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IN VITRO GROWTH AND INHIBITION STUDIES OF *CEIBA PENTANDRA* ON MONOSODIUM URATE MONOHYDRATE CRYSTALS

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Summary

Gout is disorder that results from the build-up of uric acid (MSUM crystals) in the tissues or a joint. The crystallization of MSUM in different solutions of the extracts of *Ceiba pentandra* has been investigated, focusing on the inhibition mechanism on gout formation. The growth of MSUM crystals was carried out by using single diffusion gel growth technique, which is well suited to mimic the conditions of growth of crystals in vivo. Very thin, transparent, needle type crystals are observed near the gel-liquid interface. Good quality crystals of monosodium urate monohydrate were grown which were characterized by FT-IR, Powder X-ray diffraction and Thermo-gravimetry. Aqueous, petroleum ether and methanolic extract of *Ceiba pentandra* were used for the growth inhibition study of MSUM crystals. In Vitro studies of Crystals **(**MSUM) was carried out by comparative study of different extracts which shows that Aqueous extract of *Ceiba pentandra* have the potency to inhibiting and dissolve the crystal as compared to other extract. It was concluded that Aqueous extract of *Ceiba pentandra* has shown good amount of inhibition and was considered to be statistically significant ($p<0.005$), The Result of the present study confirmed that *Ceiba pentandra* can be used as curative agents for urolithiasis

Key words: *Ceiba pentandra***,** Monosodium Urate Monohydrate Crystals, Powder X-ray diffraction, FTIR, TGA

Introduction

Gout is a medical stipulation that usually presents with recurrent attacks of acute inflammatory arthritis (red, tender, hot, swollen joint). It is caused by abnormal level of uric acid in the blood. The uric acid, i.e. monosodium urate crystallizes which resulted deposits in joints, tendons, and surrounding tissues. Gout affects 1% of Western populations at some point in their lifetimes. [1] Gout occurs when crystals of uric acid, i.e. monosodium urate precipitate on the tendons, cartilage of joints and in the surrounding tissues. Uric acid is a normal part of blood serum hyperuricemia is main cause of gout which is associated with depositions of uric acid crystal, although hyperuricemia is 10 times more common without clinical gout than with it. [2] Gout can also occur when serum uric acid is normal, and when it is abnormally low (hypouricemia). Paradoxically, acute attacks of gout can occur together with a sudden decrease in serum uric acid, such as due to use of drugs (uricosurics, xanthine oxidase inhibitors), or total parenteral Nutrition. [3] However, the sudden decrease may be a consequence of abrupt formation of crystals (removing uric acid from the serum), rather than a cause. An acute attack occurs as a result of an inflammatory reaction to crystals of sodium urate that are deposited in the joint tissue. The inflammatory response involves the local infiltration of granulocytes which phagocytise the urate crystals. Lactate is high in synovial tissues and in the leucocytes that associated with the inflammatory process which also favors the deposition of uric acid. [4, 5] Gout mainly affected 1% of the Western population at some point in their lives and is frequency increases. [6] This increases upto to 2% in men over the age of 30 and women over the age of 50. [7] Different populations have different propensities to develop gout. In the United States, gout mainly is twice prevalent than in African American as it is in European-Americans. [8] It is found in the peoples of the Pacific Islands, and the Maori of New Zealand, but rare in Australian aborigines despite the latter's higher mean concentration of serum uric acid. [9] In the United States and Italy, attacks of gout occur more frequently in the spring. Nephropathy of uric acid and urate substances is associated with the formation of Monosodium Urate (MSU), ammonium urate and uric acid in urinary tract system. The MSU is rarely observed in renal calculi in spite of human urine often being supersaturated with monosodium urate. [10-12] Abnormal level of urine uric acid can lead to uric-acid crystals precipitating in the kidneys, which may form kidney stones and lead to urate nephropathy.

Plants used in traditional medicine to treat kidney stones represent a valuable alternative for the control of this disease. *Ceiba pentandra* (L) Gaertner known as silk cotton tree and locally as “dum” belongs to the Bombacaceae family. Various parts of this plant are widely reputed in African traditional medicine. The plant has been reported to be a useful diuretic and effective remedy against diabetes, hypertension, headache, dizziness, constipation, mental trouble, fever, peptic ulcer, rheumatism, leprosy. [13-16]

The objective of present study is to growth and characterize MSUM crystals and study the inhibitive effect of different extract of *Ceiba pentandra* on its growth.

