

## CLINICAL RESEARCH

### To explore the utility of Homoeopathic medicine '*Lycopodium clavatum*' in Urinary calculi

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#### Abstract

Central Council for Research in Homoeopathy (CCRH) conducted a multicentre, observational study on Urolithiasis from 2005-2010. Results of this study showed the utility of *Lycopodium clavatum* in dissolution of urinary calculi. It is known that this medicine is efficacious for urinary calculi, but systematic and research data is not available for profession. *Lycopodium clavatum* had been in use for different purposes since many centuries in one or other form. Article is an effort to provide in-depth information of potentised *Lycopodium clavatum* in reference to the urinary calculi. The baseline symptoms score of urolithiasis was assessed before and after the treatment by using paired 't' test in SPSS (Ver. 16). A non parametric test of 'Wilcoxon rank sum' was used to analyze the changes in urinary symptoms before and after the treatment. Forty one (45.6%) patients showed marked improvement, 6 (6.7%) patients showed moderate improvement, 31 (34.4%) patients showed mild improvement, in 11(12.2%) patients there was no significant improvement and 1 (1.1%) patient did not improve.

**Keywords:** urinary calculi; *lycopodium clavatum*; lycopodiaceae; lycopodium spores; homoeopathy.

#### Introduction



***Lycopodium clavatum*<sup>1</sup>**

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#### History / Origin

*Lycopodium clavatum* (Wolf's Foot) is one of the pivotal medicines of the homoeopathic materia medica; an intimate acquaintance with its properties is essential for proper understanding of the materia medica.<sup>2</sup> This medicine belongs to an ancient family (Lycopodiaceae) with a geological history of over 380 million years. Little changed from its forebears, this diminutive fern ally bears a strong resemblance to some of the earliest plants to live on land.

Wolf's Foot is a moss like plant. It exists already for ages on this planet in an almost unaltered form; *Lycopodium clavatum* has proven to survive many changes in climate and environment.<sup>3</sup>

#### Physiological action and Content of *Lycopodium clavatum*

The physiological and medicinal powers of the potentised *Lycopodium clavatum* derives from the content of spores, and possibly also from the minerals contained in the wall of the spores. The oily contents of the spores consist of the mixture of five fatty acids: oleic, palmitic, linoleic, stearic and myristic acid. Alkaloids are present only in the herbal part of the plant, which is not used homoeopathically. They are not found in spores.

*Lycopodium clavatum* affects entire fat metabolism, a disturbing one in proving and conversely a curative or regulative effect in diseases. As an organ for assimilation of fats and formation of glycogen, the liver becomes the most important energy store of the body. Any disturbance of its function must lead to loss of physical energy, a symptom highly characteristics of this medicine.

Liver is important in protein dissimulation leading to the formation of urea, creatine and creatinine and small amount of uric acid. Slowed down protein metabolism leads to increased formation of urates, resulting in gout and development of kidney stones and producing characteristics red sediments in the urine. *Lycopodium clavatum* regulates this slowed down protein metabolism and cures gout and kidney stones.<sup>4</sup>



***Lycopodium clavatum***<sup>5</sup>

*Lycopodium clavatum* powder<sup>7</sup> has a wide range of uses:

- As an explosive mixture with air to spectacular effect in the film and entertainment industries.
- As a fine dusting on condoms and surgical gloves to lubricate the latex.
- As powder in cosmetics.
- As pill coating in the pharmaceutical industry.
- As a dry parting compound in foundry work to prevent metal from sticking to wooden molds.

