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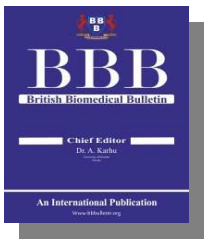

Original

Pharmacological Evaluation of Indigenous Antiurolithiatic Formulation Neeri (NRT-RF)

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A B S T R A C T

The Neeri NRT-RF was evaluated for its antiurolithiatic activity in rats. Calcium oxalate stones were induced by oral administration of ethylene glycol (0.75%) in male Wistar rats for 40 days, which is confirmed by the elevated level of several urinary parameters (calcium, uric acid, magnesium in urine and urine output), serum parameters (calcium, creatinine, phosphorus, uric acid, magnesium, blood urea nitrogen) and kidney parameters (calcium, magnesium, phosphorus). Administration of Neeri NRT-RF for 25 days significantly decreased (to normal values) the elevated levels of serum, urine and kidney parameters and also prevented the deposition of crystals in tubules. Moreover, it showed antioxidant effect. In addition to this, NRT-RF (530.55 mg/kg) shows better results as compared to the standard drug disodium hydrogen citrate. Furthermore, histopathology also revealed that NRT-RF treated group of rats retained no or very less crystals deposition in the tissue, as compared to ethylene glycol treated group.

Introduction

Urolithiasis refers to the hard crystalline minerals formed within the urinary tract[1]. It is a common chronic disorder after the hypertension[2] and affects 10-15% population worldwide[3]. Calcium containing stones (calcium oxalate, calcium phosphate) represent 80% of kidney stones while remaining 10% are composed of struvite, 9% of uric acid (UA); and the remaining 1% that of cystine[4]. Kidney stone cause disabling pain, known as renal colic, which often present when stone moves from renal pelvis into ureter[5]. Moreover stone results in bloody or foul smelling urine, nausea, vomiting like symptoms[1]. Urolithiasis is a multicomplex process that results from supersaturation of solute in urine, nucleation, growth, aggregation and retention within the renal tubules[6]. Supersaturation occurs when concentration of stone materials is higher in urine[5]. There are many substances in the body known as promoters, which promote the crystal growth, while inhibitors inhibit the crystal growth[7]. The occurrence of stone formation is also due to imbalance between the promoter and inhibitors in the kidney[8]. The most common cause of calcium oxalate stone is Hyperoxaluria[3]. In rat experimental model of urolithiasis ethylene glycol is taken as a metabolic precursor of oxalate production which results in hyperoxaluria[9]. Currently no allopathic medicine is available for the treatment of kidney stone and the option is surgical procedure or high power laser, but these procedures are costly and is remained associated with high recurrence of stone formation[10].

A number of herbs are used now a days for curing various ailments due to high benefits to risk ratio e.g. tamarind, sesame for anxiolytic & anti-depressant activity[11-13], *Premna herbacea* for antitumor and antimicrobial activity[14-16]. Likewise there is plethora of Ayurvedic plants which have been claimed for their antiurolithiatic properties[17]. Neeri (NRT-RF) is a polyherbal antiurolithiatic formulation which consists of number of principal herbs (**Table 1**). These herbs are already mentioned for their antiurolithiatic properties and the effectiveness of Neeri has been determined by *in-vivo* model of ethylene glycol induced calcium oxalate stone in Wistar rats.

Materials and Methods

Chemicals

Neeri (NRT-RF), a formulation, licensed from State Licensing Authority was procured from Aimil Pharmaceuticals India Ltd., India. Ethylene glycol (Batch no. S11S610462) was obtained from Merck Specialties private limited, India, while Citralka was procured from commercial source (Pfizer Ltd., India). Rest of the chemicals used in the experiment were of analytical grade.

Animals

Healthy adult male Wistar rats of age between 4-5 months, weighing 150-250g were procured from Panacea Biotec Ltd., Lalru (14050) India and housed in polypropylene cage. They were maintained under standard laboratory conditions (temperature 25±2º C with 12/12h night/dark cycle), fed with standard pellet diet (Sanjay Biological Museum, Amritsar) and water *ad libitum.* The experimental protocol was approved by IAEC [Protocol No. IAEC-/2012/III/0018 (PCL-M)]. The animals were acclimatized for 10 days before carrying out the experiment. The present animal experimentation work had been done as per the guidelines of CPCSEA.

Phytochemical screening

Preliminary phytochemical screening[18] was carried out to detect the presence of flavonoids (Lead acetate & Sodium hydroxide Tests), glycosides (Keller

Killani test, Borntrager test & Legal test), steroids (Salkowaski reaction, Liberman's reaction & Liberman's Burchard reaction), alkaloids (Mayer's test & Murexide test), tannins (5% $FeCl_3$ & Dilute HNO_3 tests), carbohydrates (Fehling's test & Benedict's test), proteins and amino acids (Millon's test, Xanthoprotein test & Ninhydrin test). Depending upon the plant ingredients listed on the label of the formulation.

Experimental design

Ethylene glycol induced hyperoxaluria method[9] was used to assess the Antiurolithiatic activity in Albino Wistar rats. Animals were divided into 5 groups with 5 animals in each group. Group I served as normal and received regular standard pellet diet and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was administered to group II to V for 40 days to induce renal calculi. Group III received oral administration of standard drug Citralka (137.7mg/kg b.w.) *p.o.*, from 15th day till 40th day, group IV and V received 357.7 and 530.55 mg/kg oral dose of test drug (Neeri) respectively, from 15th day[19] till 40th day. The dosages were calculated on the basis of conversion of average of human dose to rat dose. The regimen was based on human dose of neeri. Experimental design has been shown in **Table 2**.