Material & Methods

Plant material

The whole plant of *Ceiba pentandra* were identified and collected in the month of November from the local market of the Bhopal. Voucher samples were preserved for reference in the Safia College of science, (135/bot/safia/2010). The bark of the whole plant is selected for the study.

Successive Solvent extraction

The bark were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned barks were coarsely powdered in hand grinder. Powder barks were weighed (75 gm) and packed in soxhlet apparatus. The drug was defatted with petroleum ether (40^0-60^0) for about 72 hrs. Complete deffating was censured by placing a drop from the thimble on the filter paper which did not exhibit any oily spot.

The defatted material was removed from the soxhlet apparatus and air dried to remove last traces of petroleum ether, the defatted materials was subjected to extraction by methanol and water as solvent. The process was carried out for about different timings for different solvents. The liquid extract was collected in a tarred conical flask. The solvent removed by distillation. Last races of solvent being removed under vaccum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated. The yield of Petroleum ether, chloroform methanolic and aqueous extracts was found to be 1.55g, 1.06g and 1.38 and 1.54.

Qualitative analysis

The Aqueous, Ethanol extracts of *Ceiba pentandra* were screened for the presence of secondary metabolites. Two (2) milliliters of each extract was measured into a test tube for each of the tests and concentrated by evaporating the extractant in a water bath. Tests were carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes.

In Vitro experimental design

Crystal growth

The growth Monosodium urate monohydrate crystal (MSUM) was carried out by single diffusion gel growth techniques which is well suited to mimic the growth of crystals in vitro. In the present investigation, glass test tubes of 25 mm diameter and 150 mm length was used as crystal growth apparatus. Sodium Metasilicate solution of 1.05 specific gravity and 0.2 M, NaOH solutions was added in equal amount. This mixture was acidified by 2 N acetic acid in such manner that the ph value within 4.5-5.0 are obtained. This mixture was poured in equal volume in different test tubes and allowed to set into the gel forms .within 48 hours, good quality gel were set. After settling the gel, the 0.07 M uric acid solution was poured gently on that. Good quality needle shaped crystals were grown.

The probable reaction for the formation of MSUM is as follows:

$$NaOH + C_5H_4N_4O_3 \rightarrow NaHC_5H_2N_4O_3.H_2O$$

Characterization
The grown crystal were characterized by using FT-IT spectroscopy, thermogravimetric analysis(TGA) and powder X-ray diffraction method to confirm the structure and formation of crystals in the experiment

a. **Fourier's transformer – IR spectrophotometry** - FT-IR spectroscopy is an excellent tool to identify various chemical bonds in a compound.FT-IR spectrum was recorded on Shimadzu 8400 set up in 400-

b. Powdered XRD - Powder XRD was carried out on Philips X'pert using Cu Kα radiation.

c. TGA - Thermo gravimetric Analysis (TGA) is performed to assess the thermal stability of the substance. Thermo gram was recorded on Pyrif 1, Perkin Elmer

Comparative study between different extracts of *Ceiba pentandra* of growth inhibition of MSUM crystals
We were taken three test tubes having the mixtures of uric acid and solution of herbal extracts, which was used for the inhibition study. In the 1st test tube only pure uric acid was taken with the crystals, in the 2nd test tube aqueous extracts poured as supernatant solution along with the Control solution on the set gels during the crystal growth experiment., in 3rd test tube methanolic extracts poured as supernatant solution poured as supernatant solution along with the Control solution on the set gels, same as in case of petroleum ether. Result of the inhibition study of MSUM crystals in term of observed crystals after seven days of the pouring the herbal extracts solution was comes. And after 15 days see the result that crystals was dissolve completely or not.

Statistical Analysis
The statistical significance result was expressed as mean ± SEM. All data was analyzed with one way ANOVA followed by Dunnett vs. Control test and $p < 0.05$ was considered to be statistically significant.

