within the urinary tract. The disease affects 1% to 5% of the population in developed countries with a peak incidence between 20 and 50 years of age. Men are three times more likely to be affected than women and the lifetime risk of developing a calculus in a Caucasian man is nearly 20%.[1] It has been reported that 91% of the urinary calculi contain calcium in some form, while 8% and 1% are composed of uric acid and cystine, respectively. The calcium-containing calculi consist of pure or various amount of calcium components such as calcium oxalate monohydrate, apatite, calcium hydrogen phosphate, and calcium carbonate. In men, 70% to 80% of the calculi contain either calcium oxalate alone or in combination with apatite[2] *Nymphaea alba* Linn (Nymphaeaceae) is a perennial aquatic herb generally found in tanks and ponds throughout the warmer parts of India and Africa. All parts of the plants are used in folk medicine. The powder of rootstock is given to treat dyspepsia, diarrhoea and piles. An infusion of the rhizomes and stem is considered to be an emollient, diuretic and used for treatment of diseases of the urinary tract. Decoction of the flower is used in palpitation of heart and as a narcotic; syrup of the flower is used in high fever, apoplexy, inflammatory diseases of the brain as also in dysuria. Leaves are applied topically in erysipelas, whereas the macerated leaves are used as a lotion in eruptive fevers. The seeds are said to be stomachic and restorative[3-4].

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Nymphaea alba* Linn were collected from Ganesh temple, Sarasbag, Pune, Maharashtra, India during month of October-2010.The collected plant material was authenticated by Dr.P.G.Diwakar, joint director, Botanical Survey of India (BSI), Pune, India. A voucher specimen of the plant was deposited in the JSPMs Charak College of Pharmacy and Research, Wagholi, Pune as a herbarium under the number –**TTSNA-1.**

Method of Extraction

The leaves were dried in shade and were coarsely powdered (40 mesh size). The ethanolic extract (EE, 10%, w/v) of dried barks was prepared using 70% (v/v) ethanol by soxhlet method at a temperature of 60-70 ºC[5].

Animal Selection

Healthy adult male albino rats weighing between 150 and 200 g were selected for the evaluation of antilithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 2 ºC) and maintained on 12 hr light and 12hr dark cycle. They were housed in polypropylene cages and provided with regular rat chow (Bioscience Ltd, India) and drinking water *ad libitum*. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC) and were cleared by the same.

Acute Toxicity Studies

The acute oral toxicity study[6] was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose[7].

Antiurolithiatic study

Method of induction of urolithiasis by insertion of Zinc Disc

Rats were anaesthetized with intraperitoneal ketamine (50 mg/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 formed8-12.

Drug treatment

Adult albino rats of Wistar strain, weighing between 150-200 g, were selected for the study. In this study using EENA, 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+ EENA 150 mg/kg orally for 4 weeks), Group III–*Prophylactic treatment* (1% ethylene glycol+ EENA 300 mg/ 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 EENA 150 mg/kg for 4 weeks), Group VI– *Curative treatment* (1% ethylene glycol for 4 weeks followed by EENA 300 mg/kg for 4 weeks).Prophylactic

activity against urolithiasis was tested using Groups I to III in this study and after 4 weeks, animals were sacrificed and vesicle calculi were collected, weighed and statistically evaluated. Curative property was tested in using Groups IV to VI in the study and, at the end of eight weeks, animals were sacrificed and vesicle calculi were collected, weighed and statistically evaluated.

Weight of Stones

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 Statistical package for social science software.

RESULTS AND DISCUSSION

In the present study, ethanolic extract of *Nymphaea alba* Linn at the doses of 150 mg/kg & 300 mg/ was evaluated for the antiurolithiatic potential in albino rats. The method used for induction of stones in this study was zinc disc insertion technique which is followed by 1% ethylene glycol, In this study, the weight of the stones was used as key criteria for assessing the prophylactic or curative effect of the *Nymphaea alba* Linn. In both the groups, administration of the extract have resulted in significant ($p < 0.01$) 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 in prophylactic control group and 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, Lewis, Sprague-Dawley, and Wistar rats were inoculated with a host-adapted strain of *Urea plasma parvum* 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 absent [13, 14].

Table 1: Weight of stone material deposit on zinc discs in control and after treating rats with 150 mg/kg & 300 mg/kg EENA

Group	Dose	No. of animals	Weight of stone in mg (Mean ±SEM)
Group I–prophylactic control	-	6	280.18 ± 8.42
Group II–prophylactic treatment	(150 mg/kg)	6	141.7 ± 9.91*
Group III–prophylactic treatment	(300 mg/kg)	6	108.9 ± 6.56*
Group IV–curative control	-	6	272.68 ± 11.39
Group V–curative treatment	(150 mg/kg)	6	173.80 ± 13.87*
Group VI–curative treatment	(300 mg/kg)	6	116.75 ± 5.51*

* $p < 0.001$, df = 2, SEM = Standard error of mean, n = 6

In this study, the plant showed significant decrease in the weight of stones compared to control after the study period.

CONCLUSIONS

In conclusion we can say in India, ayurveda referred a system of medicines, several herbal drugs and are prescribed for reducing renal damage and to avoid kidney related complication. These can be immense value in combating renal lithiasis and subsequent damage[15]. We can also confidently confirm the possibility of antiurolithiatic activity of bark of *Nymphaea alba* Linn as there was reduction in size of the stones. Further studies are needed to prove the stone dissolving property of ethanolic extract of *Nymphaea alba* Linn (150 mg/kg & 300 mg/kg) in other animal models.

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