Urine collection and analysis

A 24 hour urine sample was collected from each group of rats using metabolic cages (Orchid scientific and innovative Pvt. Ltd. Nashik, India) on 40th day after administration of Neeri NRT-RF polyherbal formulation. Urine output was measured. The urine was centrifuged at 3000 rpm for 10 minutes and then filtered with Whatman filter paper and then urine analysis was done for estimation of uric acid (Modified Trinder Method)[20], calcium (O–Cresolpthalein Complexone Method)[21], and magnesium (Xylidyl blue Method)[22] using diagnostic kits (ERBA) and measured by semi auto analyzer (ERBA Chem Touch, Transasia Biomedical Limited, Mumbai, India).

Serum analysis

The serum was obtained for biochemical analysis on 40th day. The animals were anesthetized with diethyl ether and the blood was collected from retro orbital sinus. Serum was separated by centrifugation of blood at 3000 rpm, 4 ^{o}C for 10 minutes (VCCH-3214, Semi Elektro Technik Limited, India) and analyzed for calcium[21], creatinine (Jaffe's method)[23], uric acid[20] magnesium[22], phosphorus (ammonium molybdate method)[24], BUN (GLDH–UREASE method)[25] using diagnostic kits ERBA and measured by semi auto analyzer.

Antioxidant activity

In vivo oxidative stress was assessed by measuring lipid peroxidation levels (marker of oxidative stress) in blood[26-28] collected at the end of 40th day.

Kidney harvest and measurement

At the end of experiment, rats were sacrificed with deep anesthesia. Both the kidneys were removed. Isolated kidneys were cleaned off extraneous tissue and then weighed. Right kidney preserved in 10% neutral formalin and used for histopathological studies while the left kidney was dried at 80°C in hot air oven, after which the kidney was weighed and used for preparation of kidney tissue homogenate for estimation of calcium, phosphorus and magnesium contents in the kidney tissue[29,30].

Preparation of kidney tissue homogenate

A sample of 100mg kidney was taken and homogenized by homogenizer (Popular traders, Ambala, India) and dissolved in 1N HCl overnight. The homogenate was centrifuged at 2000 rpm for 10 min and supernatant was separated. The calcium, phosphorus, magnesium content in kidney homogenate was determined by auto analyzer[19].

Histopathological examination of kidney

The kidney was weighed and washed with cold saline and preserved in10% buffered formalin. After embedding in paraffin 4 micron sections were cut in saline and stained with hematoxylin and eosin. The slides were examined under a polarizing light microscope Olympus, India.

Statistical analysis

The results were expressed as mean ± SEM and analyzed using Dunnett's multiple comparison tests. Data was computed for statistical analysis using GraphPad Prism Software and $p < 0.05$ was considered to be statistically significant.

Results

Phytochemical analysis data

In this experimental study, qualitative phytochemical analysis NRT-RF showed the presence of glycosides, tannins, flavonoids and steroids **(Table 3)**.

Urinary data

Various physical parameters were measured including urinary output at the end of the treatment. The volume of the urine significantly lowered in the calculi induced (Group II) animals in comparison to normal control. The treatment with Neeri (NRT-RF) (IV–V Group) and standard drug Citralka (Group III) increased the volume of the urine in calculi induced rats as compared to the Group II. Gross increase in the urinary excretion of calcium and uric acid was observed in calculi control animals but on another side excretion of magnesium fell down. However supplementation with Neeri NRT-RF (357.7 mg/kg and 530.55 mg/kg) and standard drug reversed these changes in the urinary excretion **(Table 4 and Figure 1 A-D)**. Urine output and calcium level results also showed that there is insignificant difference between standard drug Citralka and Neeri NRT-RF (357.7 mg/kg) & (530.55 mg/kg) but it is important to note that the difference between Neeri NRT-RF (357.7 mg/kg) & (530.55 mg/kg) was significant ($p<0.05$). Level of uric acid result was showing Neeri NRT-RF (530.55 mg/kg) statistically better than the standard while its effect in comparison to its lower dose i.e (357.7 mg/kg) is not significant while lower dose is having no difference than standard. The level of magnesium was statistically similar in standard drug Citralka, Neeri NRT-RF (357.7 mg/kg) and (530.55 mg/kg) treatment.

Serum data

Level of serum calcium, uric acid, phosphorus, creatinine, BUN raised in calculi induced groups. Both Neeri NRT-RF (dose dependently) and standard drug treated groups, lowered the elevated level of serum parameters. Likewise magnesium level was reduced in calculi induced groups but increased in Neeri NRT-RF and standard drug treated rats. Serum data have been shown in **Table 5 and Figure 2 A-F**.

Kidney homogenate data

Table 6 and Figure 3A-C showed that the level of calcium and phosphorus increased and magnesium level decreased in calculi induced animal group. In Neeri NRT-RF and standard drug treated rats level of calcium and phosphorus decreased while that of magnesium increased.

Antioxidant study data

Antioxidant study data **(Table 7 and Figure 4)** revealed the increased lipid peroxidation and decreased level of antioxidant potential in the kidney of rats supplemented with ethylene glycol. A simultaneous dose dependent treatment with Neeri NRT-RF and standard drug protected against lipid peroxidation induced renal damage in rats of group of III-V, i.e. Neeri and standard drug treated animals.

Histopathological data

Section of kidney from ethylene glycol treated showed deposition of large sized calcium oxalate crystals in the renal tubules which cause marked dilation of proximal tubules. However kidney section of rats treated with Neeri NRT-RF (530.55mg/kg) showed the character similar to the normal. There was no presence of crystals and dilations in section of Group V NRT-RF (530.55 mg/kg). Neeri NRT-RF (357.7 mg/kg) showed the presence of dilation of tubules along with the reduction of calculi size in section Group IV NRT-RF (357. 7 mg/kg). There was no crystal deposition but inflammation of tubules was observed in kidney section of standard drug treated rats **Figure 5**.

