IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2018 Vol.:12, Issue:2

Effect of Aqueous, Ethanol and Petroleum Ether Extracts of *Amaranthus viridis* L on Nephrolithiasic Wistar Rats

IJPPR
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ISSN 2349-7203
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Submission: 22 April 2018
Accepted: 28 April 2018
Published: 31 May 2018

Keywords: kidney stone, biochemical parameters, ethanol extract

ABSTRACT

Objective: The present work was intended to evaluate the effect of aqueous, ethanol and petroleum ether extracts of *Amaranthus viridis* L on nephrolithiasic Wistar rats. **Material and methods:** Animals were divided into nine groups of six rats. Renal calculi (Urolithiasis) were induced in rats by intraperitoneal injection of sodium oxalate at dose of 7 mg/100g body weight. Group 1, 2, 3 rats served as normal, sodium oxalate untreated and standard (Cystone 7mg/100g b.w) respectively. One hour, after administration of sodium oxalate, group 4, 5 were treated orally with aqueous extracts of *Amaranthus viridis* L at dose of 200 mg/kg, 400 mg/kg respectively, group 6,7 were received ethanol extracts of *Amaranthus viridis* L orally at dose of 200 mg/kg, 400 mg/kg respectively and petroleum extracts (200 mg/kg, 400 mg/kg) of *Amaranthus viridis* L were administrated orally into group 8, 9 at dose of 200 mg/kg, 400 mg/kg respectively. On 8th day, animals were sacrificed, kidneys were removed and subjected to histopathological evaluation to observe the renal tubular damage which caused by deposition of crystals. Serum samples were estimated for biochemical parameters such as sodium, potassium, calcium, creatinine, magnesium, uric acid and oxalates. **Results:** Sodium oxalate administration crucially increased serum level of sodium, potassium, creatinine, magnesium, uric acid and oxalates but declined serum level of calcium. All treated gzzzzzzroups reinstated these biochemical serum levels towards normal. The group seven which was treated with ethanol extracts of *Amaranthus viridis* L at dose of 400 mg/kg manifested significant anti-urolithiatic activity ($p < 0.001$). Histopathology evaluation displayed that daily oral treatment with all extracts of *Amaranthus viridis* L significantly decreased calcium oxalate crystal deposition in the kidneys. The magnificent improvement in architecture of kidney was found in group treated with aqueous extracts of *Amaranthus viridis* L. **Conclusion:** The present investigation evinced that ethanol extracts of *Amaranthus viridis* L have potential anti-urolithiatic activity; hence it can be used as preventive compounds in herbal formulation against kidney stones.

INTRODUCTION

Nephrolithiasis or renal stone the formation of stones in the urinary tract that is most painful afflictions of urinary tract that can cause bleeding and moreover may lead to secondary infection. It is one of the third most common ailments found in human beings.[1] For management of nephrolithiasis, surgical operation, shock wave lithotripsy and local calculus disruption using high-power laser, medicinal treatment such as thiazide diuretics, alkali-citrate and antibiotics are extensively used to remove the calculi.[2] These procedures are relatively costly and painful[3]. with undesirable side effects such as tubular necrosis, haemorrhage, hypertension and subsequent fibrosis of the kidney leading to cell injury and recurrence of formation of renal stone is common.[2] In Ayurveda traditional system, some plants such as *Cedrus deodara*, *Paronychia argentea*, *Crataeva nurvala*, *Costus spiralis Roscoe*, *Trachyspermum ammi*, etc., were reported to be effective in diminishing the recurrence of renal calculi with less side effects.[4,5] *Amaranthus viridis* L belongs to family *Amaranthaceae* commonly, it also is called as "Jangali chaulai" and "Green amaranth" in Ayurveda and English respectively. Traditionally, *Amaranthus viridis* L was used as vermifuge, diuretic, analgesic and for improving appetite.[6,7] Hence, above evidence had prompted us to conduct investigation for anti-urolithiatic activity of aqueous, ethanol and petroleum ether extracts of *Amaranthus viridis* L in Wistar rats.