## Classification and Description

### Scientific classification<sup>8</sup>

Kingdom : Plantae  
Division : Lycopodiophyta  
Class : Lycopodiopsida  
Order : Lycopodiales  
Family : Lycopodiaceae  
Genus : *Lycopodium clavatum*

### Species : **L.clavatum**

From 54 species of Genus *Lycopodium clavatum* over 201 alkaloids have been reported. Structures of 81 new alkaloids were presented, classified and analyzed.<sup>9</sup> Some of the alkaloids showed acetylcholinesterase inhibitory activities. Acetylcholinesterase inhibitors play an important role in the treatment of Alzheimer's disease as well as Myasthenia Gravis, Glaucoma and Helminthiasis together with mechanism of action of insecticide drugs.<sup>10</sup> Acetylcholine is essential neurotransmitter in the brain and responsible for function related to cognition and memory. Loss of acetylcholine function is a primary feature of several types of brain dysfunction, including Alzheimer's disease.

A shortage of acetylcholine is considered the most common cause of memory loss, decreased learning ability and intelligence. Huperzine (one of the alkaloid) inhibits the activity of acetylcholinesterase, so the breakdown of acetylcholine is slowed and the strength and duration of the nerve impulse is improved.<sup>11</sup> One of the accidental poisoning of *Lycopodium clavatum* selago (L.species) is consistent with the anti acetylcholinesterase activities of Huperzine A.<sup>12</sup> Another alkaloid Alpha-onocerin from *Lycopodium clavatum* is effective in treatment of Alzheimer's disease (AD).<sup>13</sup> Usefulness of these alkaloids, opens a new avenue for research regarding the effectiveness of potentised *Lycopodium clavatum* in brain dysfunctions.

In one of the studies, it was found that alkaloid Lycopodine inhibits proliferation of HeLa cells (Cells involved in cancer production) which indicates its potential use in chemotherapy.<sup>14</sup> This same alkaloid was found effective as anti-inflammatory agent.<sup>15</sup>

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One of the research, showed significant reduction of tumor incidence in liver of carcinogens intoxicated mice treated with spore extract of this medicine, further validates the use of this plant extract in complementary and alternative medicine against Hepato-toxicity.<sup>16</sup>

### Doctrine of Signature



*Lycopodium Clavatum*<sup>17</sup>

1. *Lycopodium clavatum* exhibits contrariness in all its characteristics manifestations, it is symbolized by the spores, where the extraordinary hardness of capsule of spores contrasts with softness of the oily contents in it.

Contrariness presents itself in symptoms as -

“Strong mind with weak body”

Desire for solitude, but at the same time wants someone in next room.

Lack of self confidence and fear, but again anger, arrogance and dictatorial behavior.

Ravenous hunger but satiety after few bites.

Increased sexual desire and impotence.

2. The rhythm of growth proceeds from right to left

and back again in the stem; branches are always one sided. *Lycopodium clavatum* patients complaints develops from right side first than left side usually or only one sided.

3. The outstanding characteristic of plant is the enormous slowness of development, beginning with inability to achieve spermatogenesis independently, together with frequent sterility, sexual development slowed down further contrary to that 'obstinacy' to survive for millions of years on earth. This is reflected in personality as slowed down liver metabolism and other connected processes, impotency, sterility and obstinacy, persistence and adaptability to environment at mental level.

We can summed up about *Lycopodium clavatum* personality as “An inward softness with great sensitivity and an emotionality covered up by outward harshness—a soft content within a hard shell.”<sup>18</sup>

### Homoeopathic proving

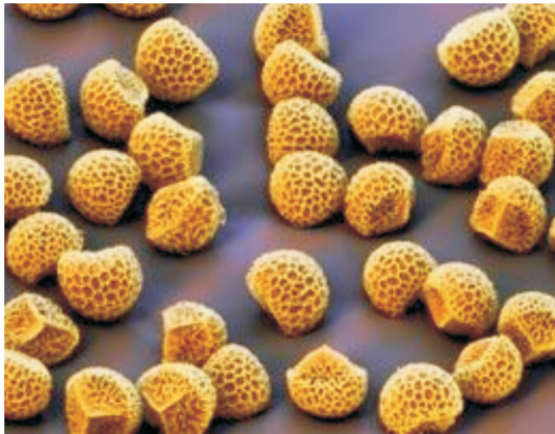
*Lycopodium clavatum* was introduced and proved by Dr.Hahnemann with seven provers. Further proving was done by Schelling, Seguin, Martin, and Huber. Small proving was done by Robinson and Epps & in higher potencies proved by Berridge.<sup>19</sup> Proving data contains 1608 symptoms from Dr.Hahnemann and fellow observers.<sup>20</sup>