In most results, Neeri NRT-RF (530.55 mg/kg) showed statistically better effect than standard drug. On the other hand lower dose Neeri NRT-RF (357.7 mg/kg) results were comparable (statistically) with standard drug.

Discussion

NRT-RF consists of number of plants which have the antiurolithiatic activity, for example *Bergenia ligulata* (BLR) inhibits the deposition of Calcium oxalate in the renal tubules. BLR also shows diuretic, hypermagnesuric effect and also have antioxidant effect[31]. *Boerhaavia diffusa* Linn. possesses antiurolithiatic activity, that possibly is mediated through diuretic and hypo-oxaluric effects. It causes diuresis and hastens the process of dissolving the preformed crystals and helps in mechanical expulsion of the stone, improve the renal function by increasing the removal of waste products and decreases the oxalate excretion probably by interfering with metabolism[32]. Aqueous extract of *Butea monosperma* shows antilithiatic effect. It reduces elevated concentration of calcium and oxalate ion and increase the concentration of magnesium in urine[33]. Yahood Hajrul Bhasam increases the level of inhibitor, decreases the level of promoters and growth of calcium oxalate crystals[34]. Administration of lupeol and betulin, isolated from *Crataeva nurvala* stem bark, to hyperoxaluric rats minimized the tubular damage and reduced crystal deposition in the kidneys. Lupeol minimizes the risk of stone formation in experimental lithogenic animals by preventing oxalate crystal induced per-oxidative in renal tissues and reversing increase urinary excretion of oxalate associated with reduction in citrate and glycosaminoglycans. Administration of *C. nurvala* decoction in experimental models reduces urinary calcium excretion[35]. The administration of *Vernonia cinerea* to mice significantly showed antioxidant effect. *Achyranthes aspera* shows its effect by decreasing the size of crystals and quick expulsion of stones[36]. The seeds of *Hordeum vulgare* decrease the elevated level of serum and urine crystalloids[37]. *M. pudica* leaf extract has been used in various urogenital infections[38]. *Tribulus terrestris* was investigated on nucleation and the growth of the calcium oxalate (CaOx) crystals, as well as on oxalate induced cell injury of NRK 52E renal epithelial cells[39]. Shilajeet has effect on calculi[40]. All the biomolecules involved in Neeri act as potential inhibitors of initial stage of mineralization and

normalize crystalloid-colloid imbalance to prevent renal calculi recurrence.

In urine there are numerous crystalloids like oxalate, uric acid, calcium, cystine which remain in solution due to the existence of colloids i.e. mucin and sulphuric acid in the urine. Increase in crystalloid and fall in colloid level creates an imbalance in crystalloid-colloid ratio, resulting in formation of stone. Furthermore, if colloid loses its solvent or adhesive property, it may also lead to calculi formation[41].

The consequence of supersaturation of these urinary crystalloids is precipitation in the form of an initial particle for crystal growth, which when trapped act as a nidus leading to subsequent crystal growth[42,43]. Biomolecules of Neeri maintain this crystalloid-colloid balance by reducing excretion of urinary calcium, phosphorous, uric acid in conditions of urolithiasis. While, magnesium concentration in kidney is increased in group treated with Neeri, which act as strong calculi inhibitor. Chronic UTI may also lead to adversely affecting colloid secretion from tubules thereby leading to poor renal functioning and calculus nucleation. In the present study, rats were selected for the induction of urolithiasis, because urinary system of rats resemble to that of humans[44]. Hyperoxaluria was induced in animals by chronic administration of ethylene glycol in the drinking water[19] for 40 days, which ultimately results in the development of kidney stones. Test drug Neeri showed antioxidant activity. It also prevented calcium oxalate crystal formation, which might be due to presence of phenol, flavonoid and saponin components from natural drugs. In case of calculi induced animals (Group II) i.e. only ethylene glycol treated group there was decline in level of magnesium but Neeri NRT-RF treated rats (i.e. Group-IV & V) and standard (Disodium hydrogen citrate) treated rats (Group-III) showed increased level of magnesium. In addition to this, the test drug Neeri NRT-RF also increased the urine volume**.** Renal damage occurred in having elevated serum level of Creatinine, uric acid and BUN (Blood urea nitrogen). However, Neeri NRT-RF caused diuresis and hastened the process of dissolving stone and stone expulsion. It was found that there was increase in level of calcium and phosphorus in the tissue of group II i.e. only ethylene glycol treated rats. Treatment with Neeri NRT-RF suppressed the intracellular accumulation of calcium and phosphorus in kidney tissue.

Exposure of renal cells to oxalate (Ox) either alone or in combination with calcium, leads to free radical generation. Free radicals cause oxidative stress, followed by renal injury and inflammation, playing major role in stone formation. Oxidative stress due to lipid peroxidation induces loss of membrane integrity, which helps in the retention of calcium oxalate crystals and growth of stones in renal tubules. Urinary excretion of malondialdehyde (MDA) is considered as a marker of renal epithelial cell injury[45]. This study revealed the increased lipid peroxidation and decreased levels of antioxidant potential in the kidneys of rats supplemented with ethylene glycol. Oxalate, the major stone forming constituent, has been reported to induce lipid peroxidation. Tannins, flavonoids, glycosides, carbohydrates, steroid compounds present in the Neeri NRT-RF may prevent the lipid peroxidation-induced renal damage caused by calcium oxalate crystal attachment as well as stone formation. Neeri NRT-RF treatment produced significant decrease in calcium, phosphorus, uric acid, creatinine and significant increase in magnesium level. In addition to this, it improves the urinary parameters and urinary output. Neeri NRT-

