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Original Article

Anti urolithiatic activity of a siddha herbomineral drug by zinc implantation method in experimental rats

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ABSTRACT

Siddha system of medicine is one of the oldest therapeutic systems prevalent predominantly in southern part of India. Siddhars have classified diseases numbering 4448 and it is mentioned in Agasthiar rathinachurukka naadi nool. Urolithiasis refers to the formation of stones in the urinary tract. It causes pain and bleeding and may lead to secondary infection. It is the third most common affliction of the urinary tract. This study aims to evaluate the anti urolithiatic activity of the drug, Kara sooda sathu parpam by Zinc implantation method in experimental rats. The anti urolithiatic activity was studied using the zinc disc implantation method in rat model. There was a significant reduction in the stone weight in both groups treated with the drug of curative regimen when compared with the curative control group. The anti urolithiatic activity by zinc implantation method revealed that the drug is effective both in preventive and curative aspects.

Key words: Siddha, Urolitiasis, Zinc disc, Stone

INTRODUCTION

Siddha system of medicine is one of the oldest therapeutic systems prevalent predominantly in southern part of India. It makes use of plant, mineral and herbomineral preparations to a larger extent [1].In Siddha system of medicine drug preparation methods, currently termed pharmaceuticals were a distinct forte. Siddha literatures carry information about selection of drugs, meticulous descriptions on drug processing, administration methods, and indications for various human ailments. The chemistry behind the preparations and the formulations in Siddha system of medicine is much deeper apart from medicine and alchemy. The diagnosis of diseases ie. noi nadal is perfectly done if only the root cause of the disease is identified. Siddhars have classified diseases numbering 4448 and it is mentioned in Agasthiar rathinachurukka naadi nool. Among them they classify **kalladaippu noi** and other obstructive uropathies come under **neerinai arukkalnoigal**. They define that dietary factors play an important role in the formation of calculi. Derangement in the pitha humor results in highly concentrated urine and accumulation of salts in the urinary tract [2].

Urolithiasis refers to the formation of stones in the urinary tract. It causes pain and bleeding and may lead to secondary infection. It is the third most common affliction of the urinary tract. Kidney stone affects 5-15% of world population not sparing any geographical, cultural or racial group [3].There are several types of calculi. Among them the most commonly occurring stones are calcium oxalate and calcium phosphate. Magnesium ammonium phosphate (struvite), uric acid and cysteine are the others types of calculi commonly seen among stone formers [4]. Stone formation occurs when urinary concentrations of stone forming salts, exceed limit of metastability for that salt in solution. This results due to excess excretion of stone forming constituents, deficient or

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inactivity of the stone inhibitors in urine or simply a low urine volume [5] In Siddha system of medicine many formulations have been indicated for urolithiasis. Herbal drugs in Siddha literature were validated to a larger extent. As already discussed, herbomineral and herbo metallic drugs add to the strength of Siddha medicine. The efficacy is more with minimum dose when compared to herbs. One of the drugs indicated for urolithiasis is **Kara soda sathu parpam**. This study aims to evaluate the anti urolithiatic activity of the drug by Zinc implantation method in experimental rats.

MATERIALS AND METHODS

Ingredients of Kara soda sathu parpam

1) Fried Vengaram (Borax / Sodium bi borate)
2) Karpoora silasathu (Gypsum)
3) Vediuppu (Potassium nitrate / salt petre)
4) Chitrandathol (Egg shell of *Gallus domesticus*)
5) Palagarai (*Cyprea moneta*.Linn)
6) Padigaaram (Alum / Alumen)
7) Lemon juice (*Citrus limon*)

Preparation of the drug [6]: The purified ingredients were ground with sufficient quantity of lemon juice and made into pellets and subjected to pudam in karchunna moosai(crucible made of limestone) with 12 – 15 numbers of cow dung cakes according to the weight of villai . The calcinized pellets were ground to fine powder and stored in an air tight glass container.

Animal selection: Adult male Wistar rats weighing between 200-250gm were used for the study. The animals were acclimatized to standard laboratory conditions (temperature: 22 ± 2ºC) and maintained on 12 h light and 12 h dark cycle. They were housed in polypropylene cages of standard size. The animals had free access to feed purchased from M/s. Provimi Animal Nutrition India Pvt Ltd, Bengaluru and purified water (Kent RO water filter cum purifier) *ad libitum*. The experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) and the registration number is 1248/AC/09/CPCSEA – 9/Dec 2013/2. . The rats were procured from, NCLAS, NIN, Hyderabad.

Preparation of zinc discs: Zinc plates were procured from local market and made in to a thin film. Discs of 2mm size were prepared which weighed around 8 – 10mg. Care was taken that the margins of the discs were smooth and rounded so that no injury will be produced on the bladder walls when implanted.

Surgical procedure: Rats were anaesthetized with Thiopentone sodium (50 mg/kg) i/p. The abdomen was opened by a suprapubic incision. After careful exposure of the urinary bladder the urine was gently aspirated with a sterile syringe. A small nick was made at the apex without causing any damage to the bladder. A disc previously weighed was carefully placed inside the bladder. The nick of the bladder was closed by a single stitch using chromic catgut (4-0), the abdominal layers with chromic catgut (3-0) and skin with silk thread. Food and water containing 0.75 % ethylene glycol was given *ad libitum*. The experiment was carried out for a period of eight weeks. After the experiment period the rats were sacrificed and the stones were harvested and weighed. The anti urolithiatic activity was assessed by comparing the weight of stones formed in the drug treated groups with control [7-11].

