CLINICAL VALIDATION OF Piper nigrum AND Nyctanthes arbor-tristis FORMULATION FOR ANTIMALARIAL ACTIVITY

N.B. GHIWARE1, T.M. NESARI2 AND N.Y. GOND3

Department of Pharmacology1 and Pharmacognosy1, Nanded Pharmacy College, Nanded-431605 Maharashtra (India) Department of Dravyaguna2, Tilak Ayurved Mahavidyalaya, Pune-411011 Maharashtra (India)

Abstract: An uncontrolled, open label clinical study was done on twenty-one smear positive patients of Plasmodium vivax malaria. High solid content deflocculated suspension of Piper nigrum and Nyctanthes arbor-tristis was given to patients after their enrollment in study. Vital signs and symptoms of malaria as body temperature, chilly feeling/rigors, headache, body ache, nausea/vomiting and anemia were recorded before treatment, after first and second week of treatment. Improvement in condition was recorded as shifting of gradation in signs of malaria. Decrease in increased body temperature was recorded at the end of first week of treatment (P<0.001). Global assessment suggests improvement within first week of treatment from chilly falling/rigors, headache and body ache. Recovery from nausea/vomiting and anemic signs was observed only after second week of treatment. Negative smear test for Plasmodium vivax was observed at the end of treatment schedule in all patients.

Keywords: Polyherbal formulation, Plasmodium vivax, Antimalarial medicine, Piper nigrum, Nyctanthes arbor-tristis, Medicinal plants.

Introduction

Malaria has been known from antiquity. Seasonal intermittent fevers with chills and shivering, recorded in the religious and medical texts of ancient Indian, Chinese and Assyrian civilizations are believed to have been malaria(1). Malaria now considered as the most important parasitic disease of humans, with transmission in 103 countries affecting more than 1 billion people and causing between 1 and 3 million deaths each year. Despite enormous control efforts, malaria has resurfaced in many parts of the world including India and other Asian countries(2,3). Occasional local transmission following importation of malaria has occurred recently in several southern and eastern areas of the United States and in Europe, indicating the continual danger to the developed countries also. Malaria remains today, as it has been for centuries, a heavy burden on tropical communities, a threat to nonendemic countries, and a danger to travelers.

There are many medicinal plants mentioned in Ayurvedic texts-Nighantu from Jwaraghna group having application in different types of fever and in malaria(4,5). Number of research scholars evaluated different herbal drugs or herbal combinations for antimalarial activity, for example, Azadirachta indica, Eurycoma longifolia, Simbaba orinocensis, Bidens pilose, Plantycladus orientalis, Quassia undulata, Psychotria klugii etc.(6).
Combinations of different medicinal plant known for their use in fever or in malaria can be tested to get safe, cost effective, easy to prepare and which is acceptable to all age group. For the present investigation polyherbal suspension of *Piper nigrum* (Piperaceae) and *Nyctanthes arbor- tristis* (Oleaceae) was prepared. This polyherbal formulation, after standardization, subjected to acute and repeated dose toxicity study according to Organization for Economic Cooperation and Development guidelines. Due to its promising activity observed during preclinical study, which motivated us to validate formulation by clinical trial in patients suffering from malaria.

**Materials and Methods**

*Plant Material-Formulation Preparation*

High solid content, deflocculated suspension was prepared. Authenticated samples of dried unripe fruits of *P. nigrum* and dried leaves of *N. arbor- tristis* are processed for milling to get 120 mesh sizes of particles of both drugs. A sorbitol solution (0.5%) was selected as a wetting agent and water used as a vehicle. Beside water, other polar liquid glycerin was used to control viscosity, solubility and stability of suspension. For good dispersion of material in the suspension, tragacanth (1.25%) was used as a suspending agent. Methyl and propyl paraben were used in a range of 0.05-0.1%. The quantity of powdered form of drugs was taken in sufficient to produce final suspension containing 250mg each of *P. nigrum* and *N. arbor- tristis* per 5 ml of suspension.