RF at dose of 530.55 mg/kg showed much better result than standard drug disodium hydrogen citrate, when compared with respect to calcium, phosphorus, uric acid, creatinine level in serum, urine and kidney. Similarly the higher dose of Neeri NRT-RF (530.55 mg/kg) has shown the effects better than lower dose (357.7 mg/kg) (equivalent in some cases) but not related to concentration level. Seeking to consideration of all findings, dosage of 357.7 mg/kg may be chosen as a drug of choice. In severe conditions increased dose of Neeri NRT-RF can be administered. The above results are further supported by histopathological examination of kidney section subjected to ethylene glycol induced urolithiasis in rat. Kidney of this group showed polymorphic irregular crystal deposition inside the tubules while the Neeri NRT-RF treated group of rats showed no or insignifican crystals deposition in the tissue.

Conclusion

Neeri NRT-RF showed significant antiurolithiatic effect in *in-vivo* studies. The observed effect of Neeri NRT-RF may be due to presence of tannins, flavonoids, glycosides, carbohydrates, steroid compounds from the natural resources having biosynergism, which may prevent the lipid peroxidation-induced renal damage caused by calcium oxalate crystal attachment as well as stone formation and may tone the tubule functioning. Neeri NRT-RF treatment significantly decreased serum calcium, phosphorus, uric acid, creatinine level and significantly increased the magnesium level in serum, which is of utmost advantage in cases of urolithiasis. Further, Neeri NRT -RF (530.55 mg/kg) results in more pronounced antiurolithiatic effect as compared to standard drug Disodium hydrogen citrate, which might be due to additive/synergistic interaction of different herbal drugs present in Neeri NRT-RF having diuretic, antiurolithiatic and antioxidant potential. The results are more significant and better tolerated in Neeri treated group than the standard drug Disodium hydrogen citrate.

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Table 1. Neeri (NRT-RF) Formulation comprises of combination of enlisted ingredients based on ancient concept of Ayurveda (As per label claim)

S. No.	Composition of Neeri NRT-RF	Latin name
1	Pashanbhed	*Bergenia ligulata*
2	Punernava	*Boerhaavia diffusa*
3	Palash Pushap	*Butea monosperma*
4	Hajrul yahood Bhasam (RTS-1)	Classical preparation
5	Swait Parpati (A.F.I.)	Classical preparation
6	Varun Ghan	*Crataeva nurvala*
7	Sahdevi	*Vernonia cinerea*
8	Appamarg	*Achyranthes aspera*
9	Gokhru	*Tribulus terrestris*
10	Sudh Shilajeet (RTS-1)	*Purified asphaltum* (black bitumen-ooze of decayed vegetable matters from rock lefts)
11	Lajaloo Mool	*Mimosa pudica* L
12	Yavakshar	Water soluble ash of *Hordeum vulgare*
13	Excipients	*q.s*
14	Processed in the decoction	*Pashanbhed, Appamarg, Gokhru, Lajaloo* Maddarmool, Kulthi

Table 2. Experimental design of ethylene glycol model

Groups	Treatment
Group I	Received regular standard pellet diet and drinking water ad libitum for 40 days.
Group II	Received, ethylene glycol (0.75% orally) in drinking water, daily for 40 days.
Group III	Received standard Antiurolithiatic drug Disodium hydrogen citrate (Di Sod. H citrate) at dose of (137.7mg/kg b.w.) *p.o.* from 15^{th} to 40^{th} day, in addition to ethylene glycol (0.75%) daily, from day 0 to day 40.
Group IV	Received test drug Neeri (NRT-RF) at a dose of 357. 7 mg/kg from 15^{th} to 40^{th} days, in addition to ethylene glycol (0.75%) daily from day 0 to day 40.
Group V	Received test drug Neeri (NRT-RF) at a dose of 530.55 mg/kg from 15^{th} to 40^{th} days, in addition to ethylene glycol (0.75%) daily from day 0 to day 40.

Each group consists of 5 rats (i.e. n = 5).

Table 3. Preliminary Phytochemical Screening of Neeri NRT-RF

S. No.	Test		Result
1	Steroids	(a) Salkowski reaction	Present
		(b) Liberman's reaction	Negative
		(c) Liberman's burchard reaction	Present
2	Glycoside	(a) Keller killani test	Present
		(b) Borntrager test	Present
		(c) Legal test	Present
3	Carbohydrate	(a) Fehling's test	Present
		(b) Benedict's test	Present
4	Alkaloids	(a) Mayer's test	Negative
		(b) Murexide test	Negative
5	Tannins	(a)5% $FeCl_3$	Present
		(b) Dilute HNO_3	Present
6	Proteins	(a) Millon's test	Negative
		(b) Xanthoprotein test	Negative
7	Amino acid	(a) Ninhydrin test	Negative
		(b) Millon's reagent test	Negative
8	Flavonoids	(a) Lead acetate	Present
		(b) Sodium hydroxide	Present

Table 4. Effect of Neeri NRT-RF on urine biochemistry of control and experimental animals

Parameters	Group I Normal control	Group II Calculi induced	Group III Di Sod. H Citrate 137.7 mg/kg	Group IV NRT-RF 357.7 mg/kg	Group V NRT-RF 530.55 mg/kg
Urine output (ml/day)	19.99±0.67	15.17±0.56	18.20±0.52*	16.40±0.63	19.10±0.5*
Calcium (mg/dl)	3.59±0.27	9.51±3.40	5.4±0.37*	6.31±0.33*	4.40±0.39*
Uric acid (mg/dl)	3.14±0.27	8.23±0.33	5.4±0.16*	5.0±0.18*	4.18±0.45*
Magnesium (mg/dl)	5.49±0.13	3.21±0.30	5.1±0.10*	4.95±0.11*	5.21±0.1*

