An exploratory study on scientific investigations in homeopathy using medical analyzer*

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Background: The action of homeopathic medicines, in ultra-high dilution, is not directly observable. An attempt was made to explore autonomic response of selective homeopathic medicines, in healthy persons, using Medical Analyzer System (Electronics Division, Bhabha Atomic Research Centre, Mumbai, India).

Objective: The objective of the study was to observe the action of homeopathic medicines on physiologic variability of heart rate and blood flow.

Material and methods: Pre- and postinterventional variability spectra of heart rate and blood flow of 77 subjects were recorded with the Medical Analyzer System, administering homeopathic preparations of Aconitum napellus (6c, 10M), Arsenicum album (200c, 1M), Gelsemium sempervirens (200c, 1M), Phosphorus (200c, 1M), Pulsatilla nigricans (200c) and Sulphur (200c, 1M) versus placebo control. The amplitude of the peaks viz. low-frequency, medium-frequency, and high-frequency was measured for postintervention analysis. An increase in the amplitude of any valid peak by 100% or a decrease by 50% was considered as significant change.

Results: Aconitum napellus produced a response in heart rate variability (HRV) with 30c potency and in blood flow variability with 1M potency. Sulphur 200c and 1M, Gelsemium 200c and Pulsatilla 200c, produced a 62.5% response in HRV against the placebo response of 16.6%. Gelsemium, Phosphorus, and Sulphur produced a response in blood flow variability with a 1M potency, similar to the response of Aconitum napellus 1M.

Conclusions: These data suggest that it is possible to record the response of homeopathic medicines on physiologic parameters of the autonomic nervous system.

INTRODUCTION

Even while constitutional medicines offered by the homeopathic system of medicine have subjectively established their curative value over the past 2 centuries, the system has not found wide acceptance, mainly due to paucity of objective scientific data. The concentration of medicinal substance in 1 g of homeopathic preparation of 12c or above is less than $1 \times 10^{-24}$ g, which is beyond the material existence as per Avogadro’s number, and hence, cannot contain a single molecule of the medicinal substance. Since analytical methods are the prerequisite for obtaining any scientific data, several attempts were made in the past to establish analytical methods on homeopathic potencies. Spectroscopic analysis with nuclear magnetic resonance or infrared1–4 did not show any significant difference in the spectrum of pure carrier solvent material (alcohol) and homeopathic preparation. Paranjpe5 gave a physicist’s view to the action of homeopathic medicines. Using antigen–antibody reaction, Davenas et al. reported a positive reaction with homeopathic dilutions of antibodies (dilution with agitation), which was absent in simple dilutions up to a concentration of $10^{-120}$. They also observed the necessity of -OH group in the solvent for obtaining a positive antigen–antibody reaction.6 However, these experiments could not be reproduced.
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A new technique, physiologic variability analysis, can study the functioning of the autonomic nervous system (ANS) through variations in a physiologic parameter in a subject resting in the supine position to estimate the input contribution of different body systems to ANS. Several other studies have indicated that diseases of different organs of the body such as diabetes mellitus, myocardial infarction, hyperthyroidism, tuberculosis, and autoimmune deficiency syndrome cause specific alterations in physiologic parameters’ variability. Jindal et al. measured central and peripheral blood flow using impedance plethysmography (IPG), before and after the administration of homeopathic medicines to patients. They recorded increase in blood flow with Sepia officinalis CM and Sulphur 1M. As a natural extension of these observations, Jindal et al. had recorded heart rate variability (HRV) and blood flow variability (BFV) after administration of Sulphur 1M, Gelsemium 10M, and Phosphorus 30c and observed unique changes.

The Central Council for Research in Homoeopathy, India, took the initiative to extend these studies with a predefined protocol and meticulous methodology at the Regional Research Institute of Homoeopathy, Mumbai. The present article describes the effect of homeopathic potencies of Aconitum napellus, Arsenicum album, Gelsemium sempervirens, Phosphorus, Pulsatilla nigricans, and Sulphur on HRV and BFV.

OBJECTIVE

The objective of this investigation was to observe the changes produced in the variability spectrum of HRV and BFV following administration of placebo and different potencies of certain homeopathic medicines.

MATERIALS AND METHODS

The study group comprised 77 healthy volunteers ages 18–35 years. Laboratory investigations such as complete blood count, erythrocyte sedimentation rate, liver function tests, renal function tests, blood glucose level, serum lipid profile, electrocardiogram, and chest radiograph were done for all the subjects whose written consent was obtained after explaining the details of the study.

Medical Analyzer System (Electronics Division, Bhabha Atomic Research Centre, Mumbai, India) records the variability spectrum of heart rate and peripheral blood flow. This system is based on the principle of IPG and records blood volume changes in any part of the body noninvasively by measuring its electrical impedance, which is inversely proportional to blood volume changes. The rate of change of impedance thus gives the rate of change of blood volume. The pulsatile blood flow during ventricular systole is reflected as well-formed peaks in IPG, and the instantaneous heart rate is derived from the time elapsed between two consecutive peaks. The blood flow index (blood flow in milliliters per 1000 cm³ of body tissue per cardiac cycle) is obtained from the amplitude of the peak and gross electrical impedance of the body segment. A 5-minute record of instantaneous heart rate and blood flow values thus obtained is interpolated to obtain periodic values of these parameters as a prerequisite before Fourier transformation. The subsequent Fourier transform depicts the contribution of various rhythms that cause variability in the physiologic parameters and represented by low-frequency (L), medium-frequency (M), and high-frequency (H) peaks (Fig. 1). “L” generally denotes sympathetic action, whereas “M” and “H”
denote parasympathetic and/or vagal action. In view of the multiple readings required in this protocol, the system software was upgraded to incorporate the average variability spectrum, for HRV and BFV obtained from multiple readings in situ, pre- and postintervention, and display these on a PC monitor.\(^{14}\)

Medicines coming in the top grade on repertory search related to cardiorespiratory functions such as variations in pulse, respiration, breathing, and so on were considered for deciding on trial drugs. For rubric selection, “Complete Repertory” by Roger van Zandvoort\(^ {15}\) was consulted. In such a group, both polychrests as well as remedies having specific action on these parameters were included. As placebo, plain globules soaked with dispensing alcohol were used. Since it was an exploratory study, two different protocols were followed for the experiments.

**Protocol 1** was designed for the *Aconitum napellus* group of 27 subjects. On the first day, pre- and postintervention data for placebo were obtained. Placebo was replaced by *Aconite* 6c, *Aconite 30c*, *Aconite 200c*, *Aconite 1M*, and *Aconite 10M* on the 2nd, 3rd, 4th, 5th, and 6th days, respectively. Data analysis was done for each of the days.

**Protocol 2** was designed for a group of 50 subjects and comprised *Arsenic 200c* (n = 5), *Arsenic 1M* (n = 5), *Gelsemium 200c* (n = 5), *Gelsemium 1M* (n = 5), *Phosphorus 200c* (n = 5), *Phosphorus 1M* (n = 6), *Pulsatilla 200c* (n = 4), *Sulphur 200c* (n = 10), and *Sulphur 1M* (n = 5). In this case, pre- and postintervention data were recorded with placebo on the 1st day and with any one of the selected medicines and potency on the 2nd day.

Since the study has been exploratory in nature, it was the intention of the authors to investigate all possibilities under this study. Protocol 1 was designed to meticulously investigate the effect of a particular medicine in different potencies. Protocol 2 was designed in such a manner that a large number of medicines could quickly be screened for their action on variability spectrum.

Data for both of the protocols were collected as follows. With the subject in the supine position, the IPG signal was recorded from the wrist of the subject for 5 minutes and three such consecutive observations were taken. The subject was then administered the desired substance (placebo or medicine) followed by recording of three similar readings again to obtain the postintervention data of HRV and BFV. The pre- and postintervention data were then analyzed using the software described above, which gave out average spectra of multiple readings.

In the postintervention analysis, a change was considered significant if the amplitude of any valid peak either increased by 100% (designated as “+”) or decreased by 50% (designated as “-”) as compared to the pre-intervention amplitude. These high thresholds were intentionally fixed in order to have reliable findings. Amplitude of the peak had been chosen for postintervention analysis in place of area under the same because the former had the advantage of noise elimination. A postintervention decrease in the first peak (L-) and increase in the third peak (H +) were discarded because these changes commonly occur due to prolonged rest.

**RESULTS**

Typical pre- and postintervention average variability spectra are depicted in Figures 2 and 3. As can be seen from Figure 2, the placebo has not elicited any response in either spectra (HRV or BFV). The medicine has produced change in both HRV and BFV as illustrated in Figure 3.
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Table 1 summarizes the results of intervention in HRV. Under Protocol 1, Aconite 30c has shown response in HRV. From Protocol 2, it is seen that the maximum response has been obtained in 200c potency, namely, Gelsemium 200c, Pulsatilla 200c, and Sulphur 200c accounting for 68.42%. The exception in this group is Sulphur 1M, which has shown some response in HRV and together, they account for 62.5% response in HRV as against 16.6% of placebo response.

Table 2 summarizes the response in BFV. From Protocol 1, Aconite 1M has shown response in BFV in 53.9% of the study sample as against placebo response of 25.92%. From Protocol 2, Gelsemium 1M, Phosphorus 1M, and Sulphur 1M have shown response in BFV, the exception here being Phosphorus 200c, which has shown a similar response. Response produced by medicines is 40% as against placebo response of 0%.

Other potencies of Aconite from Protocol 1 and both potencies of Arsenic in Protocol 2 have not shown any response either in HRV or in BFV.

Table 1 - Response in Heart Rate Variability After Intervention

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Total number of volunteers/ number responded (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconitum napellus</td>
<td></td>
</tr>
<tr>
<td>30c</td>
<td>(27 subjects) 27/12 (44.4%) 27/15 (55.5%)</td>
</tr>
<tr>
<td>Gelsemium 200c</td>
<td>Placebo 5/1 (20%)  Medicine 5/3 (60%)</td>
</tr>
<tr>
<td>Pulsatilla 200c</td>
<td>Placebo 4/1 (25%)  Medicine 4/3 (75%)</td>
</tr>
<tr>
<td>Sulphur 200c</td>
<td>Placebo 10/1 (10%)  Medicine 10/7 (70%)</td>
</tr>
<tr>
<td>Sulphur 1M</td>
<td>Placebo 5/1 (20%)  Medicine 5/2 (40%)</td>
</tr>
</tbody>
</table>

Table 2 - Response in Blood Flow Variability After Intervention

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Total number of volunteers/ number responded (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconite 1M</td>
<td>(27 subjects) 27/7 (25.92%) 26/14 (53.9%)</td>
</tr>
<tr>
<td>Gelsemium 1M</td>
<td>Placebo 5/0 (0%)  Medicine 5/2 (40%)</td>
</tr>
<tr>
<td>Phosphorus 1M</td>
<td>Placebo 6/1 (16.66%)  Medicine 6/2 (33.33%)</td>
</tr>
<tr>
<td>Phosphorus 200c</td>
<td>Placebo 5/0 (0%)  Medicine 5/2 (40%)</td>
</tr>
<tr>
<td>Sulphur 1M</td>
<td>Placebo 5/0 (0%)  Medicine 5/2 (40%)</td>
</tr>
</tbody>
</table>

Fig. 3. Pre- and postintervention average spectrum of heart rate variability (HRV) and blood flow variability (BFV) after Sulphur 1M. In HRV, the third peak is decreased in amplitude to almost half, whereas in BFV the first peak amplitude has nearly doubled.
DISCUSSION

This exploratory study on HRV and BFV with homeopathic medicines on a defined protocol was the first of its kind using average spectra of multiple readings. The results show that certain medicines and potencies have action on either HRV or BFV (Tables 1 and 2).