Post-operative care: The recovery cage was kept free of particulate bedding and lined with paper towels. To avoid cannibalism and suture chewing by cage mates in the recovery cage, rodents were housed individually. Postoperative animals were monitored at least once daily until the animal is stabilized. All postsurgical examinations were recorded, including any complications encountered.

Experimental design

Table 1: Zinc implantation method- Grouping of animals

No	Group	No of Rats
1	Curative control	6
2	Curative treatment dose 1KSSP (87.5mg/kg b.w)	6
3	Curative treatment dose 2KSSP (131.5mg/kg b.w)	6
4	Preventive control	6
5	Preventive treatment dose 1KSSP (87.5mg/kg b.w)	6
6	Preventive treatment dose 2KSSP (131.5mg/kg b.w)	6

Operated animals were randomly divided in to the following groups and the details of treatment procedure along with surgical implantation are as follows.

Group 1: Curative control (0.75% ethylene glycol for 4 weeks followed by water for 4 weeks)
Group 2: Curative treatment (0.75% ethylene glycol for 4 weeks followed by KSSP dose 1for4 weeks)

Group 3: Curative treatment (0.75% ethylene glycol for 4 weeks followed by KSSP dose 2 for 4 weeks).
Group 4: Preventive control (0.75% ethylene glycol for 4 weeks)
Group 5: Preventive treatment (0.75% ethylene glycol+ KSSP dose 1, orally for 4 weeks)
Group 6: Preventive treatment (0.75% ethylene glycol+ KSSP dose 2, orally for 4 weeks)

After a period of eight weeks, the rats were sacrificed and zinc disc implants/stones were removed from the bladder and dried. Stones taken out were weighed [6-10].

PARAMETERS FOR ASSESSMENT OF ANTIUROLITHIATIC ACTIVITY

Weight of stones formed: The dried stones were weighed and the difference between the initial and final weights of zinc discs gave the weight of the stone deposited

Serum analysis: Rat blood was collected from the retro-orbital plexus under anaesthesia. Creatinine, urea nitrogen and uric acid were estimated.

Confirmation of implantation and stone formation by radiographs: Radiographs were taken by anaesthetizing the animals and placing the animals in lateral position. The radiographs were taken using SIEMENS 500 MA make, with 80MA, 50KV, 2.5 MAS and the exposure time being 0.03 seconds. The radiographs were taken twice, once after implantation of zinc discs and the next just prior to sacrifice (i.e. after 8 weeks)

Histopathological assessment: The Urinary bladder and kidney tissues obtained from experimental animals were fixed in 10% formalin. They were dehydrated in graded alcohol and embedded in paraffin. Finally they were cut into 4–5 μm thick sections. Haematoxylin-eosin was used to stain the sections and assessed using a photomicroscope (Model N-400ME; CEL-TECH Diagnostics, Hamburg, Germany

RESULTS

Implantation of zinc disc in the urinary bladder of the animals served as a nidus to stone formation. Significant amount of stone deposition on the zinc disc was observed in the control and treated groups. The evaluation of anti urolithiatic activity was done by comparing the stone weight, renal markers, x ray and histopathology of the kidneys and urinary bladder of animals from control and drug treated groups.

Effect of KSSP on serum parameters: The urea level got significantly reduced to 36.33±0.67 and 36.40±1.63 in the drug treated curative groups when compared to curative control group (44.20±1.46). There was no significant change in the urea level between preventive control and drug treated groups. The creatinine level was 0.80±0.03 in the curative control group. The level significantly reduced to 0.48±0.02 and 0.44±0.02 in the drug treated groups treated with 87.5 mg/kg and 131.5 mg/kg KSSP when compared to control group. There was no significant change in the creatinine levels of the preventive control and treated groups. There was no significant change in the uric acid of the control and drug treated groups both in the preventive and curative regimens as shown in the table 2.

Effect on stone weight: The weight of stone formed in the curative control group was 748.2± 153.9. The stone weight significantly reduced to 147.3± 63.1 and 22.0± 0.8 in the group treated with 87.5mg/kg and 131.5 mg/kg KSSP respectively when compared to curative control group. The weight of stone formed in the preventive control group was 34.3± 2.5. The weight of stone significantly reduced to 18.9 ± 0.7in the group treated with 131.5 mg/kg KSSP when compared to preventive control group.

X ray findings: The implantation of zinc discs in the urinary bladder of the animals was confirmed by taking x rays after surgical procedure in the animals from all the groups. The presence of zinc disc in all the groups is shown in the fig 2.1 and fig 2.2. The x ray radiographs were taken in the animals of all groups at the end of the experiment period before sacrificing the animals. Significant amounts of stone deposition with appearance of multiple stones were noticed in curative control group (fig. 2.3a). The reduction in size and the number of stones were noticed in drug treated groups as shown in the fig 2.3b. Deposition of stones was noted in the preventive control and drug treated groups as shown in the fig 2.4. Noticeable change in the size and number of stones were not observed in the x rays of preventive control and drug treated groups.