Table 5. Effect of Neeri NRT-RF on serum biochemistry of control and experimental animals

Parameters (mg/dl)	Group I Normal control	Group II Calculi induced	Group III Di Sod. H Citrate 137.7 mg/kg	Group IV NRT-RF 357.7 mg/kg	Group V NRT-RF 530.55 mg/kg
Calcium	8.01±0.29*	14.1±0.56	12.5±0.25*	10.5±0.27*	9.15±0.38*
Uric acid	1.40±0.2*	4.53±0.44	1.7±0.22*	2.1±0.21*	1.1±1.0*
Magnesium	2.6±0.6*	1.62±0.8	2.3±0.09*	2.1 ±0.07*	2.0±0.09*
Phosphorus	5.03±0.57*	7.11±0.33	5.46±0.42*	6.06±0.23	4.96 ±0.45*
Creatinine	0.72±0.71*	1.61±0.26	1.0±0.04*	0.8±0.21	0.75±0.09*
BUN	32.7±1.3*	53.4±0.79	48.4±0.48*	40.6±0.65*	36.0±0.52*

Table 6. Effect of Neeri NRT-RF on kidney homogenate biochemistry of control and experimental animals

Parameters (mg/g)	Group I Normal control	Group II Calculi induced	Group III Di Sod. H Citrate 137.7 mg/kg	Group IV NRT-RF 357. 7 mg/kg	Group V NRT-RF 530.55 mg/kg
Calcium	9.76±0.16*	16.27±0.14	11.46±0.07*	11.8±0.10*	10.08±0.11*
Magnesium	3.90±0.34*	0.77±0.10	2.4±0.10*	1.9±0.15*	1.7±0.313*
Phosphorus	2.72±0.06*	3.847±0.09	3.1±0.06*	2.9±0.11*	2.74±0.08*

Table 7. Effect of Neeri NRT-RF on Lipid peroxidation level or Thiobarbituric acid reactive substances: TBARS

Parameters nano moles	Group I Normal control	Group II Calculi induced	Group III Di Sod. H Citrate 137.7 mg/kg	Group IV NRT-RF 357. 7 mg/kg	Group V NRT-RF 530.55 mg/kg
TBARS	0.101±0.86*	0.615±0.074	0.33±0.061	0.295±0.045*	0.245±0.027*

Values are expressed as mean ±SEM, (n=5), * represents P< 0.05 as compared to the calculi, Induced group, using one-way ANOVA followed by Dunnett's multiple comparison test.

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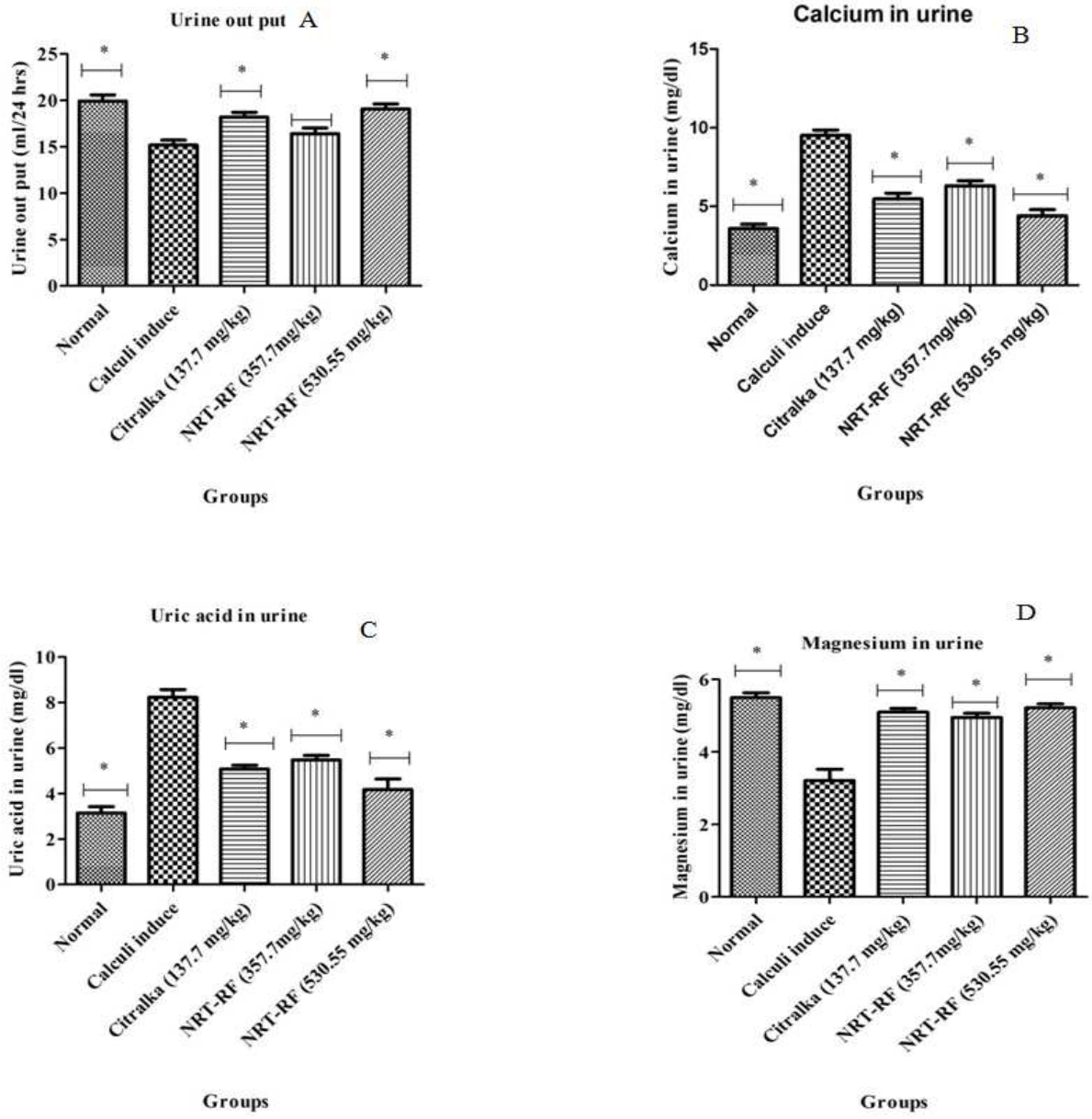

