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PHYTOCHEMICAL AND PHARMACOLOGICAL INVASTIGATION ON THE LEAVES OF *TAMARINDUS INDICA* LINN. FOR ANTILITHIATIC ACTIVITY

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ABSTRACT: The main objective of the present investigation is to evaluate the antilithiatic activity of ethanolic extract of *Tamarindus indica* L. on mice. Antilithiatic activity of the ethanolic extract of the *Tamarindus indica* leaves at a dose of 250mg/kg, 400mg/kg & 500mg/kg was evaluated against the standard drug Cystone was given orally 750mg/kg. Adult Wister Albino rats of either sex of divided in six groups of six animals each as undertaken for study and evaluated by Rat models of calcium oxalate urolithiasis induced by either ethylene glycol (EG) are most commonly used to study the pathogenesis of urolithiasis.
Ethanolic extract of *Tamarindus indica* (at 250, 400 and 500 mg/kg) exhibited a dose dependent significant anti-lithiatic activity on treatment. The extract dose of 250 mg/kg also caused partial reduction of Uria, Uric acid, calcium, Potassium, oxalates, phosphorus and creatinine in blood serum level the results were found statistically insignificant. The antilithiatic effect of ethanol extract at was found less effective than the reference standard.

INTRODUCTION: *Tamarindus indica* Linn. belonging to Caesalpinaceae subfamily of Fabaceae family. The tree is a long-lived, large, evergreen or semi-evergreen tree, 20-30 m tall with a thick trunk up to 1.5-2 m across and up to 8 m in circumference.

The trunk forks at about 1 m above ground and is often multistemmed with branches widely spreading, drooping at the ends and often crooked but forming a spreading, rounded crown. . The bark is brownish-gray, rough, and scaly. Young twigs are slender and puberulent.

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A dark red gum exudes from the trunk and branches when they are damaged [1]. India is a major producer and consumer of Tamarind in the world.-[2]. Along with culinary usage, there is a vast medicinal utility of *T. indica* L. described which are enumerated in different Ayurvedic classics. Besides fruit being an important part, Thai traditional medicine recognizes *Tamarindus indica* fruit as digestive, carminative, laxative, expectorant and blood tonic [3].

Many other properties have been also reported like Hypolipemic and antioxidant [4], anti-inflammatory [5], antimicrobial [6], cytotoxic [7], against gastrointestinal spasms [8] and modifying the complement system [9]. Pain is an unpleasant feeling often associated with tissue damage. Tissue injury is the immediate cause of pain as it releases different chemical mediators like prostaglandins, bradykinins, substance P which act on the nociceptors causing this sensation.

The nociceptive stimulus is transmitted to the CNS by small myelinated Aδ- fibres or by unmyelinated thin C-fibres [10, 11, 12].

MATERIALS AND METHODS:

Plant material & method: The leaves of the *Tamarindus indica* Linn. were collected from Jhansi. The leaves were dried in shade. The leaves were authenticated by Dr. Neelima sharma (Reasearch Investigation Officer), (Reference No. 5714) from National Botanical Research Institute, Jhansi. After authentication, fresh plant material was collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized in a mechanical grinder. The coarse powder was passing through sieve no. 40 and taken for further studies.

Preparation of Extract: For the preparation of extract 100gm of dried coarse powdered leaves were charged in to the soxhlets apparatus (hot extraction) and extracted successively with ethanol at temperature at 60°-80°C to defate it. Extracted with ethanol (95%) at 60°-80°C.The success of the extraction with ethanol is directly related to the extent that chlorophyll is removed into the solvent. The successive methanolic extract (deep brown colour) was filtered & dried under reduced pressure to get a solid mass free from the solvent. The yield was 7.2% with respect to dry starting material with characteristic odour & greasy consistency. The dried extract was dissolved in distilled water and & using 1% tween 80 as a suspending agent for the evaluation of analgesic activity.

Preliminary Phytochemical Screening: The Ethanolic extract of *Tamarindus indica*was screened by different chemical test for the identifying the basic chemical constituents present in the extract. The standard chemical tests for alkaloids, tannins, flavonoids, terpenoids and steroids were performed to get a preliminary idea of the chemical constituents [13, 14].