DISCUSSION

Zinc disc implantation method mimics the stone formation as that of humans. This foreign body implantation method is used in the induction of bladder stones [12, 13]. The foreign body when present in the bladder acts as a nidus around which stone deposition takes place. Round zinc discs of 2mm diameter were punched out with smooth margin, were used in this study for stone formation[13]. Since bladder is a place where implant can be placed compared to other parts of the urinary tract, it was chosen for studying the anti urolithiatic activity of the drug. The pathogenesis of stone formation is similar in all parts of the

urinary tract. In the present study, considerable amount of stone deposition were present in all the groups (Table 3). There was a significant reduction in the stone weight in both groups treated with KSSP of curative regimen when compared with the curative control group. The group treated with 131.5 mg/kg KSSP showed more activity than the group treated with 87.5 mg/kg KSSP. In the preventive regimen, the group treated with 131.5 mg/kg KSSP showed a significant reduction in stone weight when compared to preventive control group. From this observation it can be inferred that the dose 131.5 mg/kg KSSP is more effective in preventing and curing the stone formation.

Since ethylene glycol was administered in the drinking water for the induction of oxalate stones, the renal parameters in serum were also analyzed. The urea and creatinine levels were significantly reduced in the drug treated groups of curative regimen. No significant changes were observed in the preventive regimen. There was no significant change in the uric acid levels of drug treated groups both in the preventive and curative regimen. The implantation of zinc discs and the stone formation were confirmed by X- ray radiographs taken after implantation and at the end of the experiment in all the groups. Initially the zinc discs were visualized in all the groups (Fig 2.3). Reduction in the size of the stone was observed in the drug treated groups of curative regimen (Fig 2.3b & Fig 2.3c). Digital X-ray radiography provides a clear picture of the implants and also the crystal growth at various stages. This is a vital tool to monitor the growth stone and also helps the researcher to decide the experimental period thus preventing the mortality of animals.

The quality of water used in the animal facility was analyzed for minerals and pathogens to check if it facilitated the stone formation. By this analysis (Table 4) it is understood that water used in the animal facility didn't influence the stone growth. The histopathological study was done to observe the crystal deposition in the kidney tissue and studying the extent of injury to bladder tissue of all groups. Heavy mononuclear cell infiltration was present in the kidney curative of control group. No abnormalities with mild mononuclear cell infiltration were seen in the drug treated groups of curative regimen. Tubular cast with mild crystals were observed in the preventive control group and less tubular cast were present in the kidneys of drug treated groups of preventive regimen. Mononuclear cell infiltration was present in the bladder tissue of the curative control group. This infiltration was mild in the drug treated groups of curative regimen. The histopathology of bladder showed focal epithelial hyperplasia both in control and drug treated groups of preventive regimen.

CONCLUSION

The anti urolithiatic activity by zinc implantation method revealed that the drug KSSP is effective both in preventive and curative aspects. The drug significantly reduced the stone size and X-ray findings too revealed the same.

Table 2: Effect of KSSP on serum parameters in zinc implantation model of lithiasis induction

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1.Curative control	44.20 ± 1.46	0.80 ± .03	1.42 ± 0.13
2.Curative regimen-1 (KSSP 87.5mg/kg)	36.33 ± 0 .67 [b]	0.48 ± 0.02 [c]	1.58 ± 0.07
3. Curative regimen-2 (KSSP 131.5mg/kg)	36.40± 1.63 [b]	0.44 ± 0.02 [c]	1.50 ± 0.11
4.Preventive control	36.40 ± 1.63	0.44 ± 0.02	1.50 ± 0.11
5.Preventive regimen-1 (KSSP 87.5mg/kg)	34.17 ± 1.89	0.47 ± 0.03	1.47 ± 0.10
6.Preventive regimen-2 (KSSP 131.5mg/kg)	36.17 ± 1.01	0.48 ± 0.03	1.63 ± 0.19

Results are expressed as Mean ±SEM, [a] $p<0.05$, [b]$p<0.01$, [c] $p<0.001$, Compared to group 1. [d]$p<0.05$,[e]$p < 0.01$, [f]$p<0.001$, Compared to group 4. PR – Preventive regimen, CR – Curative regimen

Table 3: Effect of KSSP on weight of stones in zinc implantation model of lithiasis induction

Groups	Weight of stones(mg)
1. Curative control	748.2± 153.9
2. Curative regimen-1(KSSP 87.5mg/kg)	147.3± 63.1[a]
3. Curative regimen-2(KSSP 131.5mg/kg)	22.0± 0.8 [b]
4. Preventive control	34.3± 2.5
5. Preventive regimen-1(KSSP 87.5mg/kg)	24.2± 3.5
6. Preventive regimen-2(KSSP 131.5mg/kg)	18.9± 0.7[f]

Results are expressed as Mean ±SEM, [a] $p<0.05$, [b]$p<0.01$, [c] $p<0.001$, Compared to group 1. [d]$p<0.05$,[e]$p < 0.01$,[f]$p<0.001$, Compared to group 4. PR – Preventive regimen, CR – Curative regimen