MATERIALS AND METHODS

Plant material and Preparation of extracts

The roots of *Amaranthus viridis* L were collected from Chennai, Tamil Nadu, India and authenticated by Amruta herbals company, Indore, Madhya Pradesh, India, a voucher specimen (AV-GRC-010) were preserved for future references. The roots materials (500 g) were dried, powdered mechanically and the leaves powder was macerated in the solvents including water, ethanol 95% (v/v) and petroleum ether that undergoing mechanical shaking for 8 hours followed by filtration. The filtrate was evaporated at 60^{o}C in a vacuum dryer and the percentage yield of extracts is as follows: Aqueous: 23% w/w, Ethanol 10% w/w, petroleum ether: 3% w/w. The extract was suspended in distilled water using 1% acacia as suspending agent for oral administration to animals.

Animals

Healthy male adult Wistar rats weighing 200±20 g was obtained from the Central Animal Facilities of St. John's Pharmacy College, Bangalore. All the animals were maintained under standard husbandry conditions, i.e. room temperature of 25 ± 1°C; relative humidity 45-55% and a 12:12 h light/ dark cycle. The animals had free access to standard rat pellet (Pranav Agro Industries Ltd, Bangalore, India), with water supplied *ad libitum* under strict hygienic conditions. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), St. John's Pharmacy College, Bangalore and the experiments were conducted according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Treatment designed

Calcium oxalate nephrolithiasis in rats induced by intraperitoneal injection of sodium oxalate (7 mg/100g b.w daily for 7 days). Renal stone were induced in all groups except group one. Every day, one hour after administration of sodium oxalate, treatments were done in standard group as well as all extracts treated groups. Animals were divided into 9 groups of 6 rats (n=6). Group 1 rats served as normal control, group 2 rats were renal calculi untreated group. Group 3 rats were received standard drug Cystone 750 mg/kg b.w, p.o. for 7 days, group 4, 5 rats were administered, orally, aqueous extracts of *Amaranthus viridis* L at dose of 200 mg/kg b.w and 400 mg/kg b.w respectively, for 7days. Groups 6, 7 were treated orally with ethanol extracts of *Amaranthus viridis* L at dose of 200 mg/kg b.w and 400 mg/kg b.w respectively, for 7 days. Whereas group 8, 9 were received petroleum ether extracts of *Amaranthus viridis* L at dose of 200 mg/kg b.w and 400 mg/kg b.w, orally, respectively, for 7days. On 8th day, the animals were sacrificed for serum analysis and histopathology study.

Evaluation of anti-urolithiatic activity

Serum analysis

Blood was collected from the retro orbital. Serum was separated by centrifugation at 10,000 rpm for 10 min and analysed for magnesium, uric acid, creatinine, sodium, potassium, calcium and oxalates.[8]

Histopathology:

The abdomen was cut and both kidneys were removed from each animal. Isolated kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The kidney was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5µm and stained with H and E (Haematoxylin and Eosin) for histopathological examination. The slides were evaluated with low power of light microscope (10X) to study architecture of the kidney and calcium oxalate deposits.[9]

Statistical analysis

The data were exhibited as mean ± S.E.M. Statistical analysis was done by using means analysis of variance (ANOVA) followed by Dunnett' post hoc test, where the difference was considered statistically significant if $p < 0.05$.