Figure 1. Effect of Neeri (NRT-RF) on A. urine output B. level of calcium C. level of uric acid and D. level of magnesium in urine

Values are expressed as mean ±SEM, (n=5), * represents P< 0.05 as compared to the calculi induced group, using one-way ANOVA followed by Dunnett's multiple comparison test.

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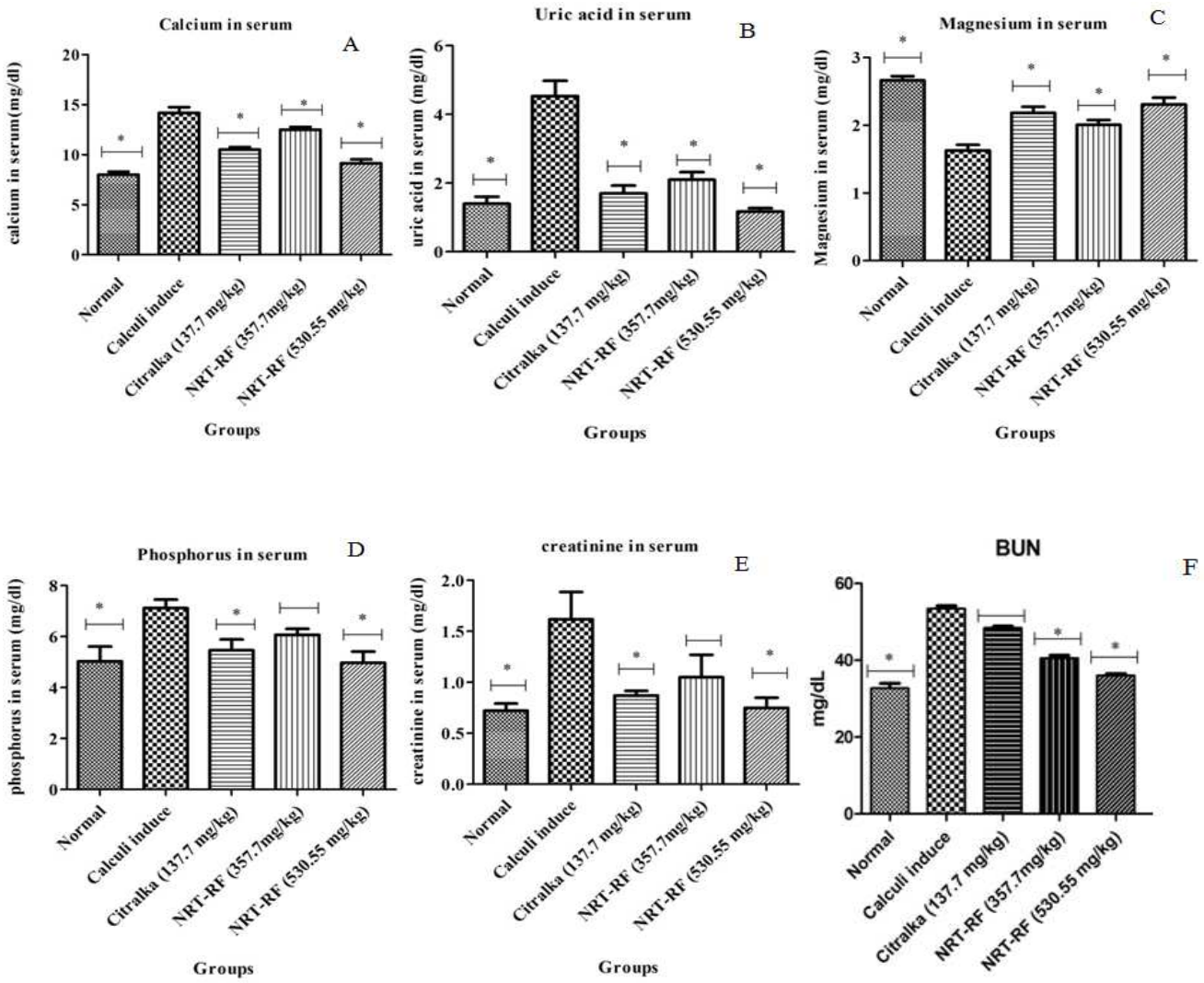

Figure 2. Effect of Neeri (NRT-RF) on level of A. calcium B. uric acid C. magnesium D. phosphorous E. creatinine F. blood urea nitrogen in serum

Values are expressed as mean ±SEM, (n=5), * represents P< 0.05 as compared to the calculi induced group, using one-way ANOVA followed by Dunnett's multiple comparison test.