### Source and Preparation

Most of the commercial drug is collected in Poland and the former USSR, but Indian and Pakistani supplies are obtaining from the Himalayas.<sup>21</sup> In July and August the cones are cut off and dried and shaken to separate the spores, which fill the sporangia in the axils of the sporophylls. Vegetable debris is removed by shifting through fine horse- hair or flour sieves and the spores are packed in sacks which are usually covered with matting.<sup>22</sup>

The spores, from which the attenuations are made, have been called “vegetable sulphur.” Medicine is prepared by Trituration of spores, tincture of spores and tincture of fresh plant.<sup>2</sup> This drug is inert until the spores are crushed. Its wonderful medicinal properties are only disclosed by Trituration and Succession.<sup>23</sup> Trituration of the powder precedes the preparation of the alcoholic tincture.<sup>19</sup>





**Lycopodium clavatum spores**<sup>6</sup>

### Characteristic symptoms of *Lycopodium clavatum* in urinary calculi

The process of forming calculi in urinary tract i.e. kidney, bladder and ureter is referred to as 'Urolithiasis'. "I find it the best medicine where the patients is suffering from an excess of lithic acid or from gravel, and look upon the copious sediments of this nature as one of the most unerring indication for its choice in dyspepsia"- Hughes<sup>24</sup>

In nearly all the cases where *Lycopodium clavatum* is indicated, evidence of urinary or digestive disturbance will be found. It corresponds to Grauvogl's carbo-nitrogenoid constitution, the non-eliminative lithemic<sup>23</sup> Carbo-nitrogenoid constitution is characterized by insufficient oxygenation; it produces diseases of retarded nutrition and creates liability for infections.<sup>25</sup>

This *medicine* covers all three miasms, though psoric manifestations are very prominent. As roots of all diseases are grown on soil of psoric miasm, remedy becomes multidimensional curative.

#### i) **Materia Medica Pura**<sup>26</sup>

1. Reddish- yellow sand in the urine. Strong smell of the urine.
2. While urinating, urine suddenly ceases, only a few turbid and mucous drops follows, with pains in urethra; then pressive pain in the groins.
3. Sharp cutting pain from the posterior termination of the urethra, obliquely up into the abdomen.
4. Frequent micturition at night.

#### ii) **Hering Guiding Symptoms**<sup>27</sup>

1. Stiffness in renal region, especially on right side; worried by noise or interruption in

business; brings up wind after eating, mentally depressed and physically weak till it comes up; passes small quantities of fine red sand. Gravel.

2. Urging to urinate: must wait long before it will pass, or inability, with constant bearing down; supports abdomen with hands.
3. Turbid, milky urine, with offensive purulent sediment; dull pressure in region of bladder and abdomen, disposition to calculi.
4. In children urging to pass water, with impossibility to pass it; cry impatiently and grasp abdomen; red sand in diaper or urine may be pale and clear.

#### iii) **Encyclopedia of Pure Materia Medica by T.F.Allen**<sup>28</sup>

1. Urging to urinate, but inability to do so, with constant bearing down feeling.
2. Urine whitish, turbid immediately after its passage.
3. Reddish- yellow sand in the urine, which remains rather clear.