**Design of Study**

Uncontrolled and pilot open labeled clinical trial was designed to screen antimalarial activity of suspension of *P. nigrum* and *N. arbor- tristis* Study was conducted on 21 numbers of diagnosed and smear positive (malarial parasite-test) for *Plasmodium vivax* patients of malaria. Patients were randomly selected from Government Hospital, Akkalkot and Seth Tarachand Ramnath Charitable Hospital, Pune.

**Ethical Clearance**

Institutional Ethical Committee of Tilak Ayurved Mahavidyalaya, Pune, approved design of study. Written consent was taken from each patient willing to participate before the start of trial study. For those patients who are unable to read or write, consent of their parents was taken. Such patients were enrolled in the study. Patients were free to withdraw their name from study at any time without asking for any reason.

**Selection of Patients**

Patients were enrolled for trials according to inclusion and exclusion criteria. A detailed proforma was prepared for signs and symptoms of malaria along with MP-test observations for each group of patients.

**Inclusion Criteria**

i) Diagnosed patients suffering from malaria with MP-test positive for *P. Vivax*.

ii) Patients with signs and symptoms of malaria.

iii) Male and Female patients from age range of 20 to 65 years.

iv) Patients willing to participate in trial and signing consent by fulfilling the conditions of proforma.

v) Patients incorporated in study randomly irrespective of sex, religion, region, occupation and family background.

vi) Patients who have not taken any trial medicines 2 months prior to the enrollment in the present study.

**Exclusion Criteria**

i) Patients with MP-test positive for other than *P. Vivax* especially like *P. falciparum* and patients with cerebral malaria.

ii) Fever associated with any other conditions like common cold, typhoid etc.

iii) Patients associated with other known critical conditions like TB, AIDS, diabetes.
Piper nigrum and Nyctanthes arbor-tristis: Antimalarial Activity

Assessment Criteria

A detailed proforma was prepared for improvement in signs and symptoms in malarial patients after first week of treatment and at the end of second week of treatment, separately. Using following criteria’s carried out assessment of efficacy-

i) Smear test for malarial parasite was taken for confirmation and recorded as either MP-positive or MP-negative, before and after treatment schedule.

ii) Change in body temperature was recorded in Degrees F.

iii) Presence of headache and body ache was recorded as 0 to 4 gradation indicating Nil, Mild, Moderate, Moderate to Severe and Severe, respectively during and after treatment schedule.

iv) Assessment of signs such as chilly feeling, nausea / vomiting and anemia were recorded either as present (1) or absence (0) of signs.

v) Signs of uneasiness or any complaints during treatment were recorded as adverse effects due to administered dosage form.

Drug delivery and Duration

Selected 21 patients were received 5ml of suspension dosage form for three times a day for 2 weeks. All the patients asked to take routine diet. As such there was no restriction on diet pattern.

Follow up of Treatment

Every patient assessed for any clinical improvement and for adverse effects regularly, where as data was recorded after first week of treatment and at the end of second week.

Criteria for withdrawal of Patients

i) Patients, who are irregular or not following prescribed treatment schedule.

ii) Patients who are absent on follow up of treatment.

iii) If signs of critical adverse effects due to test formulations was observed.

Statistical Test

Timely noted observations were subjected to statistical analysis for level of significance by Paired ‘t’ test and for final global assessment(19).

Results

Twenty-one patients were randomly selected for clinical study and were between 20 to 65 years of age. Majority of patients (61.90%) were from age group of 20-30 years of age. Seven out of twenty one patients were female patients. All the twenty-one patients with positive test for malarial parasite P. vivax were enrolled for study as per inclusion criteria.

Body temperature was recorded in Degree F before and during treatment schedule. Mean value of body temperature in patients before treatment was 102 ± 0.75. Improvement in increased body temperature was recorded at the end of first week of treatment, (P<0.001). Further improvement after second week of treatment was marginal (Table 1).