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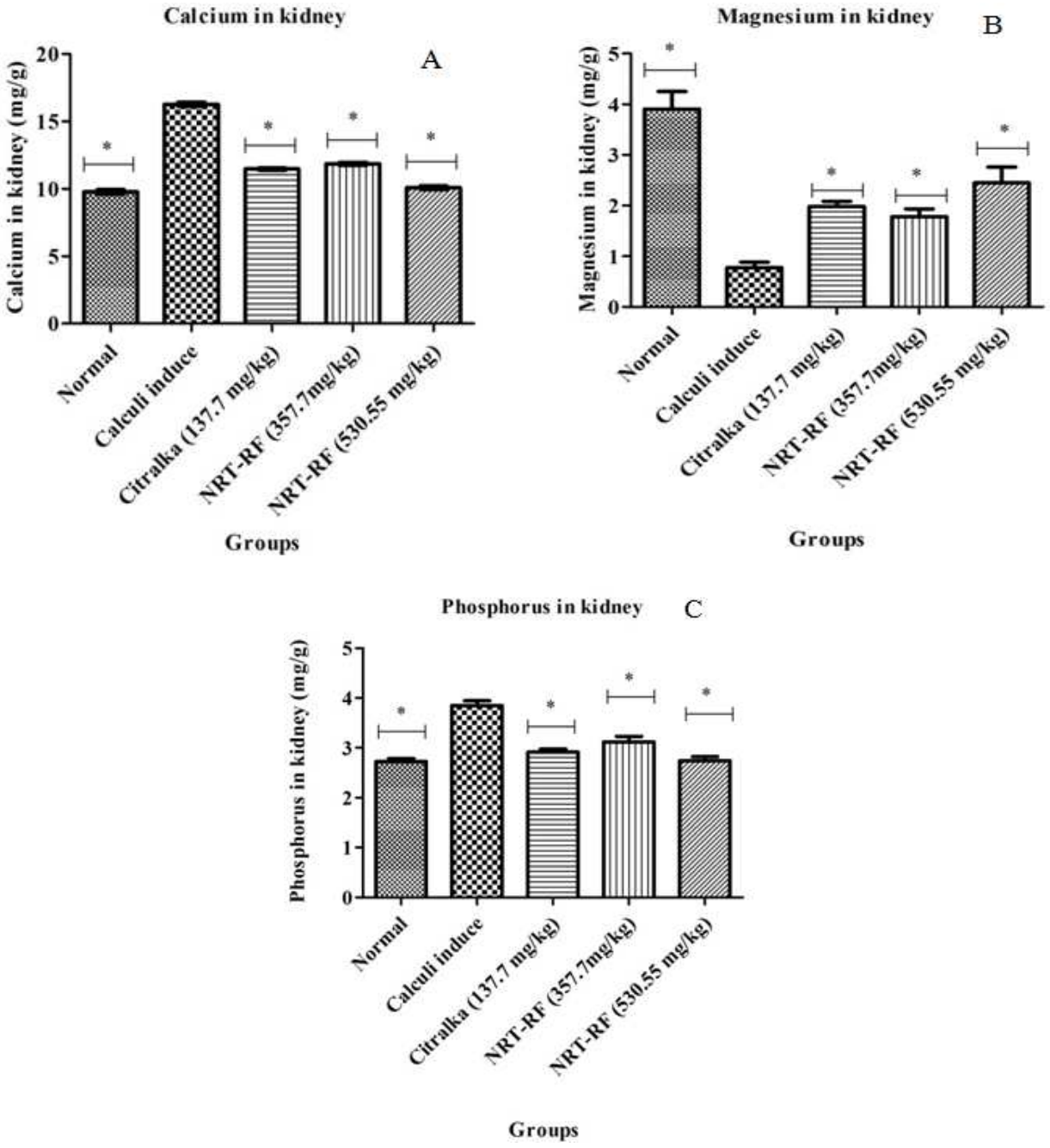

Figure 3. Effect of Neeri (NRT-RF) on level of A. calcium B. magnesium and C. phosphorus in kidney homogenate

Values are expressed as mean ±SEM, (n=5), * represents P< 0.05 as compared to the calculi induced group, using one-way ANOVA followed by Dunnett's multiple comparison test.

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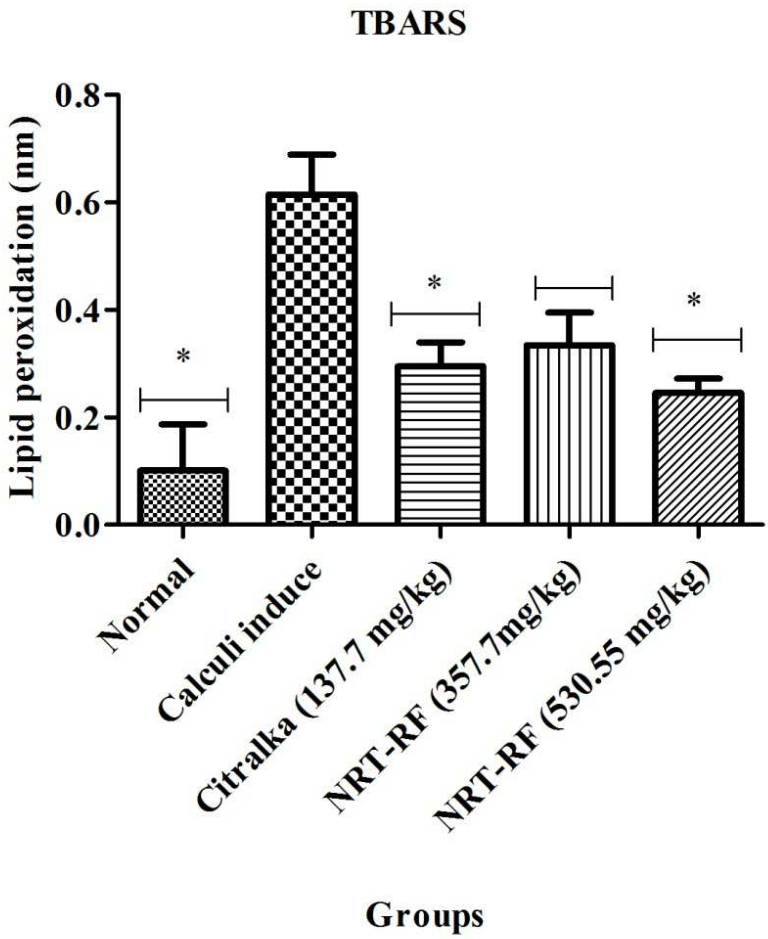