#### Important rubrics which indicate *Lycopodium clavatum* in urinary calculi

##### i) **Repertory of Hering's guiding symptoms of Materia medica-Calvin B. Kneer**<sup>29</sup>

- **Kidney, calculi.**
- **Kidney colic, with itching.**
- *Kidney colic, in right ureter to bladder.*
- *Urine sediments, brick dust with copious urination.*
- *Urine sediments, reddish white sticking to crystals.*
- **Urination, difficult.**
- *Bladder calculi, passes frequently small crystals of uric acid.*

##### ii) **Kent's Repertory**<sup>30</sup>

- **Kidney, pain, ureter, right side.**
- **Urine sediments, renal calculi.**
- *Urine sediments, red, bright.*
- **Urine, cloudy standing on.**
- **Bladder, urination, dysuria.**

##### iii) **A Concise Repertory of Homoeopathic Medicines- S.R.Phatak**<sup>31</sup>

- **Urine sandy.**
- **Urine, sediments, red brick dust.**



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- *Uric acid diathesis, lithemia.*
- **Calculi formation in general.**
- **Pain, neuralgia, in general, begins on one side goes to other and there aggravates.**
- *Urinary bladder affection in general.*
- *Cramping griping colic.*

### STUDY AND TREATMENT DETAILS

CCRH conducted an observational study on the roll of homoeopathic medicines in Urolithiasis at its different centers from 2005 to 2010. *Lycopodium clavatum* was

found most effective medicine for the dissolution of urinary calculi. Patients conforming to the inclusion and exclusion criteria were included in the study. Baseline assessment of the patient was conducted by Consultant Surgeons. Severity of illness was assessed on Urolithiasis Symptom Score (USS) index chart, developed by CCRH (Table No 1). Treatment was started after analysis, evaluation of symptoms, forming totality and repertorization of case. Final medicine was selected in consultation with homoeopathic materia medica.

**Table No.1** Urinary Symptoms Score (USS) chart for intensity assessment of symptoms.

| <b>Urolithiasis Symptoms Score (Circle relevant number on each line)</b> |                     |                          |                   |                      |
|--|---------------------|--------------------------|-------------------|----------------------|
| <b>* Urolithiasis Symptoms Score</b><br>→                                | <b>0</b>            | <b>1</b>                 | <b>2</b>          | <b>3</b>             |
| <b>Symptoms</b><br>↓   | -                   |                          |                   |                      |
| 1. Pain/colic  | No pain             | Mild pain                | Moderate pain     | Severe pain          |
| 2. Haematuria  | No haematuria       | Microscopic              | Persistent        | Gross                |
| 3. Dysuria   | No dysuria          | Mild dysuria             | Moderate dysuria  | Severe               |
| 4. Stone   | -                   | Single stone             | Multiple stone    |                      |
| 5. Size of stones  | Gravel < 03mm       | 3 mm - < 4 mm            | 4mm - < 5 mm      | 5 mm and above       |
| 6. Position of stone kidney  | no stone in kidney  | Pelvic ureteric junction | Pelvis of kidney  | Calyces of kidney    |
| 7. Position of stone ureter  | no stone in ureter  | Lower part of ureter     | Middle of ureter  | Upper part of ureter |
| 8. Position of stone bladder   | no stone in bladder | Base of bladder          | Intramural ureter |                      |

\* Maximum symptom score = 22 (Sum of 8 symptoms); Mild intensity (1-7 score), Moderate intensity (8-14 score) and Severe intensity (15-22 score).

Ninety patients were prescribed *Lycopodium clavatum*, single dose in 30 centesimal (C) potency, 4 pills (size 30) on empty stomach followed by placebo. Repetition was not made as long as the improvement continued. (Reduction in Symptoms Score). Medicine was repeated in same potency when improvement stopped. During follow up, changes in signs and symptoms of patients were noted and next higher potency (200C) of *Lycopodium clavatum* was administered in those cases who had not further improved or improvement lasted for a short duration

from previous potency. Potency of this medicine was raised up to 1000C in the same manner.

### Auxiliary management

In appropriate dietary habits, overweight and lifestyle are suggested to contribute considerably to the increasing incidence and prevalence of Urolithiasis. Dietary oxalate, calcium, protein, purines, sodium and ascorbic acid are known to be promoters of stone formation, whereas potassium and magnesium have been shown to be effective inhibitors.<sup>32</sup> Dietary

restriction and lifestyle changes were advised to patients for preventing recurrence of urinary calculi.