Improvement in other vital signs of malaria was recorded as shifting in gradations with percent reduction during treatment schedule. 100% improvement was observed in chilly feeling after first week of treatment as compared to other symptoms. However, improvement in other symptoms was observed only after second week of treatment (Table 2).

Global assessment of percent improvement in vital signs of malaria including for body temperature was carried out. Improvement in increased body temperature and
chilly feeling was excellent after first week of treatment. Improvement in headache and body ache was satisfactory at the end of first week. Complete improvement in signs of nausea/vomiting and anemia was observed only after second week of treatment (Table 3).

At the end of second week of treatment with test formulation, all twenty one patients showed negative smear test for malarial parasite P. vivax. No adverse effects of test formulation were observed during the treatment schedule.

Discussion

The present investigation was aimed to validate efficacy of polyherbal formulation in patients suffering from malaria.

The Pharmacopoeia of Ayurveda is a rich heritage of herbal practices describing medicinal uses of over 600 plants in seventy books containing 8000 recipes of drug combination. There are many medicinal plants mentioned in Ayurvedic texts—Nighantu (Antipyretic) group like P. nigrum (Maricha), N. arbortristis (Parijataka), H. antidysentrica (Kutaki), T. chebula (Hirda) etc. and having application in different types of fever and in malaria(20,21).

By considering the global need of safe and effective antimalarial medicinal preparations, scientific investigation of herbal based drugs and their formulations is the main task in front of India like developing countries to be a self dependent. Present study was also designed by considering such global need of antimalarial formulations. Standardization and preclinical study of suspension form of Piper nigrum

Table 1. Mean value of body temperature in degrees-F during treatment schedule.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Treatment</th>
<th>After First Week of Treatment</th>
<th>After Second Week of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Temp. (0°F)</td>
<td>102± 0.75</td>
<td>98.14± 0.75</td>
<td>97.57± 0.53</td>
</tr>
</tbody>
</table>

#Values are mean ± S.D., n (number of observations) =21
* P<0.001, **P<0.05 significant difference when compared with before treatment data.

Table 2. Mean values of malarial signs and symptoms as shifting in gradations with percent reduction during treatment schedule.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before Treatment</th>
<th>After First Week of Treatment</th>
<th>% Reduction</th>
<th>After Second Week of Treatment</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Headache</td>
<td>3.57±0.78</td>
<td>1.42±0.53</td>
<td>60.22</td>
<td>0.28±0.48</td>
<td>92.15</td>
</tr>
<tr>
<td>*Body ache</td>
<td>3.00±1</td>
<td>1.14±0.34</td>
<td>62</td>
<td>0.42±0.53</td>
<td>86</td>
</tr>
<tr>
<td>#Chilly feeling</td>
<td>1±0</td>
<td>00</td>
<td>0</td>
<td>00</td>
<td>0</td>
</tr>
<tr>
<td>#Nausea/Vomiting</td>
<td>1±0</td>
<td>0.28±0.09</td>
<td>72</td>
<td>00</td>
<td>100</td>
</tr>
<tr>
<td>#Anemia</td>
<td>1±0</td>
<td>0.42±0.098</td>
<td>58</td>
<td>00</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n =21 * Headache and Body ache was recorded from 0 to 4 Grade
# Recorded as either Present (1) or Absence (0) of signs
and *N. arbortristis* revealed stability, safety and effectiveness of formulations.

As there are no sophisticated chemical or analytical methods available for testing polyherbal formulations, further validation of test formulation for efficacy was carried out by uncontrolled, pilot open labeled clinical trials in smear positive patients of malaria. Statistical analysis and global assessment suggests significant improvement in vital signs of malaria within first week of treatment, beside smear negativity for *P. vivax* at the end of treatment schedule.