Though the study was on an elaborate scale with defined protocol and multiple readings per setting, it was still exploratory in nature and, therefore, the study protocol and interpretation of results were not strictly in conformity with conventional pharmacologic trials of modern medicine. Some deviations were made in the analysis of variability data. Hitherto, the variability spectrum was analyzed on the basis of area under different peaks, which represents the contribution of a particular rhythm in autonomic control. Though this approach has the simplicity of machine-generated results, it completely overlooks the amplitude of a particular peak, which signifies the coordination of a particular rhythm in autonomic control. For instance, a narrow peak with high amplitude may have the same area as that of a very broad peak with very low amplitude; in reality, the narrow peak with high amplitude represents better coordination in the ANS. This has led to using the amplitude of the peak in variability spectrum to differentiation the response. Furthermore, the spectrum was analyzed between 0.02 Hz and 0.50 Hz as compared to the 0.00–0.50 Hz conventionally used. This was because the zero frequency component of variability sometimes suppresses the higher frequency components and can lead to incorrect inference. Since our interest was to study the various rhythms controlling ANS and not the zero frequency component, the deviation was justified.

In conducting the experiments, even though a separate placebo group has not been tested, placebo was administered on the first day of the experiment and medicinal intervention on following day(s) in all the subjects, irrespective of the group or medicine they belong to. This was done so that subjects act as their own control because physiologic response of every individual is different even in similar circumstances. To keep the variables at minimum, in all the subjects the experiments were conducted under similar conditions, and for each individual, the same timing was maintained throughout the experiment.

The experiment began with the Aconite group, as per Protocol 1, and various potencies were used in ascending order in each of the individuals. The observed outcome was potency specific. Aconite 30c produced a peak in HRV (Fig. 4A) and Aconite 1M produced a similar peak in BFV (Fig. 4B). It shows that Aconite acts at one level in 30c potency and another level in 1M potency. Other potencies did not show any remarkable changes either in HRV or BFV.

The results in the second group of subjects under Protocol 2 also revealed similar observations. Gelsemium 200c, Pulsatilla 200c, Sulphur 200c, and Sulphur 1M (1M potency is an exception here) produced response peaks in HRV (Table 1) and Gelsemium 1M, Phosphorus 1M, Sulphur 1M, and Phosphorus 200c (200c potency is an exception in this group), produced response peaks in BFV (Table 2). Arsenic did not produce any response in either of the potencies. The observations were consistent with those of Aconite: lower potency produced a response in HRV and higher potency produced a response in BFV, with the exception of Sulphur 1M in HRV and Phosphorus 200c in BFV.

A pattern seems to emerge from these experiments: medium potencies such as 30c and 200c have probable action on HRV and higher potency such as 1M has probable action on BFV. It is noteworthy that HRV and BFV are derived from the same data, yet the changes produced in both are by different potencies of the same medicine. To illustrate, the change in HRV at Aconite 30c is not reflected in BFV, and change in BFV at Aconite 1M is not reflected in HRV. This may be indicative of the selective action of different potencies of the same medicine. Plotting of the same shows a peak in HRV response, with Aconite 30c (Fig. 4A) and a peak in BFV response with Aconite 1M (Fig. 4B). These peaks separate the response of Aconite 30 and 1M from the rest of the interventions in this group.

![HRV Responses in %](image1)

![BFV Responses in %](image2)

Fig. 4. A. Heart rate variability (HRV) response to various potencies of Aconite napellus. B. Blood flow variability (BFV) response to various potencies of Aconite napellus.
The response has been counted positive, irrespective of its nature. For instance, significant increase or decrease in either of the L, M, or H peaks has been counted as response. In future such studies, the authors may explore the possibility of analyzing response with respect to a particular peak or parameter.

This was an exploratory study on variability in physiologic parameters, conducted for the first time on this subject hitherto unexplored in a systematic manner with homeopathic medicines. Eliciting an autonomic tone of healthy subjects with the help of homeopathic medicine is a process similar to that of a “homeopathic pathogenetic trial” in which, when a medicinal substance is given to a group of healthy provers, some symptoms of the medicine are elicited in a number of proving subjects, yet there are few symptoms that appear in 1 or 2 subjects only. Some subjects do not prove any of the symptoms of a medicine. This perhaps explains why some of the medicines/potencies did not show any response.

A few observations emerge from the experiments. First, the autonomic nervous system is not under voluntary control, and recording signals from the ANS immediately after intervention (within 5–15 minutes of intervention) helped eliminate subjective bias and objectively demonstrated the action of the homeopathic medicines.

Second, responses were observed in 30c, 200c, and 1M potencies, which are well beyond Avogadro’s number. These are observations from exploratory experiments in emerging areas of physiologic variability and need validation by repeated experiments of this type. Detection of response was the primary objective of this study, which has been achieved. The number of subjects in each group was small; hence it was not possible to show the statistical significance of the results, an aspect that is intended to be covered in future studies.

CONCLUSIONS

Selective response has been detected with the administration of different potencies of various homeopathic medicines in HRV and BFV, and the exploratory study has provided directions for future trials. Reproducibility of these observations in a larger sample size will be necessary for validation of this study’s results.

ACKNOWLEDGMENTS

The authors are grateful to Dr. G.D. Jindal, Electronics Division, Bhabha Atomic Research Centre, Mumbai for providing the expertise needed for the study. They thank all of the students, interns, and faculty members of CMP Homoeopathic Medical College, Mumbai for enthusiastic participation in the study as volunteers. The authors acknowledge the contributions of Mrs. Uma A. Clerk and other staff of the Regional Research Institute for Homoeopathy, Mumbai.

REFERENCES

Evaluation of anti-leukemic activity of potentised Catharanthus roseus: An in-vitro study

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2 Dept. of Pharmaceutical Chemistry, MAEER’s Maharashtra Institute of Pharmacy, Pune, India

Alcoholic extract of potentised Catharanthus roseus (L.) G. Don. was evaluated for its anti-leukemic activity in in-vitro model. For this in-vitro study two cell lines namely, jurkat and K562 for promyelocytic leukemia and chronic myeloid leukemia were used. The cell lines were grown in Roswell Park Memorial Institute Medium (RPMI 1640 medium) containing 10% fetal bovine serum and 2mM L-glutamine. Doxorubicin (Adriamycin, ADR) was used as positive control drug. Growth inhibition of 50 % (GI50) was calculated from [(test growth in the presence of drug at the four concentration levels Ti – time zero Tz)/(control growth C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50 in sulforhodamine B (SRB) assay. The Catharanthus roseus in 2X potency showed maximum anti-leukemic activity in molar drug concentration of 20 µg/ml on human leukemic cell line K562 for chronic myeloid leukemia.

Keywords : anti-leukemic activity, Catharanthus roseus (L.) G. Don., chronic myeloid leukemia

INTRODUCTION

More than 100 alkaloids and related compounds have so far been isolated and characterised from the plant Catharanthus roseus.

The alkaloid contents in different parts of the plant show large variations as roots have 0.14-1.34%, stem 0.074-0.48%, leaves 0.32-1.16%, flowers 0.005-0.84%, fruits 0.40%, seeds 0.18% and pericarp 1.14%. These alkaloids include monomeric indole alkaloids, 2-acyl indoles, oxindole, α-methylene indolines, dihydroidones, bisindole and others. Dry leaves contain vinblastine (vincleucoblastine or VLB) 0.00013-0.00063%, and vincristine (leurocristine or LC) 0.0000003-0.0000153% which have anti-cancerous activity.1

Both vinblastine and vincristine at relatively low doses (<1 mg/kg/day for 10 days) can prolong the life span of leukemic animals by 100% and more. Vincristine is particularly effective against leukemia.

Chronic myeloid leukemia (CML) is a clonal disorder of pluripotent stem cell (stem cell that has the potential to differentiate into any of the three germ layers) involving myeloid, erythroid, megakaryocytic and lymphoid cells. More than 90% of cases have a cytogenetic abnormality involving reciprocal translocation between the long arms of chromosomes 9 and 22 (t9; 22) (q34:q11). The oncogene c-abl, located on the long arm of chromosome 9, get translocated to chromosome 22, where a specific gene called breakpoint cluster region (bcr) is found. Both abl and bcr form a fusion gene, abl/bcr, which encodes an unregulated, cytoplasm-targeted tyrosine kinase that allows the cells to proliferate without being regulated by cytokines.2

In contrast to normal bone marrow, which is usually about 50% cellular and 50% fat, the marrow of a patient with CML is usually 100% cellular. This increase in cellularity of CML marrow is from the maturing granulocytic precursor. Increased number
of megakaryocytes, often including small dysplastic forms, is frequently observed, whereas erythroid progenitors are usually present in normal or decreased number. Peripheral blood examination reveals a marked leukocytosis, often exceeding 100,000 cells/mm.\(^4\) The circulating cells are predominantly neutrophils, metamyelocytes, and myelocytes, with less than 10% myeloblasts.\(^2\)

**OBJECTIVES**

To evaluate the anti-leukemic activity of potentised whole plant extract of *Catharanthus roseus* in *in-vitro* model by SRB assay.

Determination of concentration or quantification of vincristine alkaloid by HPLC (High Performance Liquid Chromatography) in whole plant extract of *Catharanthus roseus*.

**MATERIAL AND METHODS**

*Plant Material:* Whole plant of *Catharanthus roseus* (Apocynaceae) was collected from the Beed district of Maharashtra state in the month of June 2009. It was authenticated as *Catharanthus roseus* (L.) G. Don. belonging to family Apocynaceae, by Prof. Dileep Pokle, Head of Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra and accession no. was 5844.

For preparation of alcoholic extract, the crude drug was dried for 15 days and then coarsely powdered. The coarse powder was loaded in thimble made up of Whatman filter paper no. 1 and extracted in the Soxhlet continuous extraction column with 3 litres of 95% alcohol for 10 cycles for each of two batches. The extract so obtained was viscous with characteristic odour. The excess of alcohol was evaporated on the water bath. The final extract was thick and was stored separately in amber colour bottle of 30 ml capacity.