Table.4 The quality of water used in the animal facility

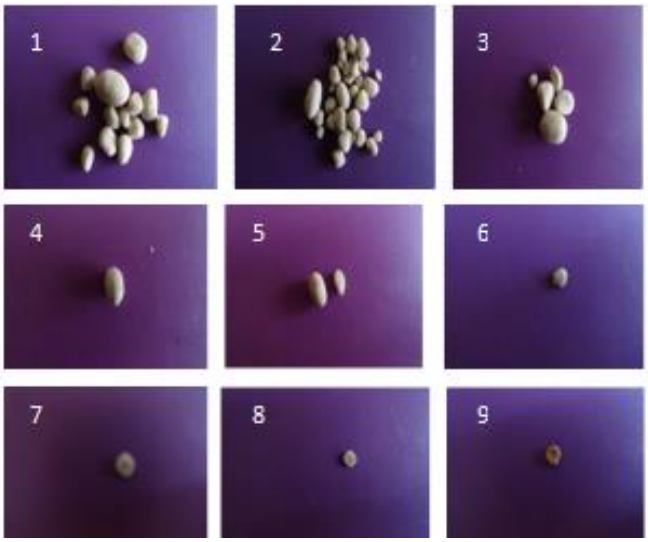
Sl.no	Parameters	Units	Result
Physical and chemical parameters			
1	Appearance	-	Slightly turbid
2	Colour	Hazen units	5
3	Odour	-	Agreeable
4	pH value at 25^0 C	-	6.62
5	Electrical conductivity at 25^0 C	Micromhos/cm	300
6	Turbidity	NTU	2.8
7	Total dissolved solids	mg/L	177
8	Chlorides as Cl	mg/L	76.4
9	Total hardness as $CaCO_3$	mg/L	16
10	Calcium as Ca	mg/L	4.4
11	Magnesium as Mg	mg/L	1.2
12	Alkalinity Phenolphthalein as $CaCO_3$	mg/L	Nil
13	Total alkalinity as $CaCO_3$	mg/L	19.3
14	Residual Chlorine	mg/L	BLQ
15	Sulphate as SO_4	mg/L	2.3
16	Manganese as Mn	mg/L	0.01
17	Nitrate as NO_3	mg/L	9.1
18	Fluorides as Fl	mg/L	BLQ
19	Copper as Cu	mg/L	0.01
20	Total iron as Fe	mg/L	0.12
Bacteriological examination			
1	Total coliforms	MPN/100 ml	< 2
2	Thermotolerant coliforms	MPN/100 ml	< 2
3	*E.coli*	MPN/100 ml	< 2

Table 5: Histopathological findings in zinc implantation model of lithiasis induction

Group	Treatment	Tissue	Observations
1	Zinc disc implantation + 0.75% EG treatment in water (week 1-8)	Kidney	Heavy mono nuclear cell infiltration present
		Urinary bladder	Mono nuclear cell infiltration present. Mild neutrophilic infiltration seen.
2	Zinc disc implantation + 0.75% EG treatment in water + KSSP 87.5 mg/kg (week 4-8)	Kidney	No abnormalities seen with presence of occasional cast
		Urinary bladder	Mild MNC infiltration seen
3	Zinc disc implantation + 0.75% EG treatment in water + KSSP 131.5 mg/kg (week 4-8)	Kidney	Moderate tubular cast seen. Moderate MNC infiltration seen
		Urinary bladder	NAD. Mild neutrophilic infiltration seen.
4	Zinc disc implantation + 0.75% EG treatment in water (week 1-4)	Kidney	Tubular cast and mild crystals present. Focal loss of tubules seen
		Urinary bladder	Focal epithelial hyperplasia seen
5	Zinc disc implantation + 0.75% EG treatment in water+ KSSP 87.5 mg/kg (week 1-4)	Kidney	Less tubular cast present
		Urinary bladder	Focal epithelial hyperplasia seen
6	Zinc disc implantation + 0.75% EG treatment in water + KSSP 131.5 mg/kg (week 1-4)	Kidney	Moderate tubular cast seen.
		Urinary bladder	Focal epithelial hyperplasia seen

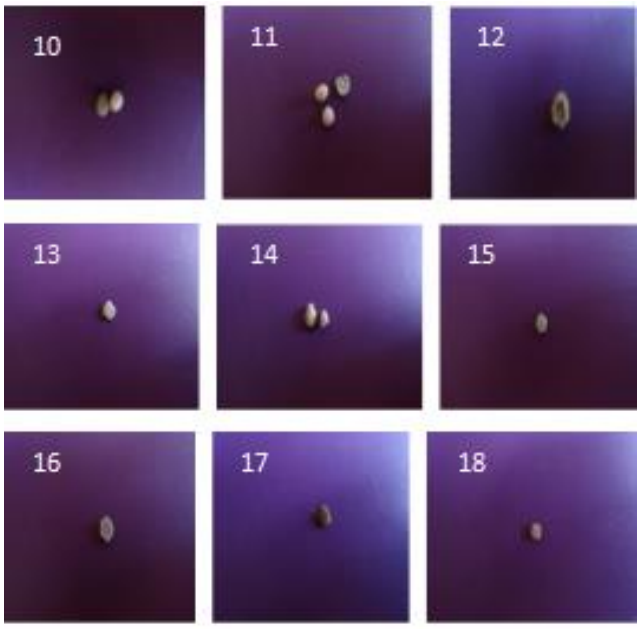
Fig 1: Stones harvested from different groups in zinc implantation method.

