

## SUMMARY AND CONCLUSION

Urolithiasis is a common clinical condition known as kidney stone and it affects 12% population of the world (Rajeshwari *et al.*, 2013). The problem of the stone formation is considered as a medical challenge due to its high rate of recurrence and also due to multifactorial etiology.

The medical management of lithiasis focuses on removal of existing stones and prevents recurrence of stones. It was reported that spontaneous passage rate of urinary stones ranges between 70-98 % for small (< 5 mm) distal ureteric calculi. Large calculi (stones larger than 5 mm) associated with unbearable pain or stones that fail to pass through should be treated by some interventional procedures. These procedures are costly, risky, recurrence is common, and also show several adverse effects like hypertension, trauma, hemorrhage and renal impairment. From the earlier studies and literature it was proved that phytotherapy has a promising role in the prevention and cure of renal calculi with fewer side effects and low rate of recurrence.

In the indigenous system of medicine, there are many reports regarding the efficacy of phyto-medicines for the treatment of urinary stones. *Tribulus terrestris*, *Aerva lanata*, *Scoparia dulcis*, *Tridax procumbens* are some medicinal weeds widely recommended by traditional healers for the treatment of urolithiasis. In the present investigation, we aim to assess the validity of this indigenous knowledge by conducting *in vitro* and *in vivo* experiments.

The research entitled “***In vitro* and *In vivo* Investigation of Antilithiatic and Antioxidant Activity of Aqueous Extract of *Aerva lanata***” was carried out and the results are summarized in this chapter.

The three phases of the study involved

- Phase I- Selection of the weeds extract with maximum efficacy against stone forming constituents *in vitro*

- Phase II- *In vivo* analysis of the selected weed extract in ethylene glycol induced lithiasis in male Wistar albino rats and further analyze the effect of the extract *in vitro* on renal epithelial cells (NRK 52E)
- Phase III- Determination of the phytochemical constituents responsible for the therapeutic action

In Phase I, in order to study the weed extract with maximum antilithiatic activity three different solvents with varying polarity, namely chloroform, methanol and aqueous extract of *Tribulus terrestris*, *Aerva lanata*, *Scoparia dulcis*, *Tridax procumbens* were used. The role of stone forming constituents in the presence and absence of the weed extracts revealed a dose-dependent inhibition against nucleation, growth and aggregation of CaOx crystals. This showed the antilithiatic activity of the extract that prevented precipitation of the stone forming constituents.

It was observed among the weed extracts selected, aqueous extract of *Aerva lanata* at the concentration of 1600µg/mL showed maximum inhibition. The maximum inhibitory effect was recorded at 1600 µg/mL after which there was no significant change in the extent of inhibition rendered by the weed extract and hence this concentration was chosen for further analysis of the study.

In Phase II, the parameters such as volume of urine, pH, calcium, oxalate, phosphate, uric acid, creatinine, magnesium and citrate were analysed in experimental rats from 0 to 28 days of treatment period at an interval of 7 days. The urine and pH analysis showed that there was a reduction in the urine volume and pH in the lithiasis induced rats when compared to the control group, an indication of calculogenesis. The urine volume and pH was restored to normal levels in the groups treated with the extract indicating that the *Aerva lanata* extract was able to generate therapeutic action against the stone forming constituents.

Ethylene glycol treatment raised the urinary crystal forming constituents level (calcium, oxalate, phosphate, uric acid and creatinine) in the lithiatic group rats. The results showed by the preventive regimen revealed that the elevated

levels of the constituents dropped significantly indicating that the extract has rendered a good protection thereby prevents precipitation of the stone forming constituents. The curative and standard drug (Cystone) treated groups showed a marked decrease in the stone forming constituents, this shows that aqueous extract has curative property that helps to disintegrate the preformed crystals. The extract control groups were comparable with the control group signifying that *Aerva lanata* extract by itself does not cause any toxic side effects to the renal cells. An inverse trend was observed in respect of inhibitors of stone formation namely magnesium and citrate levels in the rat urine. Thus the urine analysis reiterates the antilithiatic property of *Aerva lanata* extract.

The serological parameters on the 28<sup>th</sup> revealed that, on treatment with ethylene glycol, calcium, oxalate, phosphate, creatinine and uric acid levels were significantly higher than the control group, a sign of crystal deposition in the kidneys causing obstruction in the urine passage leading to the accumulation of nitrogenous substances. These levels were markedly reduced in the rats treated with the aqueous extract of *Aerva lanata*. This might be due to the flushing out of the calculogenic ions due to diuretic property of the extract.

The body weight of the animals were increased in all the groups but was much lowered in the lithiatic group. An increase in kidney weight was observed in the lithogen treated group which may be due to the deposition of the crystals in the renal cells or due to inflammation caused by the presence of stones.

ALT and AST are enzyme markers which indicate liver and kidney tissue damage. In the litholitic rats, ALT and AST levels were reduced in the liver and kidney tissue whereas there was an elevation in the enzyme levels in the serum. This might be an indication of the structural damage induced by the crystals causing seepage of the enzymes into the circulatory system. The activity of the enzyme was enhanced in the liver and kidney upon supplementation with the extract suggesting that the organ damage might be reversed due to the presence of the aqueous extract of *Aerva lanata*.

There was an increase in the calcium and oxalate deposition in the groups treated with ethylene glycol which was restored to its near normal levels by the extract treatment. The histopathological studies showed the deposition of crystals in the tubular and peritubular interstitium which was dissolved upon the addition of the aqueous extract of *Aerva lanata*.

The results of the *in vivo* analyses were further supported by the study done using NRK 52E cell lines from normal rat kidneys. NRK 52E cell lines were procured from National centre for cell science, Pune, India and maintained as monolayer in Dulbeco's Modified Eagle's Medium (DMEM) under standard laboratory conditions. Cells were subjected to oxalate-induced cell injury by incubation with sodium oxalate in the medium in the presence and absence of the aqueous extract for 72h.

MTT and SRB cell viability assays showed an increase in cell viability in the aqueous extract administered along with oxalate indicating that the extract helps in decreasing cell death by protecting the cells from oxalate injury. The LDH leakage induced by cell damage due to oxalate injury was reduced by the addition of the aqueous extract, thereby confirming the cytoprotective role of the aqueous extract of *Aerva lanata*.

In Phase III, The aqueous extract was analyzed for its enzymic (superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione S-transferase and polyphenol oxidase) and non-enzymic (ascorbic acid, tocopherol, reduced glutathione, total carotenoids, lycopene, total phenol and flavonoids) antioxidants. The results showed that aqueous extract of *Aerva lanata* was found to be a rich source of enzymic and non- enzymic antioxidants.

DPPH, ABTS, H<sub>2</sub>O<sub>2</sub> and OH<sup>•</sup> assays were performed to test the efficacy of the aqueous extract to quench an array of free radicals produced due to oxidative stress. The assays revealed that the aqueous extract of *Aerva lanata* had good free radical quenching ability, thereby reducing the risk of oxidative stress.

To determine the active components present in the aqueous extract of *Aerva lanata* responsible for its stone dissolution property, the phytochemical

screening of the extract was done. The preliminary screening assays showed the presence of major secondary metabolites like alkaloids, flavonoids, steroids, terpenoids, saponins, tannins and phenols.

Each of these fractions were prepared and subjected to UV-Visible absorption spectroscopy between 190-900nm showed multiple peaks in each fraction. The aqueous extract of *Aerva lanata* was subjected to HPTLC profiling, which confirmed the presence of major secondary metabolites.

The HPLC profile showed 9 peaks indicating the presence of major phytochemicals in the aqueous extract. The results of the FTIR peak value and functional groups of aqueous extract of *Aerva lanata* showed the presence of phenols, alkynes, alkenes and alkanes.

GCMS profile obtained also confirmed the presence of these phytochemicals. The individual fragmentation pattern revealed the presence of double bonds, methyl substitutes and COOH groups in the extract. <sup>1</sup>H NMR results also reiterates the presence of major secondary metabolites. Further studies need to be conducted to determine the structure and characteristic features of the predominant secondary metabolites present in the extract.

## Conclusion

Based on the results of the present study, it could be concluded that all the extracts tested were found to show stone inhibitory property in all stages of crystal formation *in vitro*. Aqueous extract of *Aerva lanata* was found to have maximum inhibitory potential when compared to all the weed extracts selected. Diuretic effect and antilithiatic property were also confirmed by the *in vivo* studies. Histopathological studies supported the results. The phytochemical analysis revealed that the aqueous extract is a rich source of antioxidants and secondary metabolites. The study confirmed that the bio- synergistic action of the phytochemicals confer antilithiatic and antioxidant property to the extract. Thus, the aqueous extract of *Aerva lanata* can be recommended as an antilithiatic agent to treat kidney stones.

### **Significance of the study**

- Utilization of weeds in productive ways, so that people may benefit from an aspect that has been largely ignored. 'Utilization' has been recognized as an effective means of weed management
- Use of weeds for providing effective, non harmful cure that caters to people of all groups
- NRK 52E cells can be used as alternate for the *in vivo* analyses to study the mechanism of action of the crystals in the cell
- For use as formulation in home remedies

### **Future research suggestions**

- An elaborate study is needed to identify the bioactive component that regulates the inhibitory activity in the extract against kidney stone formation
- A detailed study about the promoters and inhibitors of stone formation can be conducted using *in silico* approach
- Detailed pharmacological kinetic study in human is required to establish the use of *Aerva lanata* aqueous extract