### STATISTICAL ANALYSIS

Each enrolled case was followed up every month and, clinically assessed with Ultrasonography (KUB) and X-ray (KUB) every third month to compare the number, size and location of the calculus. Patients were grouped into mild intensity (1-7), moderate intensity (8-14) and severe intensity (15-22) on the basis of score obtained from USS index chart, according to intensity. Outcome of the study was measured in gradation as marked improvement (75 to < 100% improved), moderate improvement (50 to < 75% improved), mild improved (25 to < 50% improved), no significant improvement (< 25% improved), static (no change), and worse (increase in symptoms score).

Percentage gain in improvement was assessed as:

$$\frac{\text{baseline score} - \text{score at end}}{\text{baseline score}} \times 100$$

The symptoms score was analyzed before and after the treatment by using paired 't' test. A non parametric test of 'Wilcoxon rank sum' was applied to see the significance in each individual symptom. SPSS (Ver. 16) software was used for statistical analysis.

### RESULTS

On the basis of Totality of Symptoms and Repertorization, 90 patients were prescribed *Lycopodium clavatum* and followed up for one year. Mean age recorded was 31.20 ± 5.50 years in age group of 20-40 years. In maximum (n=50) cases, duration of illness recorded was below 6 months. (Table No. 2)

**Table No. 2** Baseline details of the *Lycopodium clavatum* patients.

|  | No. of Patients | Mean±SD       | Percentage (%) |
|--|-----------------|---------------|----------------|
| <b>Location of Institute</b>           |                 | -             |                |
| • Noida                                | 10              |               | 11.12          |
| • Kottayam                             | 2               |               | 2.22           |
| • Jaipur                               | 12              |               | 13.33          |
| • Gudivada                             | 18              |               | 20             |
| • Lucknow                              | 48              |               | 53.33          |
| <b>Sex</b>                             |                 | -             |                |
| • Male                                 | 72              |               | 80             |
| • Female                               | 18              |               | 20             |
| <b>Age group (In years)</b>            |                 |               |                |
| • 1 -20                                | 4               | 15.75 ± 2.62  | 4.44           |
| • 21-40                                | 58              | 31.20 ± 5.50  | 64.46          |
| • 41-60                                | 24              | 46.91± 5.13   | 26.66          |
| • 61 above                             | 4               | 66.5 ± 1.91   | 4.44           |
| <b>Duration of disease (In months)</b> |                 |               |                |
| • Up to 6                              | 50              | 2.14 ± 2.19   | 55.57          |
| • 6 -12                                | 11              | 10.09 ± 1.75  | 12.22          |
| • 12-60                                | 25              | 29.88 ± 11.79 | 27.77          |
| • 60-120                               | 2               | 84.63 ± 37.04 | 2.22           |
| • More than 120                        | 2               | 234 ± 48.31   | 2.22           |
| <b>Intensity</b>                       |                 |               |                |
| • Mild (1-7)                           | 4               | 6.75 ± 0.5    | 4.44           |
| • Moderate(8-14)                       | 82              | 10.75 ± 1.53  | 91.12          |
| • Severe (15-22)                       | 4               | 16.5 ± 1.73   | 4.44           |

The mean symptom score at baseline ( $10.83 \pm 2.11$ ) and at end of treatment ( $3.9 \pm 3.69$ ) was found statistically significant ( $P < 0.05$ ). Symptoms Pain, Haematuria and Dysuria were found in 89 (98.88%),

28 (31.11%) and 61 (67.77%) patients respectively at baseline. At the end of treatment pain remained in, 13 (14.44%), haematuria in 08 (8.88%) and dysuria in 04 (4.44%) patients. (Table No.3).