The potencies up to 12X of *Catharanthus roseus* were made under Class IV of Hahnemannian classification by using 95% ethyl alcohol as solvent in ratio 1:5 (Dry drug: alcohol)\(^3\) in Department of Pharmacy, Sonajirao Kshirsagar Homoeopathic Medical College, Beed, Maharashtra. The drug strength was 1:10.

Qualitative Analysis: Phytochemical evaluation of sample by Thin Layer Chromatography (TLC): 50mg of the sample was dissolved in 5ml of methanol. To 2ml of the solution was added a few drops of Dragendorfs reagent.

Quantitative Analysis: Analysis of sample by High Performance Liquid Chromatography (HPLC): 2.135 g of sample was dissolved in 10x2 ml methanol and sonicated for 30 minutes, filtered and made up volume to 25 ml with methanol.

For *in-vitro* Cytotoxicity study, total 5 compounds in coded form were submitted to Advanced Centre for Treatment Research & Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai, through Dr. A.S. Juvekar, Officer-in-charge, Anticancer Drug Screening Facility.

**Control Group:**

Dexorubicin (Adriamycin) procured from Pfizer Pharmaceuticals with batch number BQL008-B 88J004 was used as control.

For analysis by Thin Layer Chromatography: 10 mg of standard vincristine sulphate was dissolved in 1 ml of methanol.

For analysis by High Performance Liquid Chromatography: 20 mg of standard vincristine sulphate was dissolved in 10 ml of methanol and subsequent dilutions were made to get the final concentration of 100 µg/ml.

**Experimental Group:**

In experimental group, the following codes were used for the drugs:

<table>
<thead>
<tr>
<th>Code</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA-1</td>
<td>Crude Drug Substance</td>
</tr>
<tr>
<td>USA-2</td>
<td><em>Catharanthus roseus</em> Mother Tincture</td>
</tr>
<tr>
<td>USA-3</td>
<td><em>Catharanthus roseus</em> 2X</td>
</tr>
<tr>
<td>USA-4</td>
<td><em>Catharanthus roseus</em> 6X</td>
</tr>
<tr>
<td>USA-5</td>
<td><em>Catharanthus roseus</em> 12X</td>
</tr>
</tbody>
</table>

**Quality Control**

The following procedures were followed to enhance quality and reliability of experiment:

1. Tests for alkaloids
   a. Qualitative: Phytochemical evaluation of sample by Thin Layer Chromatography (TLC) by using Silica gel 60F\(_{254}\) pre-coated TLC plate (Merck) absorbent.
   b. Quantitative: Analysis of sample by High Performance Liquid Chromatography (HPLC) by using HiQ sil C18 HS 4.6mmX250mm 5µm column.

2. *In-vitro* cytotoxic studies by using sulforhodamine (SRB) assay.
**Phytochemical Evaluation of Sample by TLC**

**Sample preparation:**

50mg of the sample dissolved in 5ml of methanol
Silica gel 60F$_{254}$ pre-coated TLC plate (Merck)

**Absorbent:**

Toulene:Ethylacetate:Diethyl amine: (70:20:10)

**Chromatography solvent/mobile Phase:** Ascending

**Spraying Reagent:** Dragendorfs reagent

**Amount spotted:** 10µl

**Band width:** 6mm

**Phytochemical Evaluation of Sample by HPLC**

**Column:** HiQ sil C18 HS 4.6mmX250mm 5µm

**Mobile phase:** Acetonitrile: phosphate buffer (30:70) pH 3.6

**Wavelength:** 260nm

**Run time:** 30 min

**Sample preparation:** 2.13g of sample was dissolved in 10 X 2ml of methanol and sonicate for 30 min. filter and made up the volume to 250ml with methanol

**In-vitro Cytotoxicity Studies**

**Vehicle used for the test articles:** Ethyl Alcohol

**Source of Cell Lines:** National Cancer Institute (NCI), USA and National Centre for Cell Science (NCCS), Pune.

**Method of Testing:** Sulforhodamine B assay.

### Cell Lines details:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Cell Line</th>
<th>Human Tissue Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>jurkat</td>
<td>Leukemia (Promyelocytic Leukemia)</td>
</tr>
<tr>
<td>2.</td>
<td>K562</td>
<td>Leukemia (Chronic Myeloid Leukemia)</td>
</tr>
</tbody>
</table>

Compound with G150 ≥ 50 is considered to be active at the respective concentration

**Experimental Setup**

**Experimental Procedure for TLC**

Slurry of the stationary phase, generally in water, was applied to a glass, plastic or foil plate, generally 20 cm square, as a uniform thin layer by means of a plate spreader starting at one end of the plate and moving progressively to the other. The layer was 0.25 mm thick. Once the slurry layer had been prepared, the plates dried to leave the coating of stationary phase. Dried in an oven at 100 to 120°C served to activate Silica gel 60F$_{254}$ pre-coated TLC plate (Merck) absorbent.

The 50 mg of the sample dissolved in 5 ml of methanol was applied to the plate 2.0 to 2.5 cm from the edge by means of a micropipette or microsyringe. The solvent was removed from the spot by gentle heating or by use of an air blower. It was then possible to apply more sample to the spot if necessary. In thin layer chromatography, the sample was applied as a band across the plate rather then as a single spot.

Plate development: Separation most commonly took place in a glass tank that contained the developing Toulene:Ethylacetate:Diethyl amine: (70:20:10) (mobile phase) to a depth of about 1.5 cm. This was allowed to stand for at least 1 hour with a lid over the top of the tank to ensure that the atmosphere within the tank became saturated with solvent vapour (equilibration). After equilibration, the lid was removed, and the thin layer plate was then placed vertically in the tank so that it stood in the solvent. The lid was replaced and separation of the compounds then occurred as the solvent travelled up the plate. It was possible to develop the plate in a horizontal plane by connecting the sample end of it to a reservoir of mobile phase by means of a suitable wick. It was preferable to keep the system at a constant temperature whilst the development was occurring, to avoid anomalous solvent-running effects.

Analyte detection: Examination of the plate under ultraviolet light at 254 nm showed the position of ultraviolet-absorbing or fluorescent compounds. Spraying of plates with Dragendorfs reagent would stain certain compounds. Although the movement of compounds on TLC characterised by specific Rf (distance moved by analyte from origin/ distance moved by solvent front from origin) values, these measurements
were not always reproducible. Component identification was made based on comparison of the movement of the components with those of reference compounds chromatographed alongside the sample on the TLC plate.

On plate, quantification was achieved by using radiochromatograph scanning in the case of radiolabelled compounds or more generally by means of densitometry.

**Experimental Procedure for HPLC**

The stainless steel column of HiQ sil C18 HS 4.6mmX250mm 5µm was made to withstand pressure of upto 5.5 X 10^7 pa without cyclic variation.

Column packing: High pressure slurring technique was used. A suspension of the packing was made in a solvent density equal to that of the packing material. The slurry was then pumped rapidly at high pressure into a column with a porous plug at its outlet.

Mobile phase and pump: Acetonitrite:phosphate buffer (30:70), pH 3.6 solvent purified as traces of impurities affect the column and interfere with the detection. Solvent was degassed before use because gassing is bad for aqueous methanol, alter column resolution and interfere with the continuous monitoring of the effluent.

Application of sample: Microsyringe used to inject the 2.135 g of sample was dissolved in 10 X 2ml of methanol and sonicated for 30 minutes filtered and made the volume to 250 ml with methanol.

Detection: The detection was carried out at 260 nm wavelength by use of diode array technique, which allowed the simultaneous measurement of absorbance at many or all wavelength within 0.01s.

**Experimental Procedure for Sulforhodamine B Assay**

The cell lines were grown in Roswell Park Memorial Institute Medium 1640 (RPMI 1640 medium) containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, one 96 well plate containing 5*10^6 cells/well was fixed in situ with TCA (Trichloroacetic Acid), to represent a measurement of the cell population at the time of drug addition (Tz). Experimental drugs were initially solubilised in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells X 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percent growth inhibition was calculated as:

\[
\frac{(Ti-Tz)/(C-Tz)) x 100 \text{ for concentrations for which } Ti/>=Tz (Ti-Tz) \text{ positive or zero} \\
\frac{((Ti-Tz)/Tz} x 100 \text{ for concentrations for which } Ti<Tz (Ti-Tz) \text{ negative}
\]

The dose response parameters were calculated for each test article.

Growth inhibition of 50 % (GI50) was calculated from \([(Ti-Tz)/(C-Tz)]= 50\), which is the drug concentration resulting in a 50% reduction in the net
protein increase (as measured by SRB staining) in control cells during the drug incubation.

The drug concentration resulting in total growth inhibition (TGI) was calculated from \( Ti = Tz \). The concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning (LC50) indicating a loss of cells following treatment is calculated from \( [(Ti-Tz)/Tz] \times 100 = 50 \).

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

**Results**

**Qualitative:** Phytochemical evaluation of methanol extract sample by Thin Layer Chromatography (TLC) shows three bands giving positive tests for alkaloids at Rf 0.37, 0.33 and 0.241 of which one band corresponds to vincristine sulphate. The details are given in Figure 1.

**Quantitative:** Analysis of alcoholic extract of potentised Catharanthus roseus (L.)G. Don. by high performance liquid chromatography (HPLC) showed the presence of 0.036% of alkaloid vincristine.