RESULTS AND DISCUSSION

In predominance of urolithiasis cases, calcium oxalate is a main mineral compounds (80% <). Usually, the first symptom of a kidney stone is renal colic, which happens when a stone acutely blocks the flow of urine. The pain often starts when a stone moves in the urinary tract, if the stone is so large, blood may present in urine.[1] Ergo, in this investigation, all extracts were assessed for anti-urolithiatic activity by using experimental models of calcium oxalate induced by intraperitoneal injection of sodium oxalate. In urolithiasis, the glomerular filtration rate diminishes as a consequence of hindrance of the outflow of urine by stones in urinary system; so, the toxic compounds remarkably nitrogenous substances such as creatinine and uric acid accumulate in blood.[10] Table 1 demonstrates the effect of different extracts of *Amaranthus viridis* L on serum parameters. Sodium oxalate untreated group has exhibited increase in level of sodium, potassium, uric acid, creatinine, magnesium and oxalate in serum as compared with normal group; while the level of calcium has reduced as compared with normal group. The standard group was treated with cystone restored sodium and creatinine level in serum towards normal level. Groups were treated with the aqueous extracts of *Amaranthus viridis* L at dose of 200 mg/kg ($P<0.01$) and 400 mg/kg ($P<0.001$) and ethanol extracts of *Amaranthus viridis* L at dose of 400 mg/kg ($P<0.001$) indicated activities at per with cystone as standard. The increase in calcium level of serum was found to be 3.55 mg/dl, 4.38 mg/dl and 4.58 mg/dl in animals treated with ethanol extracts of *Amaranthus viridis* L at dose of 400 mg/kg, aqueous extracts of *Amaranthus viridis* L at dose

of 400 mg/kg and cystone at dose of 750 mg/kg respectively (P<0.001). The standard group was treated with cystone diminished magnesium, uric acid and oxalates level in serum and restored them towards normal level. Groups were received the aqueous extracts of *Amaranthus viridis* L at dose of400 mg/kg and ethanol extracts of *Amaranthus viridis* L at dose of 400 mg/kg manifested activities at per with cystone as standard and these difference were statistically significant (P< 0.001). The decrease in potassium level of serum was found to be 3.78 mEq/L, 3.51 mEq/L and 3.16 mEq/L in animals treated with aqueous extracts of *Amaranthus viridis* L (200 mg/kg and 400 mg/kg) and cystone at dose of 750 mg/kg respectively (P<0.001).

Table 1: Effect of different extracts of *Amaranthus viridis* L on serum parameters

GROUPS	Sodium mEq/L	Potassium mEq/L	Calcium mg/dl	Creatinine mg/dl	Magnesium mEq/L	Uric acid mg/dl	Oxalates mg/dl
1 .Normal	121.0 ±0.89	4.13 ±0.19	4.40 ±0.21	0.43 ±0.08	1.16 ±0.08	1.15 ±0.12	1.23 ±0.03
2. Control (NaOx)	160 ±2.06	6.01 ±0.06	0.88 ±0.11	1.28 ±0.12	1.76 ±0.05	2.2 ±0.26	2.64 ±0.20
3.Cystone 750 mg/kg	121.7 ±0.57***	3.16 ±0.09***	4.58 ±0.20***	0.44 ±0.04***	1.22 ±0.06***	0.89 ±0.13***	1.52 ±0.06***
4.AV(aq) 200 mg/kg	155.2 ±0.41 **	3.78 ±0.07 ***	1.81 ±0.13**	0.62 ±0.12**	1.47 ±0.01**	1.30 ±0.13**	1.93 ±0.10**
5.AV(aq) 400 mg/kg	143.4 ±0.82***	3.51 ±0.10***	4.38 ±0.20***	0.44 ±0.06***	1.29 ±0.09***	0.99 ±0.07***	1.64 ±0.15***
6.AV(E) 200 mg/kg	156.0 ±0.64**	5.56 ±0.11*	1.80 ±0.19**	0.71 ±0.09*	1.45 ±0.05**	1.29 ±0.20**	2.01 ±0.19*
7.AV(E) 400 mg/kg	155.2 ±0.39***	5.4 ±0.10**	3.55 ±0.16***	0.62 ±0.05**	1.33 ±0.02***	1.09 ±0.13***	1.79 ±0.11***
8.AV(PE) 200 mg/kg	158.3 ±0.26	5.67 ±0.14	1.51 ±0.15	0.84 ±0.21	1.55 ±0.05	1.67 ±0.15	2.14 ±0.17
9.AV(PE) 400 mg/kg	156.0 ±0.36*	5.56 ±0.11*	1.51 ±0.41	0.86 ±0.24	1.55 ±0.04	1.69 ±0.07	2.10 ±0.12