Figure 4. Effect of Neeri NRT-RF on Lipid peroxidation level (TBARS)

Values are expressed as mean ±SEM, (n=5), * represents P< 0.05 as compared to the calculi induced group, using one-way ANOVA followed by Dunnett's multiple comparison test.

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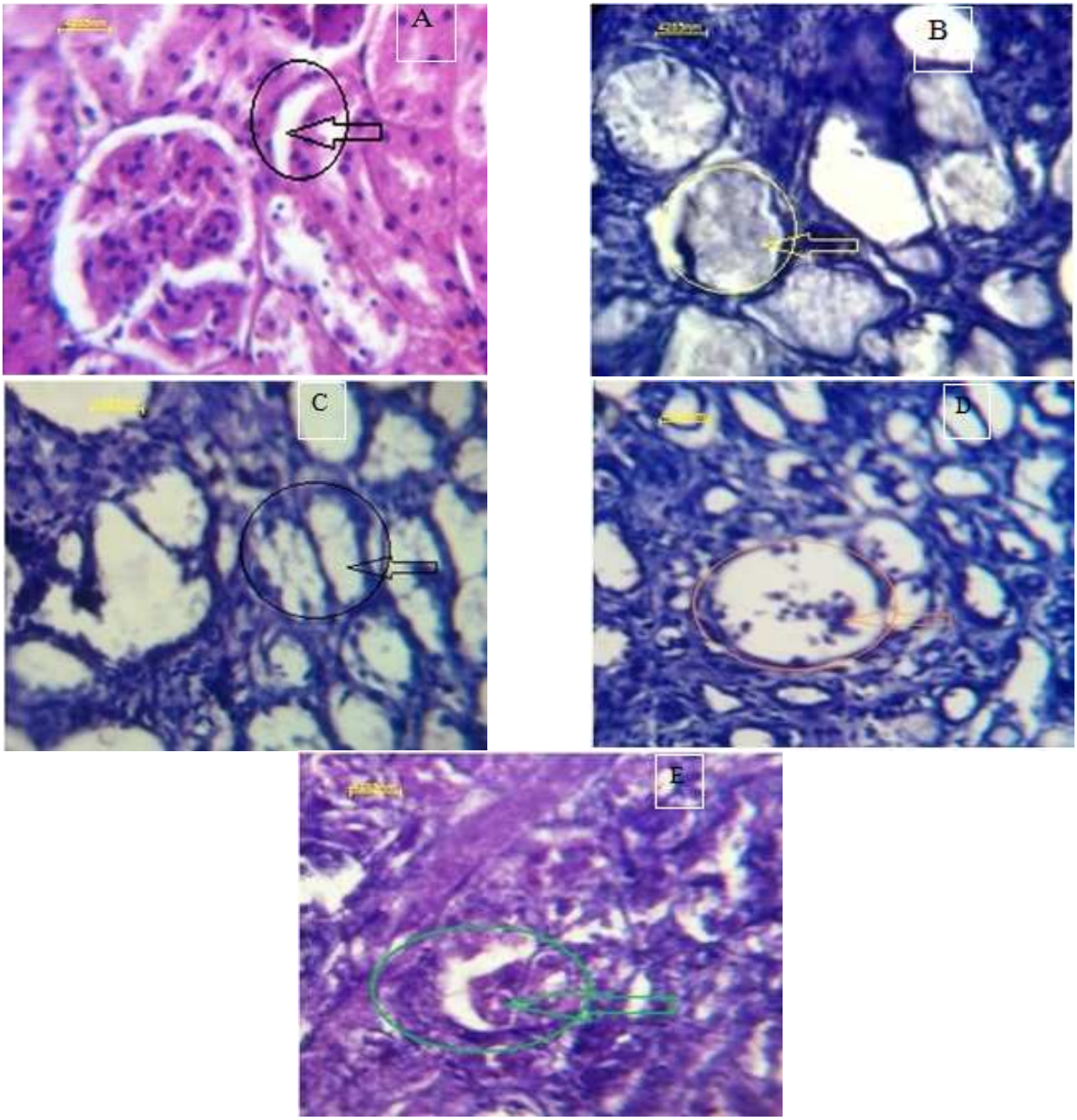

Figure 5. A. Group I Normal group showed absence of calcium oxalate (CaOX) and the normal tubular region, **B. Group II** calculi induced group showed the large size of calcium oxalate crystals in a renal tubule which cause dilation of the proximal tubule, **C. Group III** Di Sod. H. citrate (137.7) treated group showed the absence of calcium oxalate crystals but inflammation of tubules, **D. Group IV** Neeri NRT-RF (357.7mg/kg) treated group showed presence of dilation of tubules along with the reduction of calculi size and **E. Group V** Neeri NRT-RF (530.55mg/kg) treated group showed the character similar to the normal, No presence of crystals and dilation (45x)