Fig 1.1: Stone harvested from curative regimen



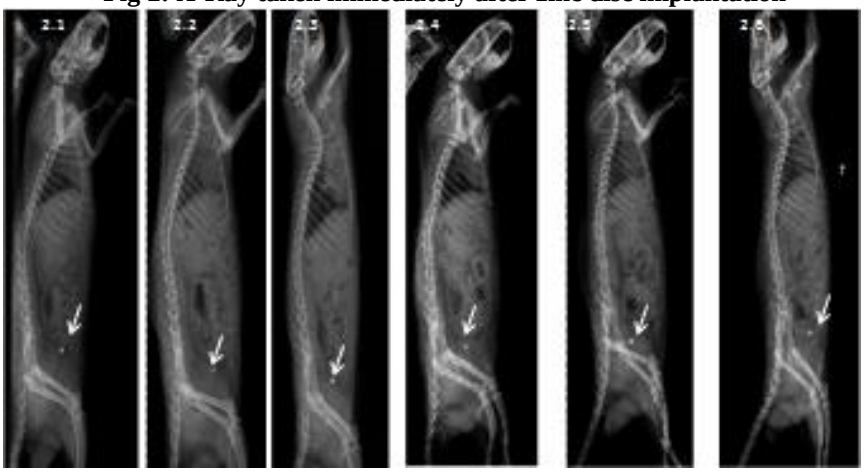
1,2,3: Stones harvested from curative control. 4,5,6: Stones harvested from curative treatment dose 1. 7,8,9: Stones harvested from curative treatment dose 2

Fig 1.2: Stone harvested from preventive regimen



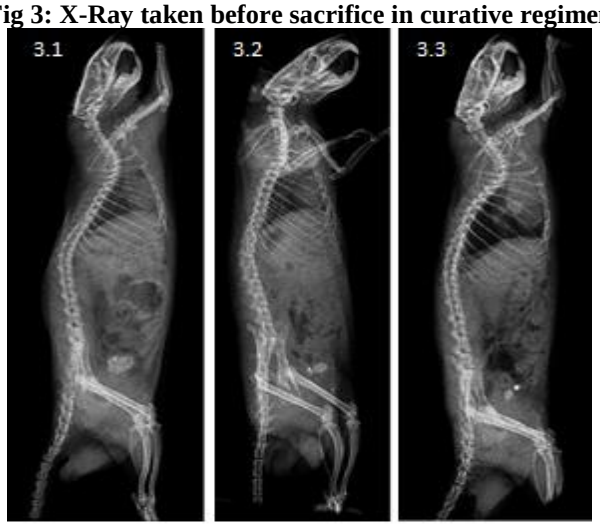
10,11,12: Stones harvested from preventive control. 13,14,15: Stones harvested from preventivetreatment dose 1. 16,17,18: Stones harvested from preventive treatment dose 2.

Fig 2: X-Ray taken immediately after zinc disc implantation



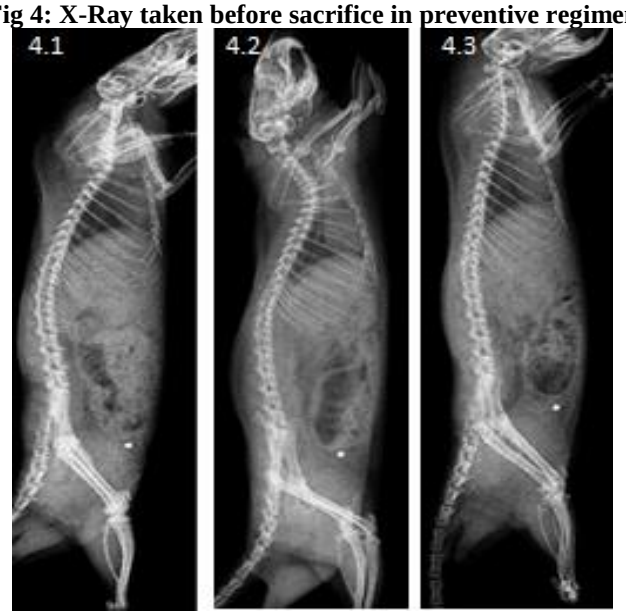
2.1: Curative control. 2.2: Curative treatment dose 1. 2.3: Curative treatment dose 2. 2.4: Preventive control. 2.5: Preventive treatment dose 1. 2.6: Preventive treatment dose 2

Fig 3: X-Ray taken before sacrifice in curative regimen



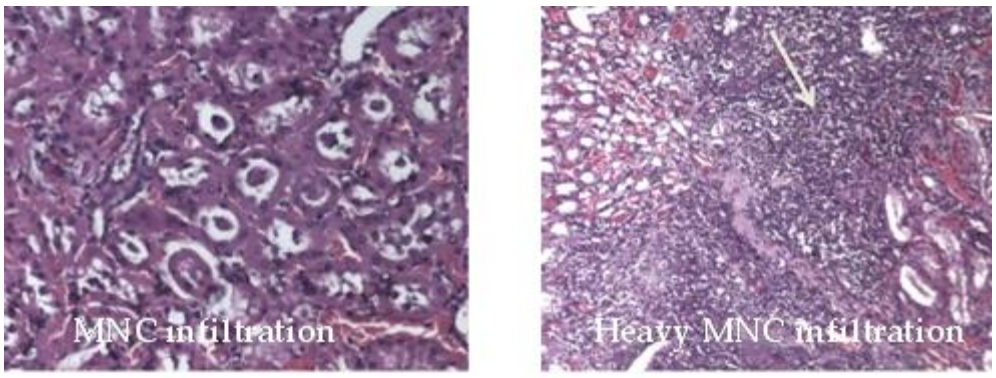
3.1: Curative control. 3.2: Curative treatment dose 1. 3.3: Curative treatment dose 2