Results and discussion

Qualitative analysis
Preliminary Phytochemical screening was performed for each extract. It was noted that Aqueous extract contain alkaloids, glycosides carbohydrates, flavonoids and tannins, petroleum ether extract contain alkaloids, flavonoids, tannins, while in ethanolic extract showed the presence of alkaloids, glycosides carbohydrates, flavonoids and tannins

Characterization of Monosodium Urate Monohydrate crystals
Joshi .et.al [17] have studied Monosodium Urate Monohydrate Crystals by Different Herbal Extracts *Routula aquatica* Lour. (Roots) and *Boerhaavia diffusa* Linn. (Roots) and *Aerva lanata* Juss ex. *Schult* (root). Joshi .et.al [18] have studied inhibit growth of

calcium oxalate monohydrate crystals. Grases *et al.* [19] have studied the structure of urate renal calculi after precipitation of ammonium and sodium urate from the synthetic urine; on the other hand, Grover *et al*. [20] have studied the *in vitro* growth of calcium oxalate crystals in the presence of preincubated seed crystals of uric acid and MSU with undiluted human urine Howell *et al*. [21] have reported the powder XRD. Patterns and d values of uric acid, MSUM and Disodium Urate Dihydrate (DSUD), Thermogravimetric Analysis (TGA) are performed to assess the thermal stability of the substance. Earlier, Schnitzler *et al*. [22] have reported TGA of purine derivatives such as aminophylline, theoophylline, caffeine and uric acid. The molecular structure of MSUM is as Shown in Fig. 3 Figure 4 shows FT-IR spectrum of MSUM crystals. Table 1 gives assignments to the peaks. From the FT-IR spectrum one can confirm the presence of carbon-nitrogen bond (C-N), ketone group (C = O), N-H stretching and rocking as well as the presence of water of hydration in the sample. Figure 5 is the powder XRD pattern and Table 2 gives the data. The samples are highly crystalline in nature. Figure 6 is the thermo-gram of MSUM, which shows that the compound is stable up to 100°C. It slowly gives up water of crystalline and beyond 170°C starts losing crystalline water immediately. The associated water molecule is completely removed at 240°C and becomes anhydrous. Subsequently, when the temperature is further increased, the mass loss occurs very sharply between 370-480°C with the release of gases like hydrogen cyanide, carbon dioxide and carbon monoxide. This is followed by a slow process between 480-750°C with loss of nearly 75-80% of original mass. Finally, it is converted into Na_2O, which remains stable up to the end of the analysis.

Figure 2 shows the test tubes having the mixture of uric acid and solution of herbal extracts, which were used for inhibition study. In the case of pure uric acid the average length of grown crystals was 0.700 cm. Result of inhibition study of MSUM crystals in terms of observed crystal length after seven days of pouring the herbal extract solutions is shown in Table 3. It was concluded that all the three extract of *Ceiba pentandra* has shown good amount of inhibition and was considered to be statistically significant ($p<0.005$) but Aqueous extract at the concentration of 40 mg/ml was more significant as compared than other extracts of *Ceiba pentandra.* Result was recorded in Table.4-5. These results were found to be encouraging for the *in vivo* studies and of the drug. This study may be helpful to design the formulation therapies for the prevention and cure of gout.

Conclusion

In Qualitative analysis, various Phytochemical were performed for the identification of common Phytoconstituents in *Ceiba pentandra* extracts. The preliminary phytochemical analysis of the all the three extract has the presence of flavonoids, which has been known for its inhibiting potential. Thus it can be indicated that possible mechanism of inhibiting and dissolving potency of *Ceiba pentandra* may be due to presence of flavonoids. The present study was done to see the inhibition and dissolving potency of different extract of *Ceiba pentandra* on Monosodium urate Monohydrate Crystals **(**MSUM). Attempts were made in the present investigation to grow MSUM crystals in vitro by single diffusion gel growth technique, which is quite suitable to mimic the growth of MSUM crystals in vivo up to a certain extent. The characterization study confirmed the formation of MSUM

crystals in the experiments. FT-IR spectrum one can confirm the presence of ketone group (C = O), carbon-nitrogen bond (C-N), N-H stretching and rocking as well as the presence of water of hydration in the sample, the powder XRD pattern shows that the samples are highly crystalline in nature. The thermo-gram of MSUM, which shows that the compounds are stable up to 100°C. The growth observations and the measurements of crystal dimensions using optical microscope suggested that *Ceiba pentandra extracts* exhibited good crystal growth inhibition results. The finding suggested that the aqueous extract shows the potency to inhibiting the crystal as compared to other extracs. *Ceiba pentandra extracts* dissolved MSUM crystals after 15 days of pouring the supernatant solution on the gel. This *in vitro* study may be helpful for *in vivo* studies, which may further lead to develop a preclinical formulation for gout treatment.

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