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CLINICAL VALIDATION OF *Piper nigrum* AND *Nyctanthes arbortristis* FORMULATION FOR ANTIMALARIAL ACTIVITY

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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 arbortristis* was given to patients after their enrollment in study. Vital signs and symptoms of malaria as body temperature, chilly felling/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 felling/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 arbortristis*, 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 resurged 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*s 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).

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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)[7,8] and *Nyctanthes arbortristis* (Oleaceae)[9] was prepared. This polyherbal formulation, after standardization, subjected to acute[10]and repeated dose toxicity[11] 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[12,13,14]. Authenticated samples of dried unripe fruits of *P. nigrum* and dried leaves of *N. arbortristis* are processed for milling to get 120 mesh sizes of particles of both drugs. A sorbital 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. arbortristis* 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. arbortristis* [15,16,17,18]. 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

mellitus, hypertension, cardiac problems.

iv) Age below 20 years and above 65 years.

v) Unwilling to participate or sign consent and not fulfilling the condition of proforma.

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*s from *Jwaraghna* (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.

Parameter	Before Treatment	After First Week of Treatment	After Second Week of Treatment
Body Temp. (^{0}F)	102± 0.75	98.14± 0.75	97.57± 0.53

#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.

Parameters	Shifting of Gradation		Shifting of Gradation		
	Before Treatment	After First week of Treatment	% Reduction	After Second week of Treatment	% Reduction
*Headache	3.57±0.78	1.42±0.53	60.22	0.28±0.48	92.15
*Body ache	3.00±1	1.14±0.34	62	0.42±0.53	86
#Chilly feeling	1±0	00	100	00	00
#Nausea/Vomiting	1±0	0.28±0.09	72	00	100
#Anemia	1±0	0.42±0.098	58	00	100

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 beneficiancy to the patients.

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

Parameters	**Treatment Schedule**	**Number of patients with % improvement in Signs and Symptoms according to shifting of gradation**			
		***Excellent**	**Very Good**	**Good**	**Poor**
Body Temperature (^{0}F)	After First Week	12 (57.14%)	09 (42.85%)		
	After Second Week	21 (100%)			
Headache	After First Week		12 (57.14%)	09 (42.85%)	
	After Second Week	15 (71.42%)	06 (28.57%)		
Body ache	After First Week		18 (85.71%)	03 (14.28%)	
	After Second Week	12 (57.14%)	09 (42.85%)		
Chilly Feeling	After First Week	21 (100%)			
	After Second Week	21 (100%)			
Nausea/ Vomiting	After First Week	12 (57.14%)			09 (42.85%)
	After Second Week	21 (100%)			
Anemia	After First Week	12 (57.14%)			09 (42.85%)
	After Second Week	21 (100%)			

* 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

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References

1. **S.A. Dahanukar** and **U.M. Thatte** : *Ayurveda Revisited*, Popular Prakashan, pp.1-28 **(1994).**
2. Government of India, National Malaria Eradication Program, Ministry of Health and Family Welfare; Malaria control-An Attempt **(1996)**.
3. **C.J. Canfield** *et al.* : Severe and Complicated Malaria, *Trans. R. Soc. Trop. Med. Hyg.* 89 (Suppl. 2): 1 **(1990)**.
4. **L.D. Kapoor** : *Handbook of Ayurvedic Medicinal Plants*, CRC Press, Florida, pp. 266 **(1990).**
5. **R.N. Khory** : *Principles and Practice of Ayurvedic Medicines*, Vol.-1, Biotech Books, New Delhi, pp. 211-219 **(2004).**
6. **P. Hyun, H.J. Myung** *et al.* : Antimalarial activity of Herbal extracts used in traditional medicines in Korea, *Biol. Pharm. Bull***.** 26(11): 1623-1624 **(2003).**
7. **P.V. Sharma** and **C. Chaturvedi** : Preliminary observations on the effects of *Piper nigrum* L. *Jour. Med. Sci.*, B. H. U. 5(1): 51-57 **(1964)**.
8. **B. Ravishankar** and **C.K. Sasikala** : Pharmacological evaluation of compound Ayurvedic preparation containing *Piper nigrum*, *Anc. Sci. Life*. 3 (1): 11-18 **(1983).**
9. **T.N. Purseglore** : *Nyctanthes arbortristis* Linn. Antimalarial activity of traditional plants against erythrocytic stage of plasmodium. *Int. Jour. Pharmacog.* 29(1): 19-23 **(1991)**.
10. Public Draft, Acute Oral Toxicity- Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals No.423 **(1996)**.
11. Public Draft, Repeated Dose - 28-Days Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals No.407 **(1995)**.
12. **H.C. Ansel** and **L.V. Allen** : *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7th ed. Lippincott, pp. 347-56 **(2000).**
13. **M.E. Aulton** : *Pharmaceutics: The Science of Dosage Form*, Churchill Livingstone, pp. 304 **(1996)**.
14. **L. Lachman** and **H.A. Lieberman** : *The Theory and Practice of Industrial Pharmacy*, 3rd edition, Varghese Publishing House, Bombay, pp.293-329 **(1990)**.
15. **T.M. Nesari, B.K. Bhagwat, J. Johnson, N.S. Bhatt,** and **D. Chitre** : Clinical Validation of Efficacy and Safety of Herbal Cough Formula: Study of Herbal Cough Syrup, *Jour.Her. Pharmaco***.** 1-12 **(2005)**.
16. The Pharmaceutical Codex- Incorporating the British Pharmaceutical Codex, Clinical Trials, 11th ed. The Pharmaceutical Press, London, pp.199-200 **(1979)**.
17. The Pharmaceutical Codex- Incorporating the British Pharmaceutical Codex, Clinical Trials, 11th ed. The Pharmaceutical Press, London, pp. 631-32 **(1979)**.
18. **H.P. Tipnis** and **A. Bajaj** : *Clinical Pharmacy*, 1st edition, Career Publications, Nashik, pp. 341-56 **(2003)**.
19. **S.K. Kulkarni** : *Hand Book of Experimental Pharmacology*, 3rd ed. Vallabh Prakashan, Delhi, pp. 172-89 **(1999)**.
20. *The Ayurvedic Pharmacopoeia of India*, Vol.1, 1st ed. Govt. of India, Ministry of Health and Family Welfare, Delhi, pp. 71-72 **(1989)**.
21. *The Ayurvedic Pharmacopoeia of India*, Vol. 1 , 1st ed. Govt. of India, Ministry of Health and Family Welfare, Delhi, pp. 137-146 **(1989)**.

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