Fig 4: X-Ray taken before sacrifice in preventive regimen

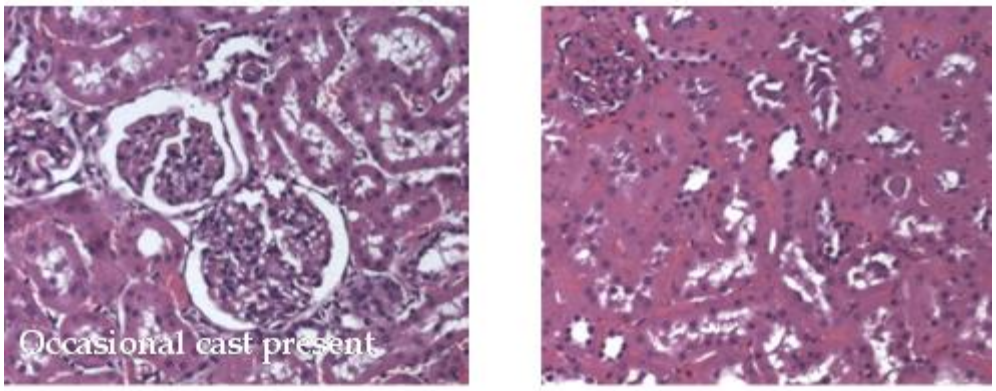


4.1: Preventive control. 4.2: Preventive treatment dose 1. 4.3: Preventive treatment dose 2

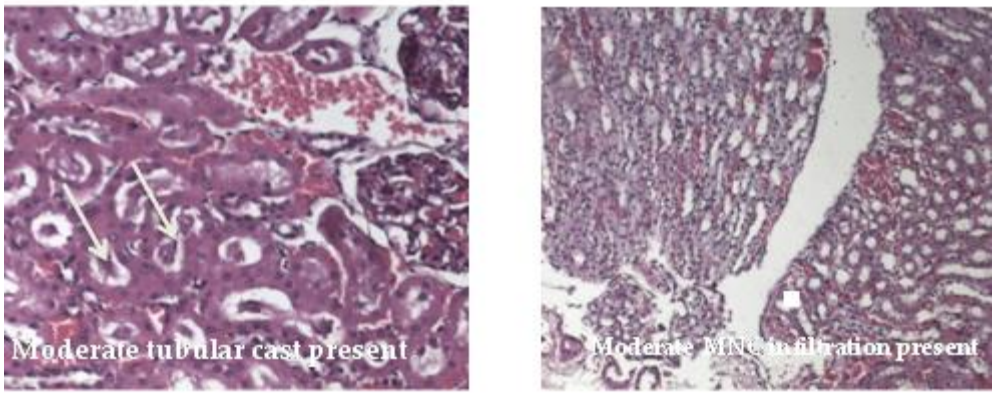
Fig 5: Histopathology of Renal tissues in curative regimen