In processed sample by HPLC, total 26 constituents are identified in which vincristine have: (Table 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>18.865 minutes</td>
</tr>
<tr>
<td>Height</td>
<td>927.8 µV</td>
</tr>
<tr>
<td>Area%</td>
<td>0.419%</td>
</tr>
<tr>
<td>Area</td>
<td>459.6 µV min.</td>
</tr>
</tbody>
</table>

Figure 1: TLC-Fingerprinting of sample
**Table 1:** 26 processed constituents of extract sample at Vincristin_Sample Run 3 9 10 94 Data- 260.00 nm

<table>
<thead>
<tr>
<th>Index</th>
<th>Name</th>
<th>Time (Minutes)</th>
<th>Height (µV)</th>
<th>Area (%)</th>
<th>Area (µV. minutes)</th>
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<td>2.346</td>
<td>121657.5</td>
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<td>54755.7</td>
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<td>3</td>
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<td>3.280</td>
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<td>8</td>
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<td>3159.7</td>
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<tr>
<td>9</td>
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<td>5.066</td>
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<td>1.373</td>
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<td>0.187</td>
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<td>15</td>
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<td>1505.9</td>
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<td>16</td>
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<td>17</td>
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<td>430.4</td>
</tr>
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<td>18</td>
<td>UNKNOWN</td>
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<td>2.059</td>
<td>2260.1</td>
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<td>19</td>
<td>UNKNOWN</td>
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<td>2500.3</td>
<td>0.850</td>
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<td>1489.4</td>
<td>0.518</td>
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<td>21</td>
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<td>1398.2</td>
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<td>15061.8</td>
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<tr>
<td>24</td>
<td>VINCRIStINE</td>
<td>18.865</td>
<td>927.8</td>
<td>0.419</td>
<td>459.6</td>
</tr>
<tr>
<td>25</td>
<td>UNKNOWN</td>
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<td>597.8</td>
<td>0.262</td>
<td>287.4</td>
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<td>26</td>
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<td>23.278</td>
<td>4214.5</td>
<td>2.195</td>
<td>2408.7</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>371076.2</strong></td>
<td><strong>100.000</strong></td>
<td><strong>109743.8</strong></td>
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</tbody>
</table>
**Evaluation of anti-leukemic activity of potentised Catharanthus roseus: an in-vitro study**

**Usha R. Kushwaha et al**

### In-vitro cytotoxic studies by using sulforhodamine assay

The extract of *Catharanthus roseus* in individual potencies used during study showed maximum cytotoxic activity at following molar drug concentration in µg/ml:

1. **Cell line K562**

<table>
<thead>
<tr>
<th>Drug used</th>
<th>Average value*</th>
<th>Molar drug concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude drug substance</td>
<td>49.4</td>
<td>80</td>
</tr>
<tr>
<td>Catharanthus roseus 1X</td>
<td>33.1</td>
<td>80</td>
</tr>
<tr>
<td>Catharanthus roseus 2X</td>
<td>12.5</td>
<td>20</td>
</tr>
<tr>
<td>Catharanthus roseus 6X</td>
<td>22.5</td>
<td>80</td>
</tr>
<tr>
<td>Catharanthus roseus 12X</td>
<td>33.1</td>
<td>40</td>
</tr>
<tr>
<td>ADR</td>
<td>9.0</td>
<td>80</td>
</tr>
</tbody>
</table>

*Catharanthus roseus* 2X showed maximum cytotoxicity of 12.5 in K562 cell line at molar drug concentration of 20 µg/ml. The details are given in Figure 3.

2. **Cell line jurkat**

<table>
<thead>
<tr>
<th>Drug used</th>
<th>Average value*</th>
<th>Molar drug concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude drug substance</td>
<td>38.6</td>
<td>80</td>
</tr>
<tr>
<td>Catharanthus roseus 1X</td>
<td>38.0</td>
<td>80</td>
</tr>
<tr>
<td>Catharanthus roseus 2X</td>
<td>6.6</td>
<td>80</td>
</tr>
</tbody>
</table>

The *Catharanthus roseus* extract in human leukemic cell line jurkat showed maximum cytotoxicity of 6.6 at molar drug concentration of 80 µg/ml in 2X potency as shown in Table 2.

### DISCUSSION

It is known that vincristine sulphate is an antineoplastic agent which may act by arresting mitosis at the metaphase. It is given intravenously in the treatment of acute leukemia of children. This experimental work evaluated the anti-leukemic activity of potentised whole plant extract of *Catharanthus roseus* (L.)G. Don. The potencies up to 12X of *Catharanthus roseus* were made under Class IV of Hahnemannian classification by using 95% ethyl alcohol as solvent.

By analysis of sample by HPLC, total 26 constituents were identified in which one alkaloid was vincristine. Therefore, it is considered that medicinal properties of the whole plant extract are better than those of the alkaloid vincristine/vinblastine alone because homoeopathic medicines are prepared using the whole plants, leaves, fruits etc. as the case may be without

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*Average value indicates the mean of all the three experiments done.*
Evaluation of anti-leukemic activity of potentised Catharanthus roseus: an in-vitro study
Usha R. Kushwaha et al

Table 2: Molar drug concentration and cytotoxic activity during study in Human Leukemic Cell Line jurkat

<table>
<thead>
<tr>
<th>Molar drug concentration (µg/ml)</th>
<th>USA-1</th>
<th>USA-2</th>
<th>USA-3</th>
<th>USA-4</th>
<th>USA-5</th>
<th>ADR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>100.0</td>
<td>100.0</td>
<td>95.1</td>
<td>88.8</td>
<td>92.5</td>
<td>7.5</td>
</tr>
<tr>
<td>20</td>
<td>100.0</td>
<td>100.0</td>
<td>77.2</td>
<td>83.7</td>
<td>90.1</td>
<td>4.2</td>
</tr>
<tr>
<td>40</td>
<td>85.3</td>
<td>59.2</td>
<td>32.4</td>
<td>58.9</td>
<td>56.3</td>
<td>1.6</td>
</tr>
<tr>
<td>80</td>
<td>38.6</td>
<td>38.0</td>
<td><strong>6.6</strong></td>
<td>42.9</td>
<td>37.9</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The details of LC50, TGI and G150 calculation are given in Table 3.

Table 3: Results of LC50, TGI and G150 calculation of human cell lines K562 and jurkat

<table>
<thead>
<tr>
<th></th>
<th>K562</th>
<th>Jurkat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC50</td>
<td>TGI</td>
</tr>
<tr>
<td>Crude drug substance</td>
<td>&gt; 80</td>
<td>&gt; 80</td>
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<tr>
<td>Catharanthus roseus 1X</td>
<td>&gt; 80</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>Catharanthus roseus 2X</td>
<td>&gt; 80</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>Catharanthus roseus 6X</td>
<td>&gt; 80</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>Catharanthus roseus 12X</td>
<td>&gt; 80</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>ADR</td>
<td>&gt; 80</td>
<td>&gt; 80</td>
</tr>
</tbody>
</table>

Figure 3: Report of in-vitro screening of USA 1-5 in human leukemic cell line K562
Evaluation of anti-leukemic activity of potentised Catharanthus roseus: an in-vitro study

Usha R. Kushwaha et al

The alcoholic extracts of potentised Catharanthus roseus 2X showed antileukemic activity in chronic myeloid leukemia on human leukemic cell line K562, with a G150< 10.

CONCLUSION

ACKNOWLEDGEMENT

REFERENCES


Pharmacognostic and physico-chemical evaluation of *Lactuca sativa* L.

P. Subramanian¹, P. Padma Rao¹, P.R. Reddy¹ and H.C. Gupta²

¹Drug Standardization Unit, Hyderabad, India  
²Central Council for Research in Homoeopathy, New Delhi, India

*Lactuca sativa* L. is an erect, annual herb belonging to the family Asteraceae. The leaves are useful in angina pectoris, ascitis, asthma, constipation, cough, diarrhea, hysteria, gonorrhoea, affections of liver and spleen and pain in pylorus and spinal cord. Leaves are long, thin, orbicular or oblong, obovate, crisp and radical. The trichomes are uniseriate filiform capitate, uniseriate macroform conical and biseriate filiform capitate types; mesophyll is undifferentiated; laticiferous ducts and prismatic crystals are present in ground tissue; midvein bundle is b collateral. The powder microscopic and organoleptic characters are provided.

Physico-chemical parameters of raw drug viz., extractive values, ash values, formulation, besides weight per ml., total solids, alcohol content along with thin layer chromatography (TLC) and ultraviolet spectroscopic (UV) studies have been undertaken for mother tincture for the first time.

**Keywords:** Homoeopathy; Lactuca sativa L.; Standardization; Pharmacognosy; Physico-Chemical; Pharmacopoeia.

**INTRODUCTION**

*Lactuca sativa* L., commonly known as ‘garden lettuce’ in English and ‘salad’ locally, is an annual herb belonging to the family Asteraceae. It originated in the warmer temperate parts of Western Asia, including Eastern Mediterranean and is also found in the Himalayas. It is widely cultivated throughout India.¹ The plant is an erect, glabrous, herbaceous annual, 0.5 – 1.2 m high. Leaves 12.5 – 25 cm long, thin, nearly orbicular, oblong or obovate, ligulate, plane, bullate or curled; flower heads of yellow, rays; achenes lenticular-oblong, dark brown or greyish brown, with slender beak and white pappus.¹

The leaves are reported to be useful in angina pectoris, ascitis, asthma, constipation, cough, diarrhea, gonorrhoea, heartburn, hysteria, lactation, affections of liver and spleen, muscae volitantes, noises in ears, pain in pylorus and spinal cord, excessive sleep, whooping cough and yawning. It is also used in poultices for burns and painful ulcers.² ³ ⁴


Chemically, lettuce is the fair source of various minerals and vitamins. The elements present in significant amounts are Mn, Zn, Cu, and Fe.⁵ Fonofos, a thiophosphonate⁶; amyrin, ergosterol, vitamin E and an anti-oxidant¹; lactucin, lactupicrin, sesquiterpene glycoside-lactucide C & A; Besides, carotenoids lactucaxanthin⁷, it also contains an alkaloid, lactucarium, which is a mixture of lactocin and three bitter principles viz., lectucin, lectopicrin and lactucic acid. Besides it also contains lectucerin, a waxy substance and a trace of hyoscyamine.²

Earlier studies on *Lactuca sativa* L. pertaining to pharmacogostic and physico-chemical parameters in general, and in homoeopathic perspective in particular, are not available. Hence, the authors have undertaken detailed pharmacognostic and physico-chemical studies for the first time as per the protocols suggested by Central Council for Research in Homoeopathy (CCRH), Government of India.
MATERIAL AND METHODS

Pharmacognosy

The leaf material of Lactuca sativa L. was supplied by Survey of Medicinal Plants and Collection Unit of CCRH, Nilgiris, Tamil Nadu. The leaves were fixed in F.A.A. (Formaldehyde: Acetic acid : Alcohol) and processed for microtomy (paraffin method), sectioned, stained and permanent slides prepared following Johansen. The epidermal peels were obtained by gently scraping and peeling with razor blade. The powder microscopy characters were studied by boiling the powder drug in distilled water, stained in saffrarin and mounted with glycerine. Photomicrography was done with Olympus CH – 2 trinocular research microscope.

Physico-chemical

The airdried sample of the leaves was coarsely powdered and was subjected to determination of moisture content (loss on drying at 105°C), total ash, water soluble ash, acid insoluble ash, extractability in water and alcohol for raw drug and weight per ml, total solids and alcohol content for the finished product. The above parameters were determined as per procedure given in Homoeopathic Pharmacopoeia of India. The mother tincture was prepared as per H.P.U.S.10

Mother tincture (alcoholic extract) was studied for its physico-chemical characters, chromatographic and spectroscopic absorbance. All chemicals and solvents used were of Analytical Grade (AR). Silica gel ‘G’ (E Merck, India) was used for thin layer chromatography (TLC) and the work was carried out at the room temperature. The TLC plate was developed using hexane : ethyl acetate (9:1, v/v) as mobile phase; vanillin - sulphuric acid was used as spraying reagent. The mother tincture was diluted with methanol and UV spectroscopy was done. The maximum absorption was recorded.

OBSERVATIONS AND RESULTS

Macroscopic

Leaves 12.5 – 25 cm long, thin, nearly orbicular, oblong or obovate, ligulate, plane, bullate or curled, crisp and radical.