Animals selection: Healthy Wistar-albino rats weighing about (150-200gm) of either sex were obtained from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The animals were housed in specific standard laboratory conditions. The conditions were kept in a temperature-controlled environment (25±1^{o}C) and with a regular 12h light/12h dark cycle. All animals were fed with commercial diet & water *ad libitum*, during the experiment. All protocols of the study was approved by the Institutional Animal Ethical Committee with reference no. BU/Pharm/IAEC/11/ 021.The IAEC is approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration no. 716/02/A/CPCSEA.

Acute toxicity studies: Acute oral toxicity of *Tamarindus indica* Linn. was performed in Swiss Albino Mice. The mice were kept for 4 hr of fasting prior to the experiment and body weight of the mice should be noted. Usually mice weighing 25-35 gm were used for acute toxicity studies. The dose was given to every mice orally according to body weight. The acute toxicity study was performed according to OECD guidelines 2001 by 'Ups and Down' method. The dose of extract was given at 50, 100, 200 300, 400, 2000 and 5000mg/kg. During the first 4 hr. after the drug administration, animals were continously observed for gross behavioral changes & then observation is continued for 24 hrs & 72 hrs in regular intervals for 14 days. The parameter such as hyperactivity, grooming, convulsions, sedation, hypothermia, change in fur colour, mortality or moribund stage or death was observed.

Drugs and Chemicals: Ethylene glycol (AR Grade) will obtain from Merck Laboratories, Mumbai, India. Cystone was procured from Himalaya Health Care Ltd. are used as standard antiurolithiatic drug.

Antilithiatic activity:

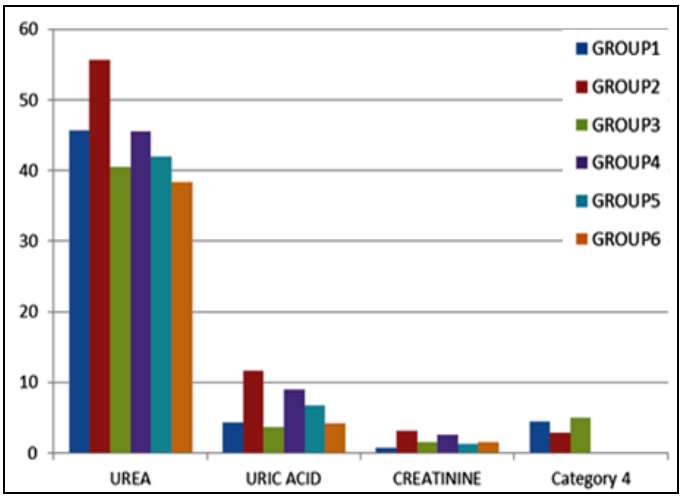
1. **Statistical analysis:** The data obtained by the various parameters was statistically evaluation by one way analysis of variance (ANOVA) followed by Student Newman Keul's test Graph Pad Prism software (Graph Pad Prism software Inc., version 4.0.0.255).the mean values ± SEM Were calculated for each parameter. The differences in biochemical parameters between the calculi induced group and standard drug treated group were considered as 100 % and the changes in biochemical parameters by the plants extracts treated groups against the calculi induced group were analyzed accordingly. Level of significance was kept at $P < 0.05$.

Table 1 and figure 1 given effect of *T.Indica* on various parameters in Serum below following 0.75% Ethylene Glycol.