Curative control

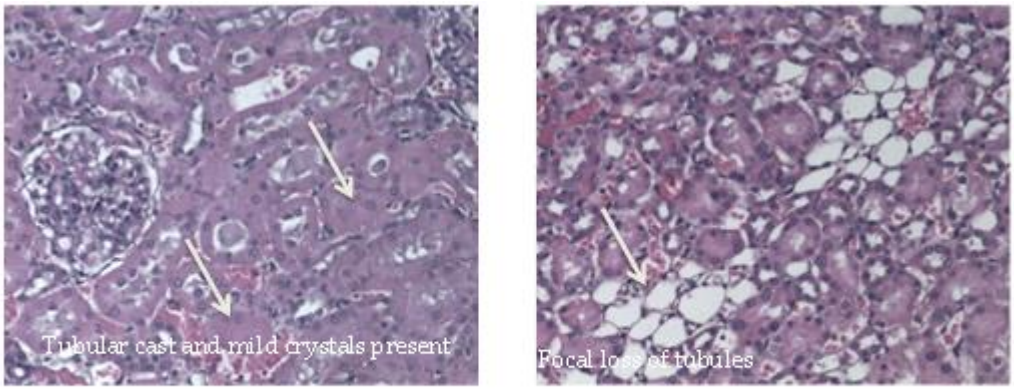


Curative treatment dose 1

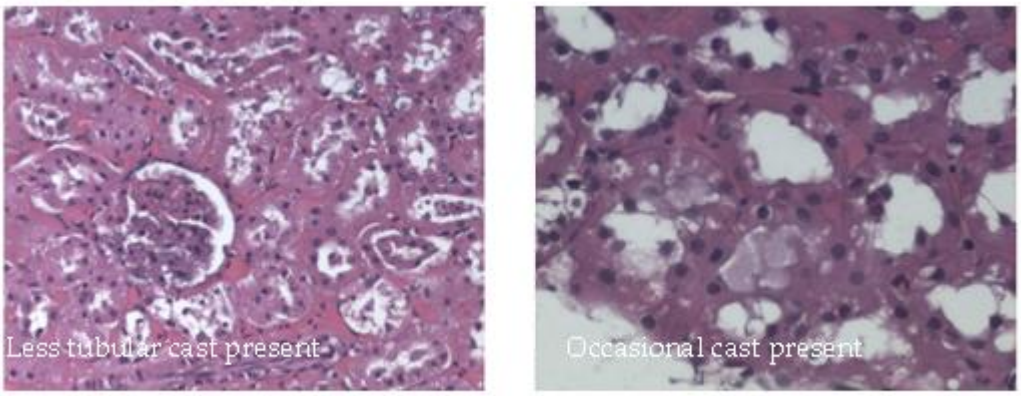


Curative treatment dose 2

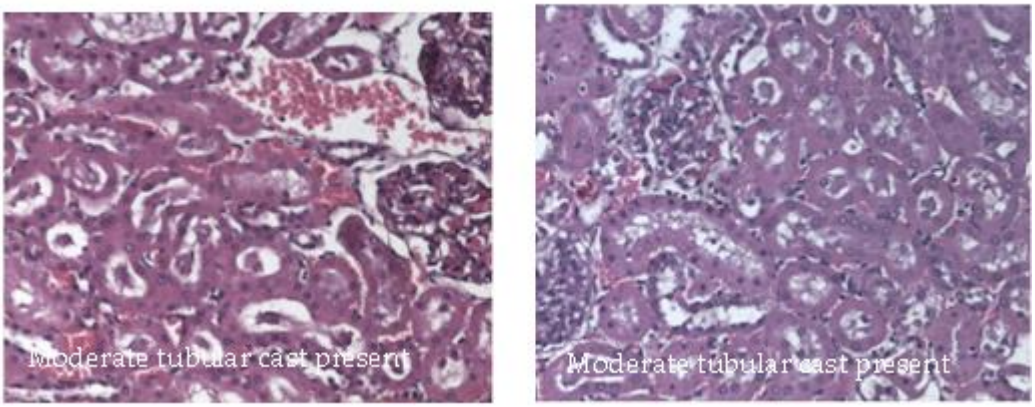
Fig 5.1: Histopathology of Renal tissues in preventive regimen



