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# STUDY OF ANTIUROLITHIATIC ACTIVITY OF *DIOSPYROS MALABARICA* (DESR) KOSTEL ON RATS

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# ABSTRACT

The purpose of this study was to investigate the antiurolithiatic activity of ethanolic extract of fruits of Diospyros malabarica (Desr.) Kostel (EEFDMDK) on rats in ethylene glycol (EG) and Ammonium chloride (AC) induced urolithiasis model. Twenty four male rats ware randomly divided in to four groups (n = 6). EG 0.75 % (v/v) and AC 2 % (w/v) in drinking water were feed to all groups of rats (Groups II, III, and IV) except normal control (Group I) rats for 10 days to induce urolithiasis. Group III and IV rats were treated with EEFDMDK at 250 and 500 mg/ kg oral (P.O.) for 10 days. Group I and Group II (positive control) rats were administered 6µl distilled water (DW) per 1g of body weight by gavage for 10 days. The change in body weight of animals was calculated (final weight on 10<sup>th</sup> day - initial weight on 1<sup>st</sup> day) and we observed that, weight of rats in positive control group was significantly decreased as compared other groups of rats. At the end of 10<sup>th</sup> day of the experimental period, blood samples were collected and analyzed for biochemical parameters i.e. serum concentration of urea, creatinine, calcium and phosphorus. The kidneys were removed and sectioned for histopathological studies. Treatment with the EEFDMDK restored all the elevated biochemical parameters when compare to positive control group. The histopathological studies confirmed the induction of urolithiasis as damages in kidney and crystal deposition was observed in section of kidney from animal treated with EG and AC. This was reduced, however after treatment with the EEFDMDK. The conclusion of this study was EEFDMDK showed significant antiurolithiatic activity and possible mechanism underlying this effect is mediated collectively through diuretic and antioxidant properties.

Keywords: Diospyros malabarica (Desr.) Kostel, antiurolithiatic, Ethylene glycol, Ammonium chloride.

# **INTRODUCTION**

Urinary stone constitute one of the commonest diseases in our country and pain due to kidney stone is known as worse than that of labour pain. Among all the pain, abdominal pain always drags not only patient's attention but also the curiosity of the surgeon. It is estimated that 12% of world population experience renal stone disease with a recurrence rate of 70-80% in men and 47-60% in women.<sup>1,2,3</sup> Kidney stone formation or urolithiasis is complex process that occurs due to imbalance

between promoters and inhibitors in the kidneys.<sup>4</sup> In the treatment of kidney stone various synthetic drugs are available in the market. Even though surgery is the treatment of choice for urinary stones, life style changes are important as the recurrence rate is high as 50% within 5 years after surgery without medical treatment.<sup>5</sup> Herbal drugs can be easily available and it does not produce any type of complications like synthetic drugs to patients. Several plant extract have been used to

treat kidney stones with promising effect in prevention and treatment. Diospyros malabarica (Desr.) Kostel (DMDK), Family - Ebenaceae is a tree distributed throughout India. The plant used in the traditional system for various clinical conditions such as liver diseases, snake bites, diabetes, diarrhea, cancer, urinary diseases and renal stone.<sup>6,7,8,9</sup> The plant possesses flavonoids, tannins, terpenoides, sugars and steroids.<sup>10</sup> In recent times, focus on plant research has increased all over the world and large body evidence has collected to show immense of medicinal plants used in various traditional systems. The present study is focused on the investigation of antiurolithiatic activity of fruits of DMDK on Ethylene glycol (EG) and ammonium chloride (AC) induced urolithiasis in rats.

# MATERIAL AND METHODS Plant Material

Fruits of DMDK was collected in bulk quantities from our college campus area and authenticated by Department of Botany, S.S.M.M. Baramati. The fruits were shade dried separately at room temperature and powder was obtained.

#### **Preparation of Extract**

The powder of fruits was subjected to successive soxhlet extraction with solvents of increased polarity. The ethanolic extract was selected for the present study. The extract was concentrated using rotary flash evaporator and stored at room temperature.

# **Preliminary Phytochemical Screening**

Preliminary Phytochemical screening was carried out on ethanolic extract of fruits of *Diospyros malabarica* (Dser.) Kostal (EEFDMDK) for detection of phytoconstituents present following the standard methods described in practical pharmacognosy book by Dr. C. K. Kokate<sup>11</sup> and K. R. Khandelwal.<sup>12</sup>

# **Acute Toxicity**

Acute toxicity study of EEFDMDK was performed on albino mice (20 - 30 g) maintained under standard conditions. Fixed dose method of CPCSEA (Committee for the Purpose of Control

and Supervision of Experiments on Animals) was adopted for toxicity studies<sup>13,14</sup> (OECD Guideline No. 420).

# Animals

Healthy, male Wistar rats each weighing between 230 to 275 g were used for the study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at  $25\pm3^{0}$  C and 35-60% humidity). They were feed with standard rat feed and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee of S.V.P.M's College of Pharmacy, Malegaon Bk II, Baramati, registered under CPCSEA, India (Registration No. 1214/ac/08/CPCSEA).

# Ethylene Glycol-Ammonium Chloride Induced Urolithiasis

Ethylene glycol-Ammonium chloride induced urolithiasis model was used for the experiments.<sup>15</sup> Twenty-four male rats were divided in to four groups six animal each. The treatment protocol

for 10 days for each group was as follows:

Group I: *ad libitum* access to regular food and drinking water and administered 6µl distilled water (DW) per 1g of body weight by gavage (normal control).

Group II, III and IV: *ad libitum* access to regular food and drinking water containing 0.75% (v/v) ethylene glycol (EG) and 2% (w/v) ammonium chloride (AC) in order to promote urolithiasis.

Group III: Rats were administered 250 mg/kg oral (P.O.) dose of EEFDMDK.

Group IV: Rats were administered 500 mg/kg P. O. dose of EEFDMDK.

Group II: Rats were administered 6µl DW per 1g of body weight by gavage (positive control). All the rats were weighed daily.

# Assessment of Antiurolithiatic Activity

At the end of  $10^{\text{th}}$  day of the experimental period, rats were anaesthetized and blood was collected from the retro-orbital region, centrifuged at 10,000 X g for 10 min. The serum was estimated for calcium, urea, creatinine and phosphorus using the respective diagnostic kits.

#### **Histopathological Studies**

The rats were killed by high dose of ether, abdomen was opened and the kidneys were removed. The kidneys were stored in 10% neutral formalin solution, fixed in bouin liquid, soaked in paraffin and section were taken using a microtome. The sections were stained with hematoxylin (H) and eosin (E) and observed under a computerized microscope (100X and 400X).

#### **Statistical Analysis**

The data were presented as men  $\pm$  standard error of mean (SEM) and analyzed using Student's "t" test and one-way analysis of variance (ANOVA) followed by Dunnett's and P< 0.05 was considered statically significant. Statistical Package for social Science (SPSS 20.0) version software was used for statistical analysis.

# **RESULT**

# Acute toxicity study

The EEFDMDK was studied for acute toxicity at dose of 2000 mg/kg P.O. The extract was found to be safe and no mortality of the animals observed. Hence 2500 mg/kg was considered as  $LD_{50}$  cut off value as per fixed dose method of CPCSEA. So, the doses selected for the evaluation of antiurolithiatic activity were 250mg/kg and 500mg/kg P.O.

# **Antiurolithiatic Activity**

The body weight of rats before experiment (initial weight) i.e. on 1<sup>st</sup> day and after experiment (final weight) i.e. on  $10^{th}$  day were compared in each group of rats and we observed that, there was a significant difference in change in body weight as shown in table number (no.)1. As shown in table no. 2 serum urea, creatinine, calcium, phosphorus level were found to be significantly increased in rats of positive control group; Whereas treatment with the EEFDMDK were found to protect the rats form elevation of serum urea, creatinine, calcium, phosphorus level . The change in body weight (final weight on 10<sup>th</sup> day - initial weight on  $1^{st}$  day) was calculated and we observed that, weight of rats in positive control group were significantly decreased as compared other groups of rats as shown in table no. 2. Similarly, as shown in table no. 3 and photomicrograph C and D glomerulopathiea, degeneration of tubules, cellular infiltration, tubular dilatation, nephrosis and dilation of tubules and crystal deposition were found to be significantly increased in rats of positive control group; Whereas treatment with the EEFDMDK were found to reduce such changes in the rats kidney histology.

# **DISCUSSION**

According to literature survey it was observed that chances of kidney stone formation are more common in men as compare to women.<sup>16</sup> So, chances of stone formation in female rats are less than male rats.<sup>17</sup> So, we had selected male rats for present study. In the present study EEFDMDK has been demonstrated for the antiurolithiatic activity against EG and AC induced urolithiasis in rats. A biochemical mechanism of EG and AC induced urolithiasis in rats is related to an increase in the urinary concentration of oxalate. EG is readily absorbed from intestine and metabolized in the liver to oxalate that further leads to hyperoxaluria.<sup>18</sup> Since most of the kidney stones composed of oxalate, EG model was selected to induce urolithiasis. Furthermore, AC has been reported to accelerate urolithiasis.<sup>18</sup> In the present investigation body weight of animal, serum concentration of urea, creatinine, calcium, phosphorus and the histopathology of the kidney are analyzed. In the analysis as shown in table no. 2 we observed that, serum concentration of urea, creatinine, calcium, phosphorus was significantly increased only in rats of positive control group; Whereas treatment with the EEFDMDK were found to reduce elevation of level of urea, creatinine, calcium and phosphorus. In the histopathology of kidney, as shown in table no. 3 and photomicrograph (A, B, G, H) no any oxalate crystals were seen in the kidney of group I and group IV rats but crystal depositions were seen in the kidney of group II (photomicrograph C, D) and group III (photomicrograph E, F) rats. In the group II rats significant loss of body weight was observed as compared to other groups of rats and this may be due to stones formation is more and this leads to obstruction in urine passage and result in pain during urination. Due to pain, food consumption may be decreased and result in