(n=6), values are Mean ± S.E.M. One way ANOVA followed by Dunnett' post hoc test. P values: *** < 0.001, **< 0.01 and * < 0.05 Control group as compared with sodium oxalate untreated group. AV(aq): aqueous extracts of *Amaranthus viridis* L, AV(E): ethanol extracts of *Amaranthus viridis* L, AV(PE) petroleum ether extracts of *Amaranthus viridis* L

Figure 1 shows the normal cellular structure of rat kidney. Figure 2 exhibits histopathology of kidney samples of sodium oxalate untreated rat that section demonstrated loss of normal architecture with presence of crystalline structure in dilated collecting tubules. Figure 3

displays the histopathology of kidney samples of rats treated with standard drug cystone which showed normal architecture of the kidney. The histopathology of kidney samples of rats treated with AV (aq) at dose of 200mg/kg indicated mild colloidal cast inside tubules and AV(aq) at dose of 400 mg/kg expressed cloudy changes and congestion of the glomeruli. However, the architecture of kidney was demonstrated almost normal architecture of the kidney (Fig 4 & 5). The histopathology of kidney samples of rats treated with AV (E) at dose of 200 mg/kg manifested more colloidal cast inside tubules and AV (E) at dose of 400 mg/kg group displayed inflammation of the pelvicalyceal system and congestion of the glomeruli (Fig 6 & 7). The histopathology of kidney samples of rats treated with AV (PE) (200mg and 400mg/kg) showed mild crystals deposition in tubules (Fig 8 & 9).

