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Antimalarial activity and clinical safety of traditionally used *Nyctanthes arbor-tristis* Linn.

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Traditional systems of medicine, such as Ayurveda and Chinese medicine in Asia have been provided novel concepts and modalities for healthcare. Critical bedside observations by astute physicians have been followed up by systematic trans-disciplinary research. Such clinical hits of novel biodynamic actions can lead to new drug candidates. In the study, this path was taken up to document antimalarial activity of *Nyctanthes arbor-tristis* Linn. (*Parijat*). *Nyctanthes arbor-tristis* Linn., a popular plant with fragrant flowers described in Ayurveda is being sporadically used for malaria by several Ayurvedic physicians. Patients with malaria were treated with the paste of five fresh leaves of *Nyctanthes arbor-tristis* Linn, Given orally three times in a day for 7-10 days. The relief of symptoms and signs of malaria and the features of *Visham jwara* were graded basally and daily. Of 120 patients, ninety two (76.7%) showed complete clinical and parasitic cure within 7 days. Other 20 patients, who then continued on the same treatment, were cured by 10 days. Those patients who did not respond clinically and by parasite clearance were treated with standard antimalarial therapy. Parasite clearance was gradual and showed a direct temporal relationship with the level of initial parasitemia. The paste was well tolerated and no severe side effects were reported. *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*

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Traditional knowledge of healing-systematized or folklore offers unique opportunity for observation of novel biodynamic activity. Ayurveda, a traditional as well as main-stream system of medicine in South Asia, has been such a source of novel concepts and products for health care[1]. Several natural products from Ayurveda have been added to the global Materia medica, over the centuries. For example, Ctesias of Cnidus- a contemporary Hippocrates (460-377 BC) translated *Charak Samhita* into Greek[2]. The Indo-European exchange on the Ayurvedic medicinal plants continued over two millennia. A major contribution on drugs of India was written by a Portuguese physician of Goa-Garcia da Orta, who described the use of several plants for the treatment of fever with rigors, much before cinchona arrived in Europe[3,4]. For his book, he was assisted by expert *vaidyas* from Goa. Malaria has been described in a fairly detailed manner in Ayurveda, emphasizing the periodicity of fever and the cardinal symptoms[5]. *Visham jwara* is analogous to the phenomenology of malaria. Both Ayurvedic pathogenesis and its reversal through drug and non-drug measures have been described and functionally utilized in day-to-day patient care even today. It is the latter that provides the chance for discovery. But as Louis Pasteur has said, “In the field of observation, chance favours a prepared mind”. Major antimalarial drugs have been of plant origin-quinine, artemisinin and their derivatives. Hence, there is a potential of looking at Ayurvedic antimalarial therapy to identify any clinical hits. This is essential because of the rapidly emerging resistance to the existing synthetic antimalarial drugs. Amongst the several complex formulations and medicinal plants described and used for malaria, attention was drawn to *Nyctanthes arbor-tristis* Linn. The plant was used by *Arya Vaidya Mayaram*, with a precise dose and duration[6]. Antimalarial potential of

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Nyctanthes arbor-tristis Linn. With traditional formulation as leaf paste was observed[7]. A combination of *Nyctanthes arbor-tristis* Linn. With *Piper nigrum* has also been described and used[8].

Nyctanthes arbor-tristis Linn. (*Parijat*) is a popular garden plant known in India since antiquity. It is a hardy large shrub or small tree grows to height 3-4.5m. with rough gray-greenish bark. The leaves are ovate, rough with hairy surface and a serrate margin. The flowers are with 3-5 milky white petals and a thin, 1.2 cm long saffron stalk. The fragrance of the fresh flowers fills the air as these drop-off in the morning[9]. The paper describes the clinical use of leaf paste of *Nyctanthes arbor-tristis* Linn. in the treatment of malaria. An attempt has been made to document the relief of the symptoms and signs and the parasitic clearance on blood smears. The tolerability of the paste has been studied with a record of side effects.

Methodology

The experiential documentation was carried out in the *Kaya-Chikista* ward of MA Podar Hospital, Mumbai. Patients with fever and rigors, suspected to have malaria were screened in the out patient clinic, during October 1997- November 1999. Patients were explained the nature of the study and consent was taken. Routine blood test (CBC, ESR) and urine tests were done at the time of admission. Totally 125 patients positive for the malarial parasite either *Plasmodium vivax* (P*v*) or *Plasmodium falciparum* (P*f*) or both (P*f*/ P*v*) were admitted for the study.

Fig. 1—*Nyctanthes arbor-tris*tis Linn:

Patients below 15 yrs and above 70 yrs, and pregnant women were excluded. Other criteria of exclusion included complicated malaria viz. cerebral, renal, hepatic involvement, Hb below 7%, severe vomiting, diarrhoea, convulsions, etc. Fresh paste of medium sized 5 leaves of *Nyctanthes arbor-tristis* Linn. was administered thrice a day for minimum 7 days. No other antimalarial or antipyretic was given. In case of high fever, tepid sponging and cold water enema was given, Patients were monitored mainly for temperature and rigors and observed daily for severity of 35 malaria-related signs and symptoms based on classical Ayurvedic texts. The blood samples were collected for thick and thin smears, stained with Giemsa, Identification of parasite and counts were made at the time of admission and on 3rd and 7th day after the treatment. Parasite count was done on thick smear based on the standard leukocyte counts (8,000/mm^3). The parasites and white blood cells (WBC) were counted simultaneously on two hand tally counters. The end point was either when the parasite count reached 500 or the WBC count reach 1,000, whichever figure was reached earlier. The parasite density was calculated as follows:

$$\text{Parasite count/mm}^3 = \frac{\text{Parasite number} \times 8000}{\text{WBC Number}}$$

The treatment was continued beyond 7 days for those who had clinically improved yet had no parasite cure. As a fail-safe procedure, patients who neither showed clinical improvement nor parasite cure up to 7 days were given standard chloroquine regimen. Parasite count was repeated on 10th day in these patients. The clinical response was monitored by assessment of the symptoms, signs and fever on day 0, 1, 3, 7 and 10. Based on temporality of clinical improvement and parasite clearance, the following groups were identified to assess the antimalarial activity of *Nyctanthes arbor-tris*tis Linn:

a.	Early complete response	Clinical cure and parasite clearance on 3rd day
b.	Early partial response	Clinical cure – 3rd day parasite clearance on 7th day
c.	Late complete response	Clinical cure and parasite clearance on 7th day
d.	Late partial response	Clinical cure and parasite persistence on 7th day
e.	No response	Neither clinical cure nor parasite clearance on 7th day

The tolerability of leaf paste of *Nyctanthes arbor-tristis* Linn. was judged from the volunteering of side effects by the patients. Thirty-two features present at the baseline were also reviewed daily. The major symptoms namely fever, chills, