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SUMMARY AND CONCLUSION

Kidney stone formation was initiated by supersaturation of urinary salts and crystal retention in the urinary tract. Although there are a few recent reports of medical treatments for enhancing the clearance of stones, there is still no satisfactory drug available for use in clinical therapy, especially for the prevention or the recurrence of stones. The focus, therefore, should be on the development of novel strategies for the prevention and treatment using plants. Natural products, due to their easy availability, low cost and lack of or less side effects, make themselves attractive candidates for drug research.

One such plant to be considered is banana and all its parts are being used in traditional medicines from ancient times. But detailed scientific data on its pharmaceutical potential with particular reference to antilithiasis have not been documented. Banana, native to India, with its vast varietal wealth is being grown round the year and hence the raw material is available in abundance.

The present study was aimed at scientifically assessing the effect of banana corm *in vitro* and *in vivo* conditions and characterizing phytochemical constituents. The banana cultivars selected for the study represents the different genome and ploidy level, including a wild species, *Musa balbisiana* clone Bhimkol. The commercial cultivars chosen for the present study includes Poovan (AAB), Red banana (AAA), Neypoovan (AB), Karpooravalli (ABB), Sannachenkadali (AA).

The research entitled, "**Evaluation of Antilithiatic Potential of Banana Cultivars of Different Genome and Ploidy"** was carried out and the results are summarized in this chapter.

The study was carried out in three phases.

In the first phase, the different corm extracts of banana cultivars were assessed for their *in vitro* antilithiatic activity. Among the cultivars tested, the banana corm with maximum antilithiatic efficacy was selected for the further studies. In the second phase,

in vivo antilithiatic potential of the selected cultivar was evaluated in ethylene glycol induced lithiatic male Wistar albino rats. An alternative system to animal model was also tested to provide an understanding of exposure of renal cells (NRK 52E cell lines) to calcium oxalate crystals *in vitro*. In the third phase, the antioxidant status and characterization of phytochemical constituents present in the selected banana cultivar were assessed.

In Phase I, in order to identify the best organic extract of banana cultivar corm with maximum antilithiatic activity, seven different extracts of increasing polarity namely petroleum ether, benzene, chloroform, ethylacetate, ethanol, methanol and water of the six banana cultivars were used for the assessement of *in vitro* antilithiatic activity. The results of the *in vitro* assays clearly indicated that all the corm extracts readily prevented crystal nucleation, growth and aggregation. The methanolic extract of Red banana corm exhibited more inhibitory potential against all the stages of stone crystallization process than other cultivars. Therefore, the methanolic extract of Red banana corm was used for further studies.

Following this, the optimum dose of the methanolic extract to be used in the assay was determined. The maximum inhibitory effect was observed at 1600µg, after which there was no further rise in the inhibitory potential and hence chosen for further studies.

In Phase II, the urinary supersaturation of stone-forming constituents was generally considered to be one of the causative factors in calculogenesis. In lithiasis-induced rats a drastic decrease in urine volume was recorded when compared to control rats. The administration of methanolic extract of the Red banana corm caused an increase in urine volume, indicating the diuretic effect of banana cultivar. The volume of urine excretion was significantly high in preventive and curative group rats upon supplementation of the Red banana corm extract. The urine pH declined in the rats treated with ethylene glycol. After administration of Red banana corm extract the pH reverted to normal.

Ethylene glycol treatment raised the urinary stone-forming constituents level (calcium, oxalate, phosphate, uric acid, and creatinine) in the lithiatic group rats. In the preventive group, the elevated levels of these constituents dropped significantly by the administration of corm extract, indicating the disruption of calcium oxalate calculi formed in the kidney. The curative group also showed a significant increase in the levels

of stone-forming constituents before the administration of the extracts; the levels, however, were reduced in the presence of the corm extracts, signifying the therapeutic properties of the banana cultivar. An inverse trend was observed in respect of inhibitors of stone formation namely magnesium and citrate levels in the rat urine.

In the lithiasis induced group, the serological parameters on the 28th day revealed that the calcium, oxalate, creatinine, phosphate and uric acid levels were significantly high, indicating remarkable renal dysfunction. These levels were markedly reduced in rats treated with the methanolic extract of Red banana corm. The levels of calculogenic ions in the preventive regime group were comparable with that of the standard drug treatment (Cystone).

The body weight of the animals increased in all the groups. The extent of weight gain however, was much low in the lithiatic animals. An increase in kidney weight was observed only in the lithiatic group, which could be attributable to the deposition of CaOx crystals.

Enzymes that are commonly employed as indicators of serum, kidney and liver damage are aspartate aminotransferase (AST) and alanine aminotransferase (ALT). In ethylene glycol-induced rats, the ALT and AST levels had increased in the serum, whereas in the kidney and liver tissues of these rats, had decreased significantly indicating cellular damage in these organs. The activities of these enzymes were enhanced upon administration of methanolic extract of Red banana corm, pointing out that the organ damage could be reverted by the corm extract.

Kidney analysis showed deposition of crystalline components namely calcium and oxalate, in the renal tissues of stone forming rats. The methanolic extract of Red banana corm considerably reduced the levels of these constituents in all treatment groups. Histopathological study of the kidney sections also supported the above findings.

Microscopic observation of the kidney tissues revealed that in all the stone forming rats damage seen in the last part of the nephron, collecting system and peritubular interstitium as compared to the architecture of normal rat kidney. Irregular crystals were present inside the tubules and in the peritubular interstitium, along the nephron and at papillary level in the lithiasis induced group when compared to control.

Following this, effect of Red banana corm extract on calcium oxalate exposed renal epithelial cells, were tested using Normal Rat Kidney cells (NRK 52E) which was procured from National Centre for Cell Science, Pune, India and maintained as monolayers in Dulbecco'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, both in presence and absence of the methanolic extract of Red banana corm for 72 hours.

The influence of the corms extract on the survival of cells was studied by MTT and SRB assays. It was evident from the data that oxalate exposure had decreased the viability of NRK 52E cells. The co-administration of the corm extract improved viability of the cells. This indicated that a significant protection was rendered by the methanolic extract of Red banana corm. The cytotoxicity was also quantified by monitoring the lactate dehydrogenase (LDH) leakage into the medium. Oxalate exposure caused a steep rise in the extent of cell damage while administration of the corm extract along with the oxalate, resulted in significant reduction in LDH release.

In Phase III, the enzymic and non-enzymic antioxidants were estimated in Red banana corm and the results revealed that the corm extract possessed greater amount of all 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, flavonoids and total chlorophyll) antioxidants. Thus, it is evident that Red banana corm is a rich source of antioxidants.

Free radical scavenging assays were performed to define the ability of the methanolic extracts of Red banana corm to quench an array of radicals generated after the induction of oxidative stress *in vitro*. The extract was tested for their ability to scavenge the oxidants like DPPH, ABTS, H_2O_2 and $OH^{\bullet}$. The results showed that the methanolic extract of Red banana corm had a good radical quenching potential.

To determine the active components present in the methanolic extract of Red banana corm, known for the stone dissolution property, the phytochemical analysis of the corm was done. In the Red banana corm alkaloids, flavonoids, steroids, terpenoids,

tannins, saponins and phenols were observed by the preliminary qualitative phytochemical screening. Each of these fractions were prepared and subjected to UV/Visible absorption spectroscopy between 190-900nm when showed multiple peaks in each fraction. The methanolic extract of Red banana corm was then subjected to HPTLC profiling, which confirmed the presence major secondary metabolites.

HPLC of the corm extract revealed 10 peaks indicating the presence of major phytochemical components. The FT-IR spectrum of the methanolic extract of Red banana corm showed the presence of several functional groups (-OH stretching, C-O-H bending and C-O stretching vibrations). It exhibited bands at 3322.53 cm^{-1} which was the characteristic of –OH stretching. This indicated the presence of –OH group. The peaks at 2941.57 cm^{-1}, 2830.66 cm^{-1} and 2521.07 cm^{-1} revealed the -C-H stretching vibrations of alkanes. Presence of -C≡C- strong stretching vibrations of alkynes at frequency of 3322.53 cm^{-1} and 2239.77cm^{-1} was observed. A band at 1183.38 cm^{-1} indicated the presence of –CO group. A peak at 1442.82 cm^{-1}, 1022.32 cm^{-1} and 652.93 cm^{-1} indicated =C-H broad and strong bend.

The GC-MS analysis also further supported the results obtained from other phytochemical analysis. The results of the ^{1}H NMR also reiterates the presence of major secondary metabolites which might be phenol/flavonoid.

Conclusion

Based on the results of the present study, it could be well concluded that all the banana corms tested were found to show antilithiatic property in all the stages of stone formation. Among them, the methanolic extract of Red banana corm had the maximum potential to inhibit the CaOx formation at all the three stages of the stone formation. Diuretic effect and antilithiatic property of Red banana corm were also confirmed by the *in vivo* studies. Histopathological studies as well supported the results. The phytochemical characterization also revealed that Red banana corm is a rich source of major secondary metabolites and antioxidants. In short, the corm of Red banana can be recommended as a potent antilithiatic agent to treat kidney stones.

Significance of the study

Banana corms, which otherwise go as waste (upto 25 tons/ha) can effectively and commercially be used for medicinal purposes. The study also provided evidence for prevention of kidney stones by the use of banana corm juice as home remedy. Finally a folk information is validated scientifically.

Future research suggestions

- Structural confirmation of the isolated compounds need to be studied in detail and i*n silico* approach can be carried out in the isolated compounds for drug designing
- The role of bioactive component isolated from methanolic extract of Red banana corm in humans need to be examined in detail, with special reference to its pharmacological kinetics
- Screening and comparison of many other banana germplasm in comparison with Red banana can be done for its antioxidative and antilithiatic properties and such a study would be optimistic.