Microscopic

Epidermal cells in surface 5 or 6 sided, polygonal, isodiametric to anisodiametric, sides straight to curved and wavy on adaxial, and sinuate on the abaxial side; surface smooth and at places striated (adaxial). Contents slightly dense. Costal cells parallally oriented, on primary and secondary veins (adaxial) also on tertiary veins (abaxial). Stomata anomocytic, tetracytic and a few anisocytic, Stomatal Index (SI) 6.25 (adaxial) and 11.76 (abaxial), size 22-33µm (27) long and 16-25µm (20) wide (abaxial) and 19-27 µm (23) long and 16-22µm (19) wide (adaxial). Trichomes: (1) Uniseriate macroform conical, few, more on veins on adaxial surface; (2) Uniseriate filiform glandular capitae, few, all over, more on veins; (3) Biseriate filiform glandular capitae, few, all over (Figure.1, 1-5).

In vertical section midvein is shield-like, tapering, ribbed on either side, more prominently on abaxial and as a small cone on the adaxial. Secondary and tertiary veins also ribbed. Midvein 962 – 1104µm (1040) thick. Lamina uneven, undulated, 140 – 216µm (168) thick (Figure.2).

Epidermis is 1-layered and 2-layered in some places at midvein; cells adaxially large, radially long and few papilllate near midvein, cells over the lamina tabular to barrel-shaped and spherical, walls slightly thick on adaxial; oval to spherical, few barrel-shaped, interspersed with stomata on abaxial (Figure.2,1-2).

Mesophyll is poorly differentiated and 6-8 celled, with 3-4 layers of closely packed cells towards adaxial and spongy-like on abaxial; cells oval, oblong and dumbbell-shaped containing chloroplasts. At some places appear as palisade (Figure.2.2).

The ground tissue of midvein consists of collenchyma, parenchyma and sclerenchyma tissues. Collenchyma as a group of cells in the adaxial and abaxial hypodermis at the tip, cells lamellar, angular, 16 – 33µm (24) in diameter. Parenchyma on adaxial side is 6-8 layered and 10-12 layered on the abaxial side; cells polygonal to spherical, 11-41µm (28) in diameter, contents scanty with prismatic crystals of calcium oxalate in a few and interrupted by laticiferous ducts. Sclerenchyma as a cap enclosing vascular bundles. Centrally, a single vascular bundle is ovate, 764-934µm in diameter, endarch, conjoint, bicollateral; secondary vein bundles 538-651µm in diameter. A 2-4 layered vascular cambium is present. Xylem elements in rows, interspersed with fibers and xylem parenchyma. Tracheary elements 8-35µm (21) in diameter. Secondary walls of vessels/tracheids mostly helical, a few scalariform. Phloem is scanty with phloem parenchyma, bast fibers and sieve elements (Figure.2).

Powder microscopy

Pieces of adaxial epidermis with straight to curved sides and stomata. Leaf fragments, light brown with attached epidermis with conical trichomes and mesophyll; Vessel elements with helical thickenings. Prismatic crystals of calcium oxalate. Chlorenchyma tissue of mesophyll. Fragments of lower epidermis with sinuate sides and anomocytic stomata. Some capitile hairs.
Pharmacognostic and physico-chemical evaluation of lactuca sativa L.
P. Subramanian et al

Figure 1
1. Adaxial epidermis in surface X 536.
2. Biseriate capitate hair X 573.
3. Abaxial epidermis in surface X 565.
4. Uniseriate capitate hair X 555.
5. Adaxial epidermis with uniseriate macroform conical hair X 93 bh – biseriate capitate hair;
mch – uniseriate macroform conical hair;
uh – uniseriate capitates hair.

Figure 2
1. V.S. of leaf midvein X 57.
2. V.S. of leaf lamina X 342 C – collenchyma;
e – epidermis; le – lower epidermis; ph – phloem;
Ue – upper epidermis; um – undifferentiated mesophyll; vb – vascular bundle; X – xylem.
**Table 1:** Physico-chemical Standardisation of Raw Drug of *Lactuca sativa*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Quantitative values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture content (Loss on drying at 105° C)</td>
<td>Not more than 11.8 % w/w</td>
</tr>
<tr>
<td>2</td>
<td>Total ash</td>
<td>Not more than 19.98 % w/w</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>Not more than 2.76 % w/w</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>Not more than 7.1 % w/w</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol soluble extractive</td>
<td>Not less than 7.5 % w/w</td>
</tr>
<tr>
<td>6</td>
<td>Water soluble extractive</td>
<td>Not less than 27.5 % w/w</td>
</tr>
<tr>
<td>7</td>
<td>Extractive values in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Hexane</td>
<td>Not less than 0.3 % w/w</td>
</tr>
<tr>
<td></td>
<td>b. Chloroform</td>
<td>Not less than 0.75 % w/w</td>
</tr>
<tr>
<td></td>
<td>c. Methanol</td>
<td>Not less than 19.25 % w/w</td>
</tr>
</tbody>
</table>

**Table 2:** Formulation of mother tincture of *Lactuca sativa* (by Percolation technique)

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>55% v/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug strength</td>
<td>1/10</td>
</tr>
</tbody>
</table>

**Preparation:**

*Lactuca sativa* leaves in coarse powder 100 g

Strong alcohol 578 ml

Purified water 450 ml

To make one thousand milliliters of the mother tincture

**Table 3:** Standardisation of Mother Tincture

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organoleptic profile</td>
<td>clear, non-viscous, producing foam</td>
</tr>
<tr>
<td></td>
<td>a. appearance</td>
<td>Reddish brown</td>
</tr>
<tr>
<td></td>
<td>b. colour</td>
<td>pleasant and aromatic</td>
</tr>
<tr>
<td></td>
<td>c. odour</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sediments</td>
<td>absent</td>
</tr>
<tr>
<td>3</td>
<td>Weight per ml</td>
<td>Not more than 0.91 g</td>
</tr>
<tr>
<td>4</td>
<td>Total solids</td>
<td>Not more than 2.8% w/v</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol content</td>
<td>52 - 55% v/v</td>
</tr>
<tr>
<td>6</td>
<td>pH</td>
<td>4.0 - 4.5% v/v</td>
</tr>
<tr>
<td>7</td>
<td>λ max</td>
<td>231.244, 295 and 326 nm</td>
</tr>
<tr>
<td>8</td>
<td>Refractive index at R.T</td>
<td>1.411</td>
</tr>
</tbody>
</table>
Table 4: Chromatographic results of *Lactuca sativa*

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Detecting agent</th>
<th>No. of spots</th>
<th>Rf values</th>
<th>Colour of spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane : Ethyl acetate (9 : 1, v/v)</td>
<td>Vanillin-sulphuric acid</td>
<td>4</td>
<td>0.26</td>
<td>Purple</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.47</td>
<td>do</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.66</td>
<td>do</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.94</td>
<td>do</td>
</tr>
</tbody>
</table>

Organoleptic characters

- Colour - light green
- Touch - smooth
- Odour - not characteristic
- Taste - slightly bitter

Physico-chemical studies

The determined data under the physico-chemical study for the raw drug is summarised in Table 1 and that of mother tincture preparation and its standardisation in Table 2 & 3 respectively. The results of TLC studies are presented in Table 4.

TLC was carried out on silica gel ‘G’ coated plates (E Merck, India) and hexane : ethyl acetate (9 : 1, v/v) was the developing solvent system, the spots were detected by spraying with vanillin-sulphuric acid reagent.

Pharmacognosy

General features

*Lactuca sativa* L., popularly known as ‘garden lettuce’, is an erect, annual herb, belonging to the family Asteraceae. Morphologically the leaves are 12.5 – 25 cm long, thin, oblong, obovate, plane, curled, crisp and radical.

Epidermal cells in surface have thin straight to curved or wavy sides on adaxial and sinuate on abaxial. Leaves are amphistomatic and possess anomocytic, tetracytic and a few anisocytic stomata. The stomatal index (S.I.) is 6.25 on adaxial and 11.7 on abaxial. Trichomes are uniseriate macroform conical, uniseriate filiform glanular capitate and biseriate filiform glanular capitate hair types. The presence of glandular hairs in *Lactuca* as reported earlier is presently confirmed.

In V.S. leaf, midvein is shield-like, 764 – 934μm in diameter, ribbed on either sides, more prominently on abaxial and tapering below. Lamina is uneven, undulated, 140 – 216μm thick. Mesophyll is poorly differentiated, 6-8 layered and at places appears as palisade.

The ground parenchyma is dispersed with prismatic crystalliferous cells and laticiferous ducts.

Centrally, an ovate vascular bundle, 764-934μm in diameter, is present, which is bicollateral. A 2-4 layered cambium is present. The secondary walls of vessels/tracheids have helical and scalariform thickenings.

The powder microscopic features and organoleptic characters along with the anatomical studies are diagnostic and establish the standards for the drug.

Physico-chemical

The observed physico-chemical data for the raw drug and finished product are summarized in Tables 1-3. Under the raw drug studies, the higher total ash content recorded indicates the presence of various elements in significant proportion. The rest of the values falls under acceptable range.

The results of TLC studies presented in Table 4 and Fig.3 reveal four distinct spots and UV spectrophotometric study exhibits (maximum absorption) four prominent peaks which can be taken as characteristic standards for the drug.
The determined physico-chemical data, macro and microscopical characters and the methodology employed in the study will help in identification, authentication and ensures quality, purity and efficacy of the drug.

References


**Bacopa monnieri - A multicentric, randomized, double-blind homoeopathic pathogenetic trial**

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1 Central Council for Research in Homoeopathy, New Delhi, India
2 Drug Proving Research Unit (H), Kolkata, West Bengal, India
3 Drug Proving Research Unit (H), Midnapore, West Bengal, India

**Objective:** To elicit the pathogenetic response of the drug Bacopa monnieri in homoeopathic potencies on healthy human beings.

**Methodology:** Drug *Bacopa monnieri* was proved by the Central Council for Research in Homoeopathy through randomized, double-blind, placebo-controlled method. The study was conducted at two centers. The drug was proved in three potencies (6C, 30C and 200C) on 32 apparently healthy volunteers who were selected after conducting pre-trial medical examination by the medical specialists and routine laboratory investigations. In the first phase volunteers were given 56 doses (04 doses per day for 14 days) of placebo. In the next three phases 56 doses (04 doses per day for 14 days) of each potency or placebo were consumed. The symptoms generated during the trial period were noted by the volunteers and elaborated by the Proving Masters. The data obtained from both the centers was compiled at proving-cum-data processing cell at CCHR headquarters after de-coding.

**Observations:** Out of the 20 provers who were on actual drug trial, 07 manifested symptoms. Drug was able to produce symptoms in all three potencies more or less related to every part of the body.