decrease in body weight of animal. In kidney histology of positive control rats showed marked changes and damages but all such histological changes were significantly reduced in rats treated with EEFDMDK. In the present study possible mode of action of EEFDMDK may be due to diuretic property of DMDK. This property favours antiurolithiasis by hastening the process of dissolving or by flushing of the performed stones or by preventing the new stone formation in urinary system. The other possible mode of action of EEFDMDK may be due to its antioxidant effect of DMDK<sup>19</sup> because there is evidence that hyperoxaluria induced per oxidative damage to the renal tubular membrane surface provides a favorable environment for calcium oxalate crystal attachment and development of kidney stone.<sup>20</sup> In the present investigation Preliminary Phytochemical studies of EEFDMDK gave positive test for tannins and flavonoids and there is evidence that, presence of these phytoconstituents (specially flavonoids) by virtue of their antioxidant potential are suggested to important role in antiurolithiatic play a effects.<sup>21,22</sup>

# CONCLUSION

From the obtained data it is conclude that administration of EEFDMDK reduced and prevented the growth of urinary stones. From the data we also observed that both low dose and high dose of EEFDMDK showed significant antiurolithiatic effect at various stages. The possible mechanism underlying this effect is mediated collectively through diuretic and antioxidant properties and lowering the concentration of urinary stone forming constituents. Further work is necessary to isolate the active constituents responsible for the antiurolithiatic activity and studies on larger animal models and on human is warranted to draw final conclusion

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Groups	Body weight (gm)					
	Initial		Final			
Ι	253.66±3.84	257.	.83±3.82			
II	259.16±3.51	246.	.33±3.87***			
III	252.50±3.81	245.	.33±3.63***			
IV	253.33±3.33	248.	.66±3.42***			

# **Table 1:** Effect of EEFDMDK on body weight in urolithiatic rats

Values are expressed as mean ± SEM, n=6, \*P<0.05, \*\*P<0.01 \*\*\*P<0.001compaired with initial weight versus final weight in each group (student's "t" test).

Tuble 2. Effect of EET Dribte of various of other parameters in aronanatic rais								
Groups	Change in body weight (gm)	Creatinine (mg/dl)	Urea (mg/dl)	Calcium (mg/dl	Phosphorus (mg/dl)			
Ι	4.16±0.30	0.42±0.02	19.34±0.74	6.43±0.05	6.27±0.02			
II	-12.83±0.47	0.75±0.02	45.24±0.59	13.36±0.04	12.18±0.02			
III	-7.16±0.40***	0.58±0.01***	34.90±0.48***	10.69±0.03***	10.28±0.02***			
IV	-4.66±0.21***	0.50±0.01***	22.09±0.69***	7.72±0.03***	6.87±0.02***			

#### **Table 2:** Effect of EEFDMDK on various biochemical parameters in urolithiatic rats

Values are expressed as mean ± SEM, n=6, \*P<0.05, \*\*P<0.01 \*\*\*P<0.001 compaired with positive control (one -way ANOVA followed by Dunnett's test).

# Laxmikant Maruti Purane *et al. / Pharmacophore* 2015, Vol. 6 (6), 299-305 Table 3: Histopathological features of the kidney of different treatment groups

Group	Glomerulopathiea	Degeneration of	Cellular	Tubular	Nephrosis and	Crystal
		tubules	infiltration	dilatation	dilation of tubules	deposition
Ι	-	-	-	-	-	-
II	++++	++++	+++++	+++++	++++	+++
III	++	++	+++	++	++	+
IV	+	+	+	+	++	-

- : No abnormality detected; +: Damages/active changes up to less than 25%; ++: Damages/active changes up to less than 50%; +++: Damages/active changes up to less than 75%; ++++: Damages/active changes up to more than 75%













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**Figure:** A: Photomicrograph of Group I rat kidney, H & E stain, 100X; B: Photomicrograph of Group I rat kidney, H & E stain, 400X; C: Photomicrograph of Group II rat kidney, H & E stain, 100X; D: Photomicrograph of Group II rat kidney, H & E stain, 400X; E: Photomicrograph of Group III rat kidney, H & E stain, 100X; F: Photomicrograph of Group III rat kidney, H & E stain, 400X; G: Photomicrograph of Group IV rat kidney, H & E stain, 400X; H: Photomicrograph of Group IV rat kidney, H & E stain, 400X.

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