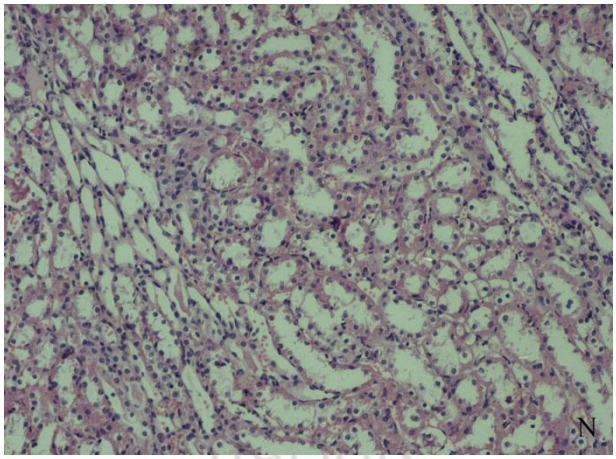

Figure 1: Normal cellular structure of rat kidney

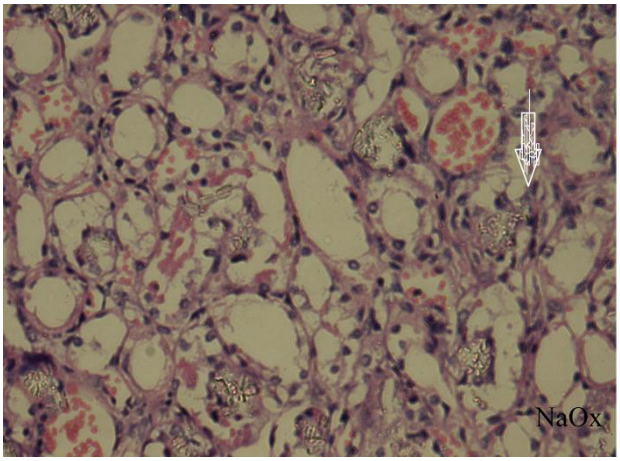

Figure 2: Histopathology of kidney samples of sodium oxalate untreated rat

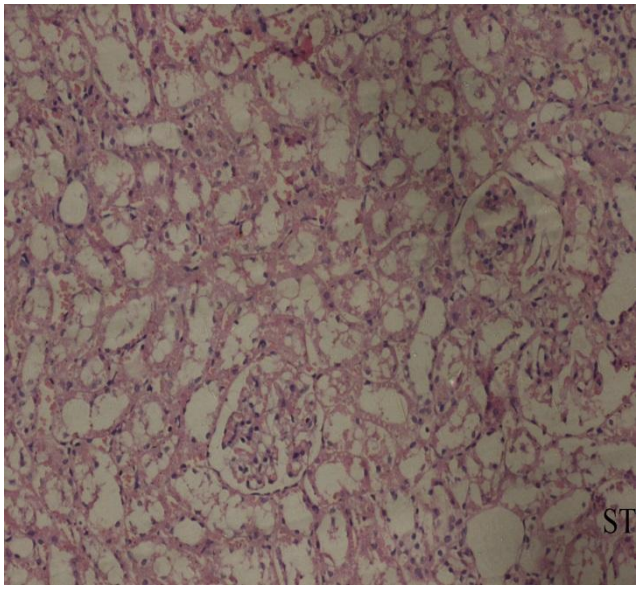

Figure 3: Histopathology of kidney samples of rats treated with standard drug cystone

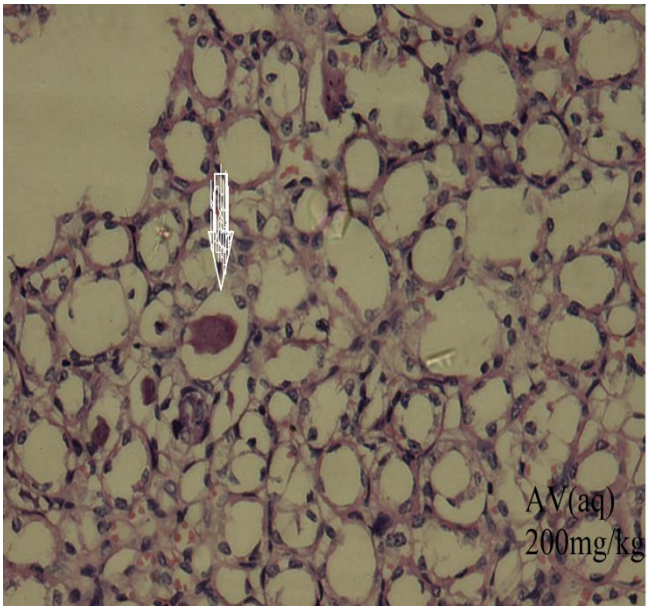

Figure 4: Histopathology of kidney samples of rats treated with aqueous extracts of *Amaranthus viridis* L AV(aq) at dose of 200 mg/kg

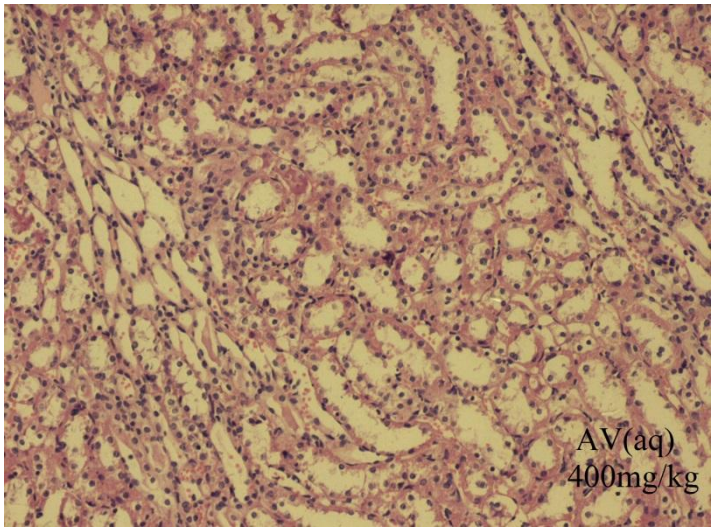

Figure 5: Histopathology of kidney samples of rats treated with aqueous extracts of *Amaranthus viridis* L AV (aq) at dose of 400 mg/kg