**Conclusion:** The pathogenetic responses elicited during the proving trial expands the scope of use of the drug *Bacopa monnieri* and will benefit the research scholars and clinicians. These symptoms will carry more value when verified clinically.

**Keywords:** homoeopathy; pathogenetic effect; homoeopathic pathogenetic trial; drug proving; *Bacopa monnieri*

**INTRODUCTION**

This is an annual creeping plant, found in moist places near streams or on the border of tanks throughout India.1,2 Leaves are tiny and thick. Flowers are white, sometimes slightly bluish in colour, appearing in spring and summer. Taste of the plant is slightly bitter.1 The root as well as the stalks and leaves are used in the Hindu medicine. It is considered to be a nerve tonic, and useful in insanity and epilepsy.2

It is also useful in asthma and hoarseness. Stem and leaves are used in snake bite.3 Leaves are used as a diuretic and aperient.4 Standardized Bacopa monnieri extract is efficacious in subjects with age-associated memory impairment with significant improvement on mental control, logical memory and impaired associated learning.5 The studies provide further evidence that Bacopa monnieri has potential for safely enhancing cognitive performance in aging.6 Bacopa monnieri has been used in the Ayurvedic system of medicine for centuries. The methanol extracts of the plant were found to have potent antioxidant, antimicrobial and anti-inflammatory properties. These active crude methanol extracts were also assayed for cellular toxicity to fresh sheep erythrocytes and found to have no cellular toxicity.7

The alkaloid obtained from *H. monniera* for which the name ‘brahmine’ is suggested, has been studied by Bose and Bose (1931). They find that it is highly toxic. Frogs are killed within 10 minutes with a dose of 0.5 mg. per 100 gm. body weight. Rats and guinea pigs...
Bacopa monnieri - a multicentric, randomized, double-blind homoeopathic pathogenetic trial

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are killed within 24 hours with a dose of 25 mg. per kilo body weight. A dose of 0.5 mg. per kilo body weight of cat produces a fall of blood pressure. In smaller doses, however, there is a slight rise of blood pressure due to vaso-constriction and stimulation of the cardiac muscles. The respiration is stimulated in small doses. Plain muscles like that of the small intestines, uterus, etc., are stimulated in dilutions of 1 in 2,00,000 to 1 in 5,00,000. In therapeutic doses, the alkaloid resembles strychnine in action. Bose has used powdered dried leaves of the Brahmi plant with very satisfactory results in cases of asthenia, nervous breakdown and other run-down conditions.²

No literature related to homoeopathic proving of Bacopa monnieri was found. Therefore, a systematic Homoeopathic Pathogenetic Trial (HPT) of the drug in homoeopathic potencies was necessary to elicit its pathogenetic power which was carried out by Central Council for Research in Homoeopathy as per its approved protocol.

Botanical Name³ : Bacopa monnieri (Linn.) Pennell
Family⁸ : Scrophulariaceae

Common names³,⁸:
- Hindi : Brahmi, Barami
- Sanskrit : Brahmi
- Bengali : Brihmi-sak
- Kannad : Nirubrahmi
- Tamil : Nirpirami
- Malayalam : Nirbrahmi
- Telugu : Sambrani chettu
- Marathi : Jahnaveri
- English : Thyme-leaved Gratiola

Description

A small, glabrous, somewhat succulent creeping herb, rooting at the nodes, with branches 10 to 35 cm long, creeping and ascending. Leaves 6 to 25 mm by 2.5 to 10 mm, sessile, opposite, decussate, obovate-oblong or spatulate, rather fleshy, very obtuse. Flowers axillary, solitary, bluish-white or yellowish, bracteolate, pedicels long, slender. Bracteoles ², linear, just adjacent to calyx; calyx 2+3, outer² broad, long, 5 to 7 X 2 to 33 mm; inner ³, short, linear, about 1 to 2 mm broad; corola ⁵, gamopetalous, bluish-white, yellowish, 8 mm long, lobes sub equal, upper³ long, broad than lower², all with shining dots; stamens didynamous, included; style dilated at the top, 2-lobed or entire. Fruit a capsule, ovoid, acute, ² grooved.⁹

Distribution

Throughout India in wet, damp and marshy areas.³

Part used in Homoeopathy

Whole plant.⁹

Potencies used

6C, 30C & 200C

Objective

To elicit the pathogenetic response of the drug Bacopa monnieri on apparently healthy human volunteers in homoeopathic potencies.

MATERIALS AND METHODS

Study Design

The study was a randomized, double-blind, placebo controlled trial.

Location and duration of study

The proving was conducted at Drug Proving Research Unit (Homoeopathy), Midnapore and in Drug Proving Research Unit (Homoeopathy), Kolkata from 2005-06.

Participants

Total 32 apparently healthy volunteers from above mentioned centers, between the age group of 18 to 50 years, comprising of 29 males and 03 females, were enrolled in this study. Pre-trial Medical Examination (PME) and Terminal Medical Examination (TME) of the volunteers were carried out by General Physicians, Psychiatrists, Cardiologists, Ophthalmologists, ENT Specialists, Dermatologists, Gynaecologists, Radiologists and their routine laboratory investigations at the centers were done to ascertain their health status. After recommendation of experts, healthy volunteers were enrolled in the Homoeopathic Drug Proving Programme.

‘Written informed consent’ from each volunteer was obtained before starting the proving. PME was conducted to confirm health status of the volunteers. Volunteers declared healthy, were enrolled in the study. The study was conducted at two centers. Out of total
32 volunteers, 20 were kept on drug (verum) and 12 were on placebo (control) in all four phases. All the volunteers were assigned code numbers and the coded drugs of different potencies (including placebo) which were supplied in separate glass phials bearing code numbers of the respective volunteers; keeping both provers and proving masters blind about what provers were consuming (drug or placebo).

**Drug**

Bacopa monnieri was procured in 6C, 30C and 200C potencies from M/s. Dr. Willmar Schwabe India Pvt. Ltd., NOIDA, in 100 ml. sealed phials of each dilution. Globules (number 30) were medicated with these attenuations at the Council’s headquarters office and sent to Drug Proving Research Units in coded phials (verum) along with placebo (control).

**Placebo**

Placebo was made up of unmedicated globules (number 30) moistened with unmedicated dispensing alcohol (unsuccussed) and was therefore indistinguishable from verum.

**Procedure of proving**

Before commencing the study, all volunteers were screened strictly by the experts and apparently healthy provers between the age group of 18-50 years, both males and females were included in the drug proving trial. Pregnant and lactating mothers were excluded.

The study consisted of four phases. Each phase consisted of 56 doses of drug or placebo.

**Phase-I**: Placebo phase. It is useful in generating prover’s response to placebo and therefore symptoms generated by the prover in this stage act as control for subsequent phases.

**Phase-II**: In 2nd phase, the proving was conducted with 6C potency.

**Phase-III**: In 3rd phase, the proving was conducted with 30C potency.

**Phase-IV**: In 4th phase, the proving was conducted with 200C potency.

The volunteers were asked to take 4-6 globules of a particular potency of the coded drug, four times a day, dry on tongue.

They were instructed to note down the details of their feelings/changes in mind and body, after taking the coded drug/placebo in ‘Prover’s Day Book Proforma’ daily.

- **If symptoms(s)/sign(s) appeared**

  The volunteers were asked to stop taking the drug/placebo as soon as any symptom(s)/sign(s) developed during the trial.

  The volunteer noted down the sequence of the appearance of new sign(s) and/or symptoms(s), their progress and the number of doses after which such sign(s) and/or symptoms(s) appeared with date, time of onset and duration for which they persisted. Intake of drug remained suspended till the sign(s) and/or symptoms(s) totally disappeared. Any change in normal routine of the prover in respect of daily habits pertaining to diet, living conditions etc./any treatment taken was also noted in the Prover’s Day Book Proforma.

  After disappearance of symptom(s) and/or sign(s) produced by the drug, the volunteer had to wait for a further period of 07 days before taking the remaining doses of that potency following the same dose schedule as stated above. In case of further appearance of new sign(s) and/or symptom(s), the same procedure as stated above was followed till the consumption of 56 doses of that potency by the volunteer.

  If the prover was experiencing the same symptom(s) what he/she had already shown, he/she was asked to stop the current quota and to switch over to the next quota after a washout period of 14 days.

  Each prover was interrogated everyday by Proving Master about the appearance of new symptom(s) or progress of symptoms and noted those in ‘Symptom Elaboration Proforma’ with respect to appearance and dis-appearance of symptoms, their location, sensation/character, modalities, concomitants, extension of symptoms, causation, clinico-pathological findings

- **If no symptoms(s)/sign(s) appeared**

  The volunteers noted down as ‘No Symptom’ with date and time of intake of the respective dose of the drug/placebo.

  Before commencing the administration of subsequent potencies (subsequent phase) of the drug, the volunteers remained on a washout/rest period (it should be a symptom free period between two phases of drug proving in which a volunteer does not take drug) for 14 days and started taking next potency in the same procedure as mentioned above, till completion of 56 doses.
The same procedure was followed for the 3rd & 4th phases.

Each volunteer was interrogated by the Proving Master to verify the sign(s) and/or symptom(s) recorded by the volunteer. The symptoms recorded in ‘Prover’s Day Book Proforma’ were verified by the Proving Master and completed through further interrogation with the provers in respect to their location/ sensation/ modalities and concomitants if any, in ‘Symptoms Elaboration Proforma’.

During the course of proving, the volunteers were referred for specific laboratory investigation(s) to rule out any pathological cause of appearance of symptom(s). Since laboratory tests were performed to identify any correlation between the subjective and objective changes during the course of proving, the expert opinion of the honorary consultant(s) was obtained, wherever needed.

After completion of trial of all potencies, the volunteers underwent TME.

On completion of all the phases of the proving, the compilation of data recorded in ‘Prover’s Day Book Proforma’, ‘Symptoms Elaboration Proforma’, ‘Pathological Report Sheets’ and ‘Terminal Medical Examination sheets’, was done at the Council’s headquarters by the Drug Proving-cum-Data Processing Cell. After decoding, the sign(s) and/or symptom(s) generated by the volunteers kept on the drug were separated from those generated by the volunteers kept on placebo. The sign(s) and/or symptom(s) which were common to both the groups i.e. placebo as well as drug groups were not taken into consideration while compiling the symptomatology of the drug.

Management of adverse effects:

A vial of antidote is sent with each quota to each center. In this trial homoeopathic potencies of Camphora were used as Antidote as it is believed that Camphora can antidote nearly every vegetable medicine. Proving master gives antidote to the volunteer if symptoms continue for a long time or intensity is much to cause discomfort. Proving Master is also directed to take advice of honorary consultants and to get laboratory investigations done, if required.

Pathogenetic effects

Pathogenetic effects (Proving symptoms) are defined as all changes in clinical events and laboratory findings reported by the volunteers during a Homoeopathic Pathogenetic Trial and recorded in the final report. The incidence of pathogenetic effects per volunteer is defined as the total number of findings observed in the trial divided by the total number of provers.