Drug resistance to synthetic antimalarials is an important problem faced by the clinician in treating malaria patients. So use of such polyherbal formulation in the treatment of malaria may prove in the long run beneficiary to the patients.

**Table 3. Global assessment of percent improvement in patients suffering from malaria.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment Schedule</th>
<th>Number of patients with % improvement in Signs and Symptoms according to shifting of gradation</th>
<th>*Excellent</th>
<th>Very Good</th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Temperature (°F)</td>
<td>After First Week</td>
<td>12 (57.14%)</td>
<td>09 (42.85%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Second Week</td>
<td>21 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>After First Week</td>
<td>12 (57.14%)</td>
<td>09 (42.85%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Second Week</td>
<td>15 (71.42%)</td>
<td>06 (28.57%)</td>
<td>18 (85.71%)</td>
<td>08 (14.28%)</td>
<td></td>
</tr>
<tr>
<td>Body ache</td>
<td>After First Week</td>
<td>12 (57.14%)</td>
<td>09 (42.85%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Second Week</td>
<td>21 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chilly Feeling</td>
<td>After First Week</td>
<td>12 (57.14%)</td>
<td>09 (42.85%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Second Week</td>
<td>21 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/ Vomiting</td>
<td>After First Week</td>
<td>12 (57.14%)</td>
<td>09 (42.85%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Second Week</td>
<td>21 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>After First Week</td>
<td>12 (57.14%)</td>
<td>09 (42.85%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Second Week</td>
<td>21 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Improvement in Signs and Symptoms:
  Excellent- Above 75%, Very Good- 51-75%, Good- 35 to 50%, Poor- Less than 35%

# The values in lower brackets were percent patients
Acknowledgement

The authors gratefully acknowledge all patients for their willingness and full cooperation during study. The authors also thank Dr. D.P. Puranik, Principal, Tilak Ayurved Mahavidyalaya, Pune.

References
Nyctanthes arbor-tristis L. (Oleaceae), a plant widely used in the traditional medicinal systems of India, has recently been reported to possess hepatoprotective, antileishmanial, antiviral and antifungal activities. In the present study strong stimulation of antigen specific and non-specific immunity, as evidenced by increases in humoral and delayed type hypersensitivity (DTH) response to sheep red blood cells (SRBC) and in the macrophage migration index (MMI), has been demonstrated in mice fed with 50% ethanolic extract of seeds, flowers and leaves of this plant. Maximum activity was found in Nyctanthes arbor-tristis Linn, with the dose used showed significant clinical antimalarial activity and good tolerability. A standardized formulation has to be prepared for further studies with critical markers of disease severity as well as parasite clearance. Keywords: Antimalarial activity, Ayurvedic drugs, Traditional medicine, Nyctanthes arbor-tristis Linn, Visham jwara, Parijat. IPC Int. Cl.8: A61K36/00, A61P29/00, A61P33/06. 8 Ghiware NB, Nesari TM & Gond NY, Clinical validation of Piper nigrum and Nyctanthes arbor-tristis formulation for antimalarial activity, J Res & Edu Ind Med, 13(1) (2007) 33-38. 9 Gogte VM, Dravya Guna Vigyan, 1st edn, 566, 1982. 10 Srimal SC, Personal communication. Clinical activity of N. arbor-tristis was evaluated by monitoring pyrexia, parasitemia and morbidity score (MS) in twenty patients. In addition, immune and biochemical markers and organ functions were monitored for objective markers of response. Student’s paired-â€”tâ€” test was applied to assess statistical significance. Further studies with a standardised formulation for dose-searching and optimizing the treatment schedule are needed in a larger sample size. Clinical trial registration no: The process of trial registration had not begun when the study was conducted in 2000. Keywords: Antimalarial Nyctanthes arbor-tristis Ayurveda. Traditional medicine Reverse pharmacology Drug resistance Plasmodium vivax Plasmodium falciparum Cytokines TNF-a. 1. Introduction.