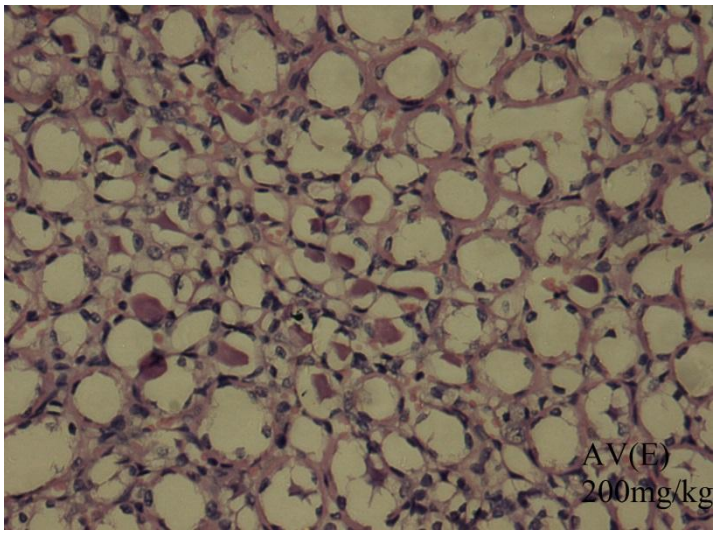

Figure 6: Histopathology of kidney samples of rats treated with ethanol extracts of *Amaranthus viridis* L AV (E) at dose of 200 mg/kg

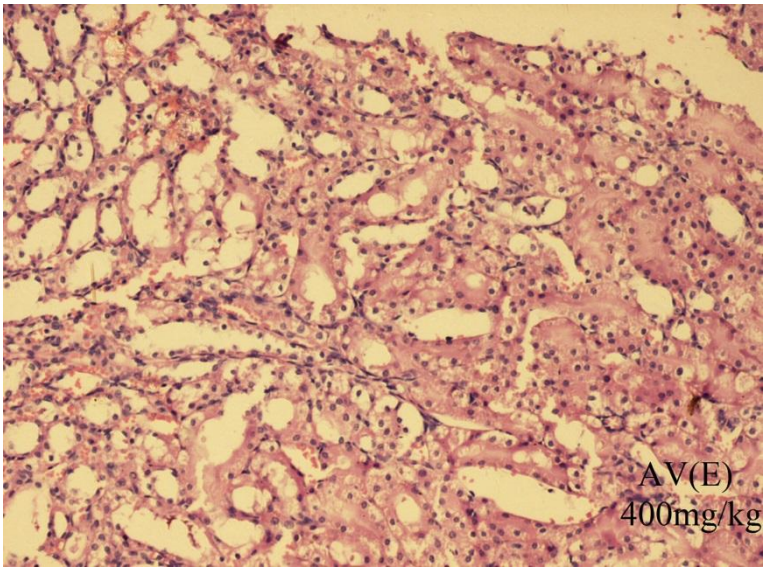

Figure 7: Histopathology of kidney samples of rats treated with ethanol extracts of *Amaranthus viridis* L AV (E) at dose of 400 mg/kg