Pathogenetic effects were deduced

(i) from comparison of symptoms developed in placebo phase with symptoms during intervention phases (Intraprover comparison)

(ii) from comparison of symptoms developed by provers on control (for all phases) with provers on actual drug trial (Interprover comparison)

RESULTS

During the pathogenetic trial, out of 20 volunteers who were in verum group, only 07 volunteers reported symptoms consequent upon the administration of the drug. A total no. of 22 symptoms were produced by the drug so the incidence in this proving was 1.1 findings per volunteer. Any adverse effects were not observed during the trial, so antidote (Camphora) was not used.

The following symptoms were observed during the drug proving:

Information regarding the parenthesis:

- In the first parenthesis, the 1st number given after every symptom denotes number of volunteers who produced that particular symptom and 2nd number denotes potency used.
- In second parenthesis, the 1st number denotes number of doses after which symptom was produced and the 2nd number denotes the duration (in days) for which the symptom lasted.

Head

- Congestive pain in temporal region and thirst with coryza. (1,6C) (20,3)
- Throbbing pain in head with heaviness, more on left side, agg. stooping, moving about, light; amel. cold application, light pressure. (1,30C) (41,2)
- Catarrhal headache. (1,30C) (12,1)
- Pain with heaviness in head and eyes and discomfort in neck agg. bending head forward while reading, light amel. pulling hair, massage, lying down, closing eyes. (1,200C) (44,4)
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- Frontal headache, agg. morning, amel. lying down. (1,200C) (28,3)

**Eyes**
- Burning in eyes. (1,6C) (40,7)
- Pain and heaviness in eye balls extending to back of head and sides of head, amel. sleeping. (1,200C) (20,4)

**Throat**
- Dryness of throat with pricking pain in left side, as if something is lodged in throat which is not relieved after drinking water, agg. empty swallowing, during sleeping, amel. hot tea and food. (1,30C) (12,3)

**Abdomen**
- Cramping pain around umbilicus with loose stool, agg. day time. (1,200C) (20,2)

**Rectum**
- Burning pain in rectum; no relief even after cold application. (1,6C) (44,2)
- Frequent loose stools with pain in right side of abdomen, agg. morning. (1,200C) (40,3)

**Stool**
- Watery, yellowish-brown, offensive. (1,200C) (20,2)
- Mixed with mucus. (1,200C) (40,4)

**Female**
- Menses with slight pain, red small clots. (1,6C) (12,5)
- Menses with pain in lower abdomen, back and both legs; flow delayed, profuse, black, clotted. (1,200C) (30,8)

**Chest**
- Palpitation, feeling of whole body moving with beating of heart in morning after profuse menses. (1,200C) (32,4)

**Back**
- Tearing pain in nape of neck, radiating towards shoulder, agg. keeping head straight. (1,200C) (42,4)

**Extremities**
- Red eruptions on thighs and elbows with itching and burning, amel. by scratching. (1,30C) (32,3)

**Sleep**
- Sleepiness. (1,6C) (44,1)

**Fever**
- Fever with headache and running nose. (1,30C) (32,3)

**Skin**
- Itching without eruptions, agg. covering, evening, night, amel. scratching, cold application. (1,30C) (24,3)

**Generalities**
- Restless, changes position and moves about, agg. at night (1,200C) (44,2)

**DISCUSSION**

Drug was able to produce symptoms in 6C, 30C and 200C potencies. 22 symptoms were produced by the volunteers in verum group in 2nd, 3rd & 4th phases. 11 symptoms were produced in 200C potency, 6 symptoms were produced in 30C potency and 5 symptoms were produced in 6C potency.

The pathogenesis of the drug was produced in almost all organs and systems of body. During pathogenesis drug produced various types of headache; dryness and pricking pain in throat etc. There was burning sensation in eyes, burning pain in rectum and reddish eruptions with itching and burning. Some other symptoms like cramping pain in umbilicus with loose stool and palpitation were also produced during the study.

These symptoms may help in clinical application of the medicine.

**CONCLUSION**

The symptoms appeared during the trial will add to the available literature on this medicine and benefit the research scholars and clinicians. These proved symptoms need further verification through clinical application in different clinical settings.
ACKNOWLEDGEMENTS

The authors are grateful to Dr. Alok Kumar, Director General In-charge, CCRH for his persistent encouragement and enthusiastic support for the preparation of the article. We also acknowledge Prof. C. Nayak, former Director General, CCRH headquarters, for providing valuable guidelines for conducting and supervising the study.

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5. Raghav Sangeet, Singh Harjeet, Dalal P.K. et. al. Randomized controlled trial of standardized Bacopa monniera extract in age-associated memory impairment.
A Case of Multiple Urinary Calculi treated with Homoeopathy

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A case of multiple urinary calculi, with size varying from five to seven millimeters in the left kidney and four to six millimeters in the right kidney, a thirteen millimeter stone in the left lower part of the ureter and a 20 x 13 millimeter stone in the base of the urinary bladder presenting complaints of burning micturition and pain in the lumbar region reported at the O.P.D of the Central Research Institute for Homoeopathy at Kottayam, Kerala. Patient was having urolithiasis complaints since four years and had undergone both allopathic and ayurvedic treatment, but the response was not satisfactory. After repertorisation, Nitric acid 30C was prescribed. Two doses of this homoeopathic medicine proved to be effective.

Keywords: Homoeopathy; Nitric acid; Urinary calculi

INTRODUCTION

Urolithiasis affects 5-15% of the population worldwide.1 One of the important phenomena that characterize urolithiasis is its high recurrence.2 Recurrence rates are close to 50%,3 and the cost of treatment of urolithiasis to individuals and society is high. Shock wave lithotripsy and ureteroscopy are effective instrumental treatments for ureteral stones. However, the possible morbidity, significant cost and the need for highly specialized equipment and special expertise raise the question whether these treatments are indeed the most attractive options to meet the increasing demand.4 Although shock wave lithotripsy is the most common treatment for urolithiasis, it can cause acute renal injury.5 Computed tomography and magnetic resonance imaging have demonstrated renal injury in 63-85% of patients treated with shock wave lithotripsy.6 A retrospective study showed that ureteroscopy is useful when lithotripsy fails; when complex or lower pole renal calculi are present.7 Ureteroscopy is less expensive than Extracorporeal Shock Wave Lithotripsy but is more time consuming and technically demanding.8

Since non-interventional treatments are the most appealing to patients, however, there is a large interest in alternative medical treatment modalities.4 Hence it would be worthwhile to look for an alternative, by using homoeopathic medicines for the treatment of urolithiasis. The efficacy of a single homoeopathic medicine in the treatment of multiple urinary calculi is highlighted in the present case. This case assumes a great deal of significance especially in the context of a large interest in alternative medical treatment modalities in recent times. This case would help to sustain this interest and also contribute towards encouraging researchers to undertake similar studies to highlight the usefulness of homoeopathic medicines in urolithiasis.

CASE PRESENTATION

A thirty six year old housewife presented with burning micturition and pain in the lumbar region. She was diagnosed to have urolithiasis since four years and was under allopathic and ayurvedic treatment, (Sodium Acid Citrate liquid for one year and Cystone tablets for three years), but with unsatisfactory response. No known family history of urolithiasis. The dietary habit was mostly rice, tapioca and dried fish.

The totality of symptoms arrived were pain in the iliac region, pain in the kidney region, frequent urge to urinate at night, painful urination, slow urination, retarded urination, thin stream of urine, sediments in the urine with red colour, eructation eating after, desire for cold, salty food and cold drinks, aversion to indigestible food, painful mouth ulcers, tongue with cracked centre and trembling extremities which increases with anger. She was highly anxious, easily irritable, curses others and gets angry very often.

Ultrasonography – KUB (Fig.2) revealed a 20 x 13 millimeter stone in the base of the urinary bladder, a thirteen millimeter stone in the left lower part of the ureter and multiple stones in both the kidneys with sizes varying from five to seven millimeters in the left kidney and four to six millimeters in the right kidney. On thorough physical examination no abnormal findings
were observed. The Serum Uric Acid and Serum Calcium levels were not normal viz. 11 mg/dl and 7.6 mg/dl.

Regular assessments of severity of disease condition, which was moderate at the entry level, were also done during each of the twenty one follow up visits (Table 1) as per the Urolithiasis Symptom Score (USS) Chart\textsuperscript{12}. The patient scored a total of 14 at the time of entry.

Table 1: Follow up

<table>
<thead>
<tr>
<th>Date</th>
<th>Main symptom</th>
<th>Laboratory findings</th>
<th>Symptom score</th>
<th>Medicine prescribed</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.12.07 (first visit)</td>
<td>Moderate pain in iliac region, Frequent urination at night and must wait for urine to start, Moderate dysuria</td>
<td>USG (KUB): Multiple calculi with sizes varying from 5-7mm in the left kidney and 4-6mm in the right kidney; a 13 mm calculus in the left lower part of the ureter and a 20 x 13 mm calculus in the base of the urinary bladder</td>
<td>14</td>
<td>Nitric acid 30/1 dose &amp; Placebo tds for 15 days</td>
</tr>
<tr>
<td>15.01.08 (first follow up)</td>
<td>Moderate pain in left iliac region, mild painful micturition</td>
<td>Urine Microscopic Examination: Pus cells and epithelial cells 0-2/HPF</td>
<td>–</td>
<td>Placebo</td>
</tr>
<tr>
<td>28.03.08 (sixth follow up)</td>
<td>Moderate pain in left iliac region which aggravated before urination and painful eruptions in the mouth</td>
<td>USG (KUB): Bilateral multiple renal calculi 4-5mm in the right kidney and 5-6mm in the left kidney</td>
<td>10</td>
<td>Nitric acid 30/1dose; Placebo tds for 30 days</td>
</tr>
<tr>
<td>25.07.08 (tenth follow up)</td>
<td>No symptoms</td>
<td>USG (KUB): Normal scan; no impression of any renal calculi</td>
<td>0</td>
<td>Placebo</td>
</tr>
<tr>
<td>23.01.09 (sixteenth follow up)</td>
<td>No symptoms</td>
<td>USG (KUB): Normal scan; no impression of any renal calculi</td>
<td>0</td>
<td>Placebo</td>
</tr>
<tr>
<td>26.06.09 (twenty first follow up)</td>
<td>No symptoms</td>
<td>USG (KUB): Normal scan; no impression of any renal calculi</td>
<td>0</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood Investigation: Serum Calcium 9.1 mg/dl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The repertorisation was done using Hompath Classic 8.0 (Kent Repertory). Nitric acid was the highest scoring medicine by covering maximum rubrics (17) and also scoring highest points (38) (Fig.1). Single dose of Nitric acid 30C was prescribed, with placebo for fifteen days. The patient was advised to avoid oxalate foods such as spinach, chocolates, nuts, whole cereal flours, milk, etc. She was also advised to avoid overeating and was asked to take plenty of water. During the first follow up the patient felt mentally better, but complained of moderate pain in left iliac region and mild painful micturition. Placebo was continued. In next four follow ups, no new symptoms were reported and there was consistent improvement in symptoms.