Preventive control

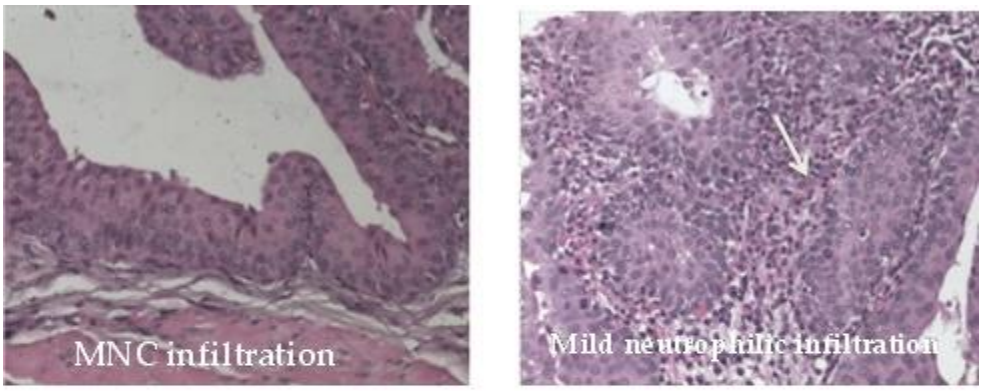


Preventive treatment dose 1

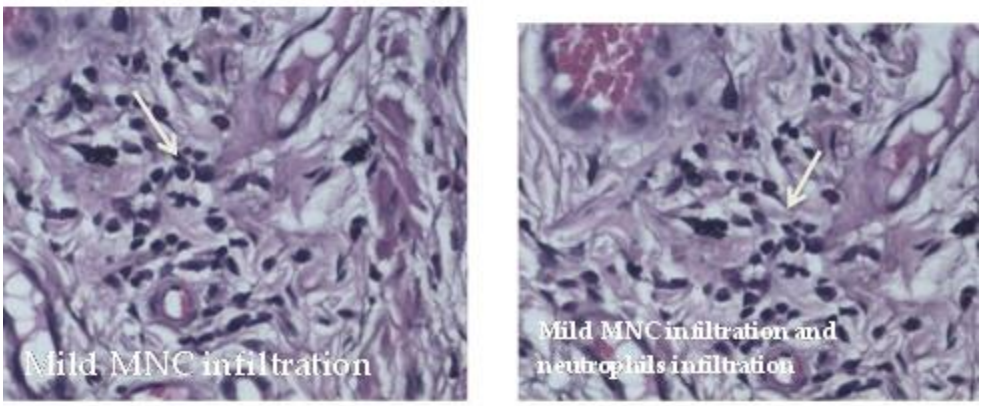


Preventive treatment dose 2

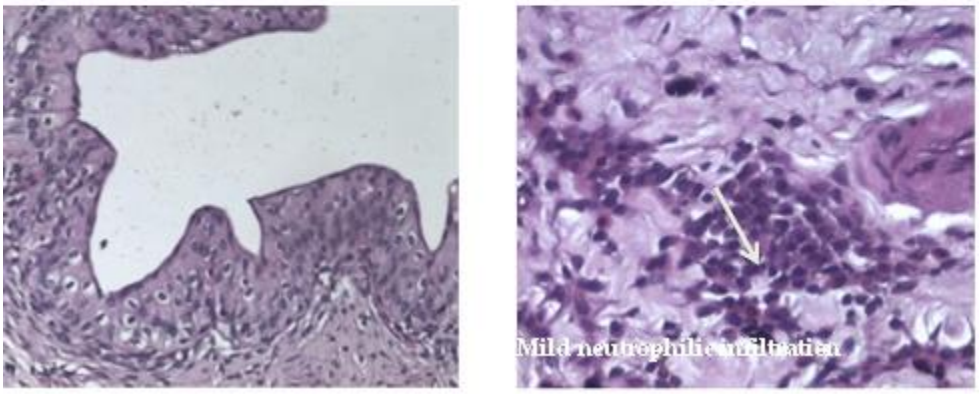
Fig 6: Histopathology of Bladder tissues in curative regimen



Curative control

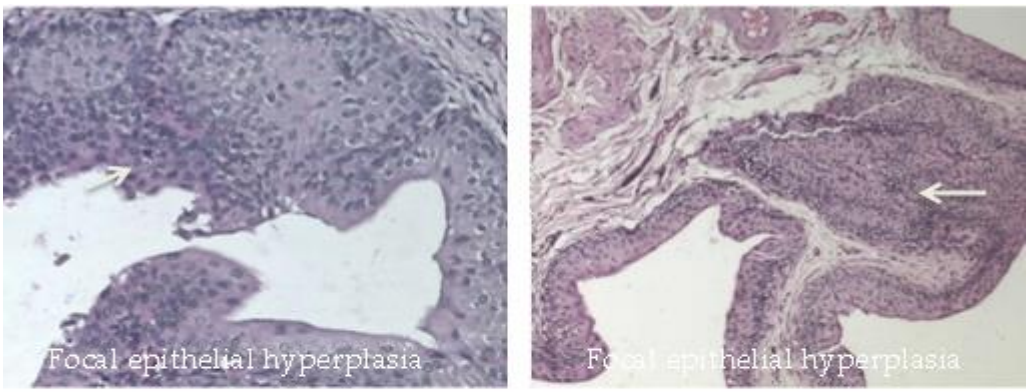


Curative treatment dose 1

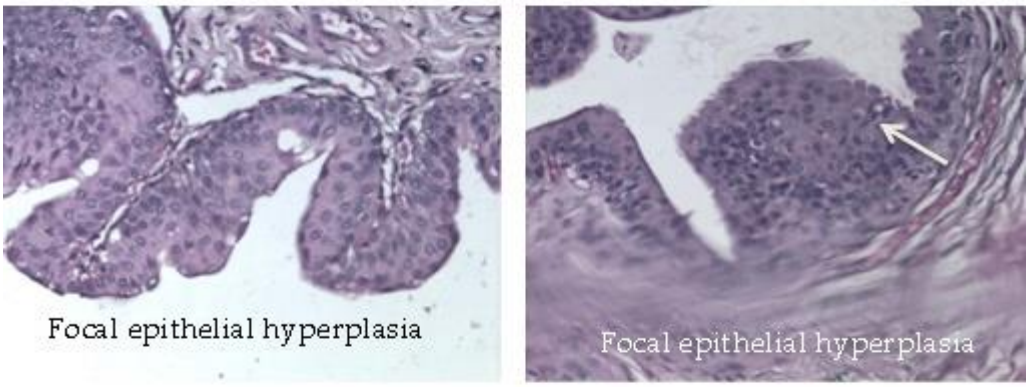


Curative treatment dose 2

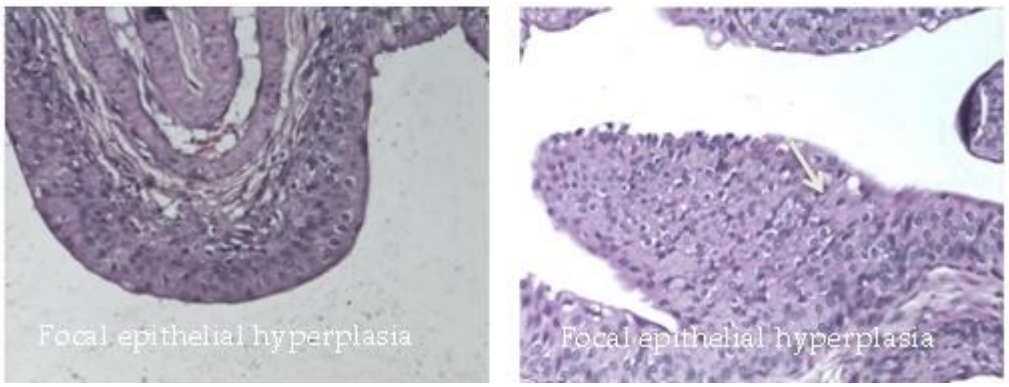
Fig 6.1: Histopathology of Renal tissues in preventive regimen



Preventive control



Preventive treatment dose 1



Preventive treatment dose 2

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