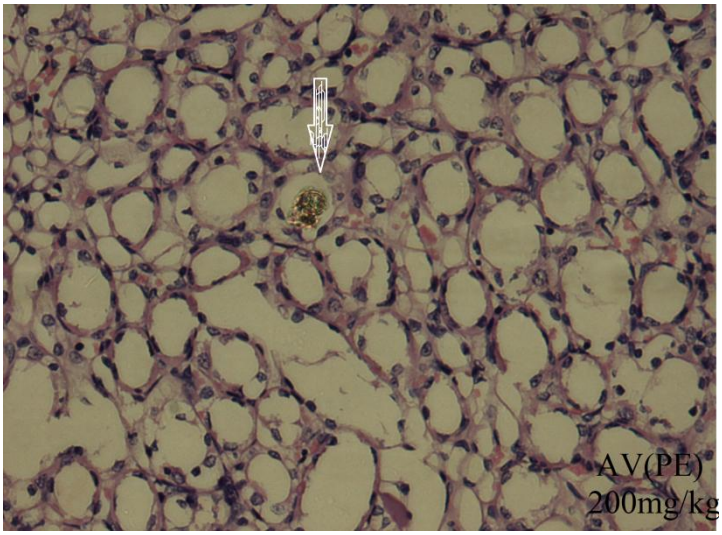

Figure 8: Histopathology of kidney samples of rats treated with petroleum ether extracts of *Amaranthus viridis* L AV (PE) at dose of 200 mg/kg

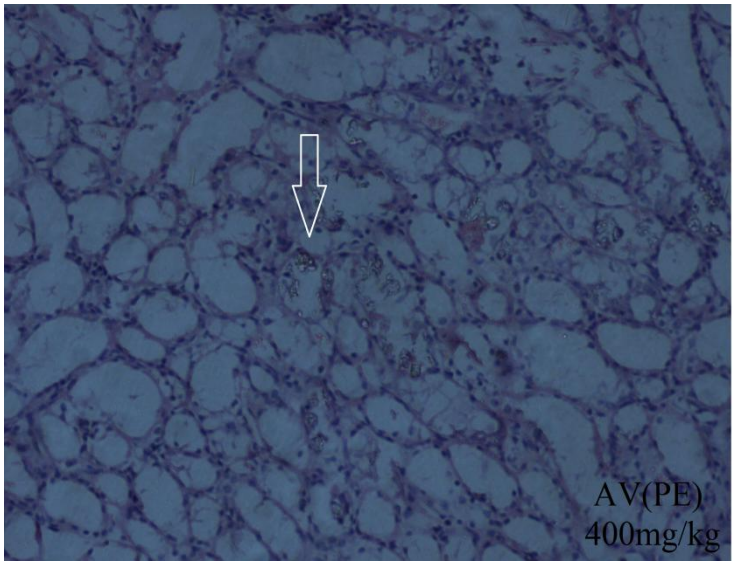

Figure 9: Histopathology of kidney samples of rats treated with petroleum ether extracts of *Amaranthus viridis* L AV (PE) at dose of 400 mg/kg

The previous phytochemical analysis of *Amaranthus viridis* L indicated the presence phenolic compounds such as flavonoids and tannin, saponins and volatile oils. Anti-urolithiatic activity could be correlated with the inhibition of oxalate levels and free radical production as well as diuretic activity.[11] Reduction in the level of oxalate supersaturation in tissue which is usually coordinated with volatile oils and tannins by way of their diuretic activity or cytoprotective effect by manifesting protection against peroxidative changes by imparting cellular membrane stability.[12] The fee radical scavenging (antioxidant activity) is related with phenolic compounds which can halt the lipid peroxidation-induced renal damage caused by calcium oxalate crystal deposition in the kidney.[11] This antioxidant activity cause to disintegration of mucoproteins and complexation with calcium which is responsible to prevent the formation of renal calculi (stone).[13]

CONCLUSION

Consequently, data obtained from investigation can prove the anti-urolithaitic activity of aqueous, ethanol and petroleum ether extracts *Amaranthus viridis* L. Present study, serum parameters analysis and histopathological evaluation showed the maximum prevention of crystal deposition by treating of aqueous and ethanol extracts of *Amaranthus viridis* L.

ACKNOWLEDGMENT

The authors are thankful to Amruta herbals company, Indore, Madhya Pradesh, India, for technical support current investigation.

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