During the sixth follow up, the patient complained of moderate pain in left iliac region which aggravated before urination and also of painful eruptions in the mouth. The ultrasound report showed the presence of bilateral multiple renal calculi of 4-5mm size in the right kidney and 5-6mm size in the left kidney. The stones in the base of the urinary bladder and the left lower part of the ureter had either been eliminated or completely dissolved (Fig.3). On assessment, the patient scored a total of 10. Another dose of Nitric acid 30 was repeated. This was followed by placebo. In the subsequent follow ups, a significant improvement in symptoms was observed and hence placebo was continued.

In the tenth follow up, no stones were found in the kidney, ureter and bladder (Fig.4). The level of Serum Uric Acid and Serum Calcium was found to be in the normal range i.e. 4.6 mg/dl (Female Normal range 2.0 – 6.0 mg/dl) and 9.1 mg/dl (Normal range 8.5 – 10.5 mg/dl). The other clinical investigations also confirmed a normal study. On assessment, the patient scored zero. In the subsequent follow ups, no new symptoms were reported. (Fig.5&6).

**DISCUSSION**

Patient responded positively to the homoeopathic treatment and was relieved of her urolithiasis, with the dissolution or expulsion of the stones. Before resorting to homoeopathic treatment, the patient was under allopathic and ayurvedic treatment for four years with out much improvement. With homoeopathic treatment the patient responded positively. The patient had shown marked improvement in symptom score from baseline score i.e. from 14 to 0. The time period taken for improvement was seven months. This case highlights the usefulness of homoeopathic medicines in the treatment of urolithiasis.

The selection of remedy viz. Nitric acid was based on the highest scoring on repertorisation. It was also verified with Kent’s Materia Medica. Lycopodium, Natrum muriaticum, Pulsatilla, Sulphur and Sepia were the other scoring medicines. In this particular case, Nitric acid covered the symptoms such as painful mouth ulcers, cracked tongue fissured centre and anger trembling after; but these symptoms were not covered...
A case of multiple urinary calculi treated with homoeopathy
P Paul Sumithran

The 30th potency of Nitric acid was found to be effective in the dissolution and expulsion of the stones and also in the marked improvement of symptoms. Only two doses were required for the effective management of the symptom. The USG (KUB) report confirmed the dissolution and expulsion of the 20 x 13 mm stone from the urinary bladder and the thirteen millimeter stone from the ureter. Interestingly, in the tenth follow up i.e. after seven months of treatment, the ultrasound report revealed no stone in the kidney, ureter or bladder.

Dr J H Allen mentioned that throughout the whole urinary tract, we find latent symptoms of all miasms. Of the true chronic miasms, psora and sycosis take an active part in the production of diseases in these organs.9 According to Miasmatic Prescribing10 and Chronic Disease – Its Cause and Cure,11 Nitric acid covers both psoric and sycotic symptoms. This case confirms the usefulness of Nitric acid in psoric and sycotic miasms. Nitric acid belongs to the inorganic or mineral acid group. According to the Universal Mineral Materia Medica, this group has a sphere of action in the genito-urinary tract and is effective in urinary calculi.13

This case assumes importance since there were multiple stones in the kidneys, a thirteen millimeter stone in the ureter and a 20 x 13 millimeter stone in the urinary bladder; all of which were either eliminated or completely dissolved in seven months. This highlights the fact that the patient who had urolithiasis for the last four years had shown better response to homoeopathic medicine.

This case demonstrates the role of Homoeopathic constitutional remedy in minimum dose, selected on the concept of totality and prescribed on the principles of
A case of multiple urinary calculi treated with homoeopathy
P Paul Sumithran

Fig. 2 Before treatment
Fig. 3, 4, 5, 6 After treatment;
Fig. 4, 5, 6 Normal study

figure 6
individualization in expulsion or dissolution of multiple urinary calculi.

References


BOOK REVIEW

Homoeopathic Materia Medica of Indian drugs

Homeopathic materia medica of the Indian drugs\(^1\) published by CCRH is the only source book now available which portrays the picture of 52 commonly used and available Indian drugs in a most systematic and methodical manner. An excellent addition to the book “Drugs of Hindoostan”\(^2\) by S. C. Ghosh and “Special Symptomatology of New Remedies” by E.M. Hale\(^3\).

India being a tropical country is endowed with a treasure of medicinal herbs which are used as folklore medicines, phytotherapy, constituents of Ayurvedic & Naturotherapy. The vegetation of this country is said to be so rich in medicinal herbs that its Materia medica hardly could be equalized in any other country. The plants grow in a particular type of soil and environment and bear remarkable affinity to the temperament and constitution of the individual inhabiting in that locality and found to be more suitable for disease of that region. The saints of ancient India, who are very thorough and meticulous on the medicinal virtues of indigenous plants of India, believed that there was a remedy for disease rampant in the universe. So they consequently toiled themselves assiduously in probing into the medicinal virtues of the plants and eventually discovered a great number of medicines. So rightly written by Hunter\(^4\), in his Indian empire “The Materia Medica of Hindus embrace a vast collection of drugs belonging to the mineral, vegetable and animal kingdoms, many of which have been adopted by European and American physicians.” It is much to be regretted that homoeopath’s contribution on indigenous drugs is still knee-high though; they depend much on plant kingdom in the field of treatment. In recent past Drs. P. N. Ray, P. C. Mazumdar, S. C. Ghosh, P. P. Biswas, K. K. Bhattacharyya added a few provings on indigenous drugs. There are still many herbs and plants in India whose therapeutic virtues and identities are insufficiently known to us and if they will be thoroughly prepared & proved may turn to be very useful drugs of Homoeopathic Materia medica.

Many more instances can be cited from traditional medicine practice which vindicates that these indigenous drugs could no longer be brushed aside and their curative effect could no longer be overlooked. These drugs should adorn the pages of Materia Medica and should have an abiding place there. Many homoeopaths from the inception of this science are using these medicinal herbs gathering their knowledge from different journal, some valuable books, and also from some haphazard sources. But unfortunately a qualitative, updated literatures on this aspect for homoeopathic doctors are very much lacking.

Some time past for a proper drug proving, the essential conditions like, properly equipped laboratories, well versed, dedicated provers and an organizational set up to conduct proving were not available. Now in India, CCRH has undertaken this aspect in a very systematic, methodical, updated way to remove this long felt want & already proved some drugs. Homoeopathic Materia medica of Indian drugs published by CCRH comprises of some of these drug provings of their own and compilation from other source books.

Publication of this resource material will help the physicians by enlightening the knowledge on Indian plants which are not adequately available in the existing literature in one place. The readers will see, it as an excellent reference book & is very valuable for practitioners. A group of experts from the field of Homoeopathy, Botany, Chemistry and Pharmacology have contributed & enriched for the corresponding sections of the book.

The book is organised well with chapters on contents, a very brief but well informative, comprehensive introduction & lastly description of fifty two drugs. In each drug, attempt has been made to provide latest & exhaustive information on taxonomy, pharmacology, chemical & toxic effects of the drugs in addition to symptoms of each drug.

Each drug started with an introduction which is very fascinating: lucrative such as in Cassia sophera it is mentioned that the drug is named as “Kashmarda” in Sanskrit which means destroyers of cough. In Hydrocotyle asiatica the trial of this drug for leprosy by this medicine is completely summarized. So this part is as good as a comprehensive review of literature for the drug in discussion and helps to memorize the keynote...
symptom and use of the drug.

Next to introduction to the drug, unique description on pharmacy part of the drug is delineated which comprises of botanical name, abbreviations, family, common names, distribution or habitat, description of the plants, parts used, chemical constituents, pharmacological action, toxic effects and preparation of the plant. This chapter of the book is most valuable, much informative justifying its uniqueness because of addition of some valuable data.

In this chapter, the information on botanical name, abbreviation, family, habitat and description of the plant makes the reader to recognize the plant, if not known earlier. But the uniqueness in this section of the writing, is exact information on common names or region wise local name of the plant written in thirteen languages, will help the reader to familiarize with the exact species in their locality. Another singularity in this section which comprises chemical constituent of the drug under discussion. Most of materia medica books, except a few are lacking in this aspect which are very essential for an assiduous reader to know the alkaloids to get information on action of drugs. The pharmacological action mentioned under the heading of Pharmacy is an unusual collection and helps the reader as a gate way to the inside story of the detailed proving symptoms. The toxic effect and a detailed preparation of the drug added under this heading gives a complete shape to the pharmacy section of the drug. In the available literatures on indigenous drug, these unparallel, exhaustive and latest collections like chemical constituents, pharmacological action, history and authority are not suffice in comparison to this book.

The other two top stories in each drug are clinical conditions and leading symptoms of the drug. In each drug a schematic presentation of the symptoms are portrayed in detail which is very difficult to remember. So leading symptoms will keep abreast with the memory of the practitioner for ready reference of the drug. So also clinical conditions which are very carefully abstracted will help in recapitulating a drug while prescribing for a patient.

These two sections will help the readers to put them in use as though mentioned in some other materia medica books but not found place in any book of materia medica on indigenous drug.


The relationship to other medicines presented at the end, helps to remember the symptoms of the drug, co-relating to other known symptoms of polychrest drugs.

References added to each drug will immensely help the reader to probe more and more in to detailed information of the drug when they need it.

Last but not least each drug is not only enriched with proving symptoms but also with clinical verified symptoms of different practitioners and also some valuable observations of the experts involved in writing this book, are immensely beneficial resource material for a practitioner. Every serious practitioner, whether novice or experienced belonging to India or Abroad are now inquisitive and eager to be conversant with the action of these indigenous drugs of India. I am sure this book will certainly gratify their passion in prescribing these fifty two indispensable drugs for their patients.

These exhaustive compilations may be very difficult to memorize but very essential for ready reference.

References


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**SCOPE**

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The names of homoeopathic medicines, books and journals appear in italics. The binomial system and abbreviations are used for homeopathic medicines e.g. Nat-m, Kal- ar. Homoeopathy potencies are indicated as 6x, 30c, 1M 10M (or dH, ch, MK etc where the method of dilution is specified). Names of homoeopathic remedies should be written in italics (Aconitum napellus).

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<thead>
<tr>
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<th>Source</th>
</tr>
</thead>
</table>
| 1       | Randomized controlled trials (RCTs) | • CONSORT- [http://www.consort-statement.org](http://www.consort-statement.org)  
• Reporting Data on Homeopathic Treatments (RedHot): A Supplement to CONSORT may be followed |
| 5       | Studies on diagnostic accuracy | STARD - [http://www.consortstatement.org/stardstatement.htm](http://www.consortstatement.org/stardstatement.htm) |

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