TABLE 1: EFFECT OF *T.INDICA* ON VARIOUS PARAMETERS IN SERUM FOLLOWING 0.75% ETHYLENE GLYCOL FOR 28 DAYS

Parameters	Group I Normal control	Group II EG control	Group III Standard drug cystone	Group IV *T. indica* (250mg/kg)	Group V *T. indica* (400mg/kg)	Group VI *T. indica* (500mg/kg)
Urea (mg/dl)	45.70±2.05	55.57±1.87*	40.50±3.07**	52.57±2.30**	45.70±2.52**	42.30±2.68**
Uric acid (mg/dl)	4.27±0.68	11.63±0.54*	3.70±1.19**	9.00±0.22**	6.67±0.57**	4.15±0.41**
Creatinine (mg/dl)	0.68±0.14	3.13±0.30*	1.46±0.24**	2.53±0.07	2.12±0.28**	1.52±0.30**
Calcium (mg/dl)	5.55±1.11	14.70±0.80*	6.34±0.33**	11.08±0.38**	9.60±1.60**	7.45±0.52**
Magnesium (mEq/L)	1.48±0.09	2.74±0.08*	1.94±0.12**	1.98±0.19**	1.87±0.04**	1.79±0.08**
Potassium (Mmol/L)	6.51±0.59	11.94±0.82*	6.95±1.67**	10.63±2.26	8.59±1.24**	5.68±0.85**
Phosphorus (mg/dl)	5.02±0.20	7.15±0.32*	4.94±0.50**	5.82±0.22**	5.43±0.15**	5.15±0.26**

Values are expressed as mean ± SEM, n=6 as compared to group 1 and 2, P<0.05 as compared to control group.



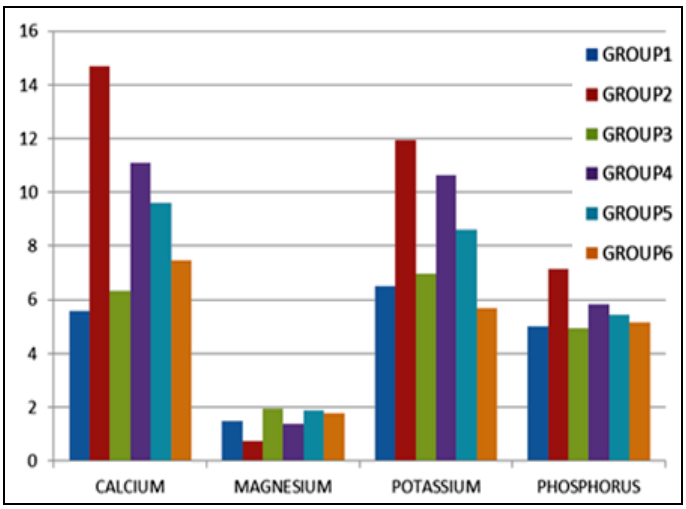


FIGURE 1: ANTILITHIATIC ACTIVITY OF *TAMARINDUS INDICA* LINN. n= 6, Values are expressed as Mean±SEM P< 0.05 when compared with control group

RESULTS: Ethanolic extract of *Tamarindus indica* Linn. in experimentally induced urolithiasis in rats. Rat models of calcium oxalate urolithiasis induced by either ethylene glycol (EG) are most commonly used to study the pathogenesis of urolithiasis. This study is an accelerated model, where rats are treated with 0.75% EG for 28 days.

A marked rise in Uria, Uric acid, calcium, Magnesium, Potassium, oxalates, phosphorus and creatinine in blood serum level observed in lithiatic control compare to normal control rats. Ethanolic extract of *Tamarindus indica* (at 250, 400 and 500 mg/kg) exhibited a dose dependent significant anti-lithiatic activity on treatment. The extract dose of 250 mg/kg also caused partial reduction of Uria, Uric acid, calcium, Potassium, oxalates, phosphorus and creatinine in blood serum level the results were found statistically insignificant. The antilithiatic effect of ethanol extract at was found less effective than the reference standard.

Ethanolic extracts of *Tamarindus indica* Linn. Significantly reduced the elevated level of calcium oxalate ions which is consider as one of the inhibitor of crystallization. The histopathological findings also show sign of improvement after treatment with extract. All these observation provided the basis for the conclusion that *Tamarindus indica* L. leaves extract inhibit the stone formation induced by ethylene glycol treatment.

DISCUSSION: Antilithiatic activity of the ethanolic extract of the *Tamarindus indica* leaves at a dose of 250mg/kg, 400mg/kg & 500mg/kg was evaluated against the standard drug Cystone was given orally 750mg/kg. Adult Wister Albino rats of either sex of divided in six groups of six animals each as undertaken for study and evaluated by Rat models of calcium oxalate urolithiasis induced by either ethylene glycol (EG) are most commonly used to study the pathogenesis of urolithiasis.

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