**Table No. 3** Changes in each symptom score before and after treatment.

| Symptoms of urinary calculi.         | Before Treatment No. of patients (%) | After Treatment No. of patients (%) | Mean $\pm$ SD of Symptom Score |                   | Z*    | 'P' value |
|--------------------------------------|--------------------------------------|-------------------------------------|--------------------------------|-------------------|-------|-----------|
|                                      |                                      |                                     | Before treatment               | After treatment   |       |           |
| <b>Pain /Colic</b>                   | 89(98.88)                            | 13(14.44)                           | 2.12 $\pm$ 0.596               | 0.18 $\pm$ 0.464  | 8.244 | 0.00      |
| <b>Haematuria</b>                    | 28(31.11)                            | 8(8.88)                             | 0.33 $\pm$ 0.519               | 0.09 $\pm$ 0.286  | 3.664 | 0.00      |
| <b>Dysuria</b>                       | 61(67.77)                            | 4(4.44)                             | 1.31 $\pm$ 1.035               | 0.06 $\pm$ 0.275  | 6.777 | 0.00      |
| <b>Stone (single, multiple)</b>      | 90(100)                              | 47(52.22)                           | 1.31 $\pm$ 0.466               | 0.69 $\pm$ 0.744  | 4.675 | 0.00      |
| <b>Size of calculi</b>               |                                      |                                     |                                |                   |       |           |
| <b>calculi &lt; 03mm</b>             | 0                                    | 2(4.25)                             | NA                             | NA                | 6.590 | 0.00      |
| <b>3 mm - &lt; 4 mm</b>              | 3 (3.34)                             | 4 (8.53)                            | 2.733 $\pm$ 0.639              | 1.24 $\pm$ 1.392  |       |           |
| <b>4mm - &lt; 5 mm</b>               | 11(12.22)                            | 6 (12.76)                           | 2.787 $\pm$ 0.495              | 1.425 $\pm$ 1.427 |       |           |
| <b>5 mm and above</b>                | 76(84.44)                            | 35 (74.46)                          | 2.811 $\pm$ 0.471              | 1.428 $\pm$ 1.441 |       |           |
| <b>Position of calculi in kidney</b> | 65(72.22)                            | 39(43.33)                           | 2.31 $\pm$ 1.205               | 1.43 $\pm$ 1.484  | 3.430 | 0.00      |
| <b>Position of calculi in ureter</b> | 17(18.88)                            | 3(3.33)                             | 0.50 $\pm$ 0.951               | 0.16 $\pm$ 0.598  | 3.430 | 0.01      |

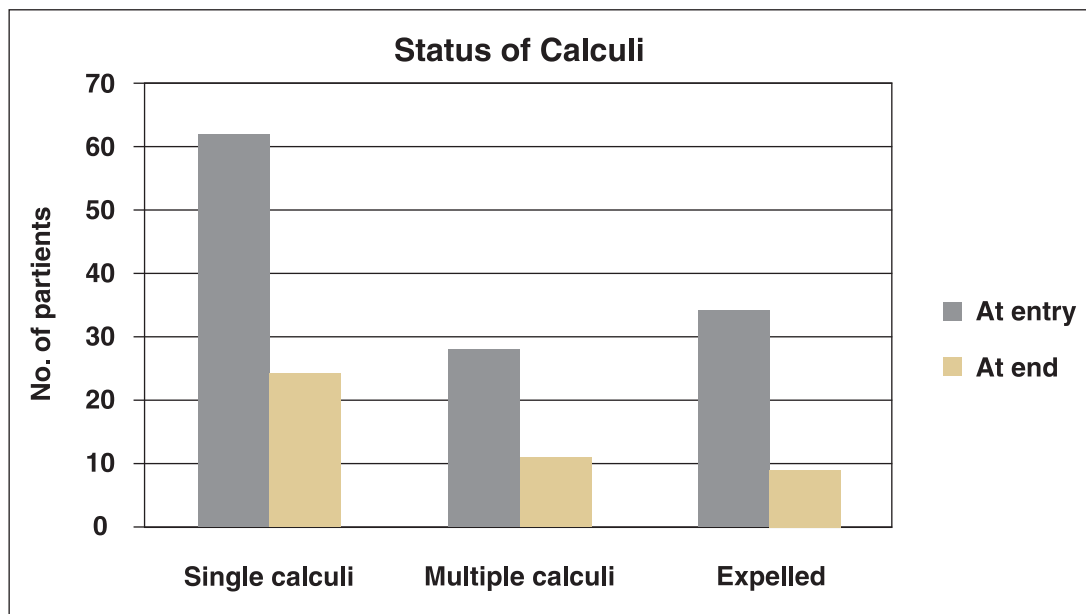
\* Wilcoxon rank sum test significant at  $P=0.001$

At baseline, single calculi was present in 62 patients (minimum size 6 mm & maximum size 22 mm). In 34 patients, calculi get expelled (minimum size 6 mm & maximum size 12 mm) and in 04 patients, single calculi converted into multiple calculi. In remaining 24 patients, there was no change at the end of treatment.

Multiple calculi were present in 28 patients at baseline (minimum size 2.8 mm & maximum size 28 mm) and at the end of treatment, calculi were expelled in 09 patients (minimum size 3 mm & maximum size 11.3 mm). In 08 patients, multiple calculi were reduced to single (minimum size 04 mm & maximum 08 mm) and in remaining 11 patients there was no change. (Figure No.1)



**Figure No.1:** Status of single and multiple calculi at entry and at end of treatment.



*Lycopodium clavatum*, could expelled calculi (single and multiple both) in 24 patients within three months, in 32 patients within 06 months, in 36 patients within 09 months and in 43 patients within 12 months. At

baseline, kidney calculi were present in 65 patients; calculi in ureter were present in 17 patients and after the treatment calculi remained in kidney 39 patients and in ureter 03 patients. (Table No.4)

**Table No. 4** Position of calculi in urinary system at entry and at end of treatment.

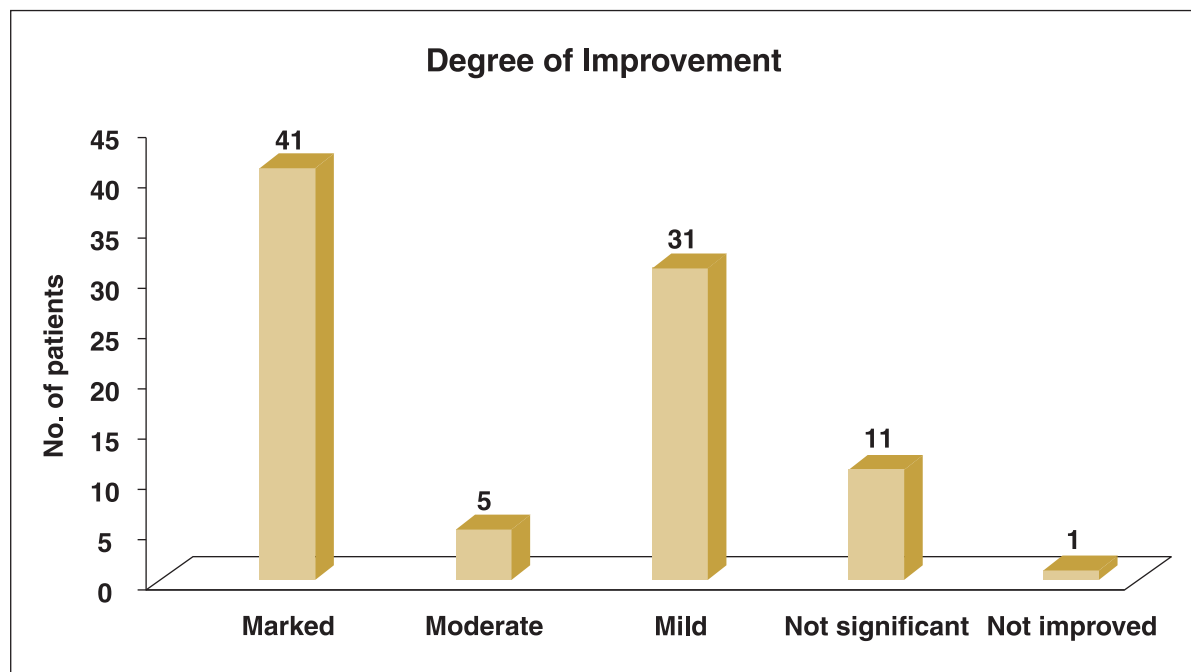
| Position of stone               | No. of Cases at Entry (n) | Expulsion of stone | Size Increased | Size Decreased | Statuesque |
|---------------------------------|---------------------------|--------------------|----------------|----------------|------------|
| <b>Kidney</b>                   | <b>65</b>                 |                    |                |                |            |
| Calyces of kidney               | 58                        | 22                 | 3              | 7              | 26         |
| Pelvis of kidney                | 6                         | 3                  | 0              | 0              | 3          |
| Pelvi ureteric junction         | 1                         | 1                  | 0              | 0              | 0          |
| <b>Ureter</b>                   | <b>17</b>                 |                    |                |                |            |
| Lower part of Ureter            | 8                         | 7                  | 0              | 1              | 0          |
| Middle part of Ureter           | 4                         | 4                  | 0              | 0              | 0          |
| Upper part of Ureter            | 5                         | 3                  | 0              | 0              | 2          |
| <b>Calculi at two positions</b> | <b>8</b>                  |                    |                |                |            |
| Calyx of kidney+ Bladder        | 1                         | 0                  | 0              | 0              | 1          |
| Calyx of kidney+Ureter          | 6                         | 2                  | 0              | 1              | 3          |
| Bladder+ureter                  | 1                         | 1                  | 0              | 0              | 0          |

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41(45.6%) patients showed marked improvement, 06(6.7%) patients showed moderate improvement, 31(34.4%) patients showed mild improvement. In 11(12.2%) patients there was no significant improvement and only 01(1.1%) patient did not

improve. There was expulsion of calculi in 41 patients, with reduction in Symptoms Score and in 02 patients calculi were expelled but no improvement in Symptoms Score, hence not considered in marked improvement. (Figure No.2)

**Figure No. 2** Degree of improvement after treatment with *Lycopodium clavatum* (n=90).



## DISCUSSION

Similar to previous studies, in our study, male dominance and prevalence in urolithiasis in age group of 20-40 was recorded<sup>33</sup>. Male 72 (73.2%) Female 18 (26.8%) and maximum patients 58 (64.46%) were in age group of 20- 40 years.

After completion of study, when duration of illness is compared with outcome of study, it has been observed that cases enrolled with early duration of complaints viz. duration of illness up to 6 months (n=117) responded to *Lycopodium clavatum* ( $P= 0.000$ ) and also cases with long duration of illness responded well as shown statistically significant ( $P=0.001$ ).

In one of the similar kind of research study on urinary calculi, most useful medicine was *Lycopodium clavatum*.<sup>34</sup> our study supports these result.

As per the principles of Homoeopathy, one dose of *Lycopodium clavatum* was prescribed in 30C potency followed by placebo to each patient. In 05 patients,

30C potency was repeated at 3<sup>rd</sup> month. In 02 patients potency was raised to 200C because of slow improvement. 84 patients were prescribed this medicine as first prescription and 06 patients as second prescription.

The follow up period was only one year, so no case could be labeled as 'Cured'. Little more period of follow up of the study could have yield better results.

## CONCLUSION

This was an observational study with positive results in dissolution of urinary calculi with *Lycopodium clavatum* and these results need further validation by suitable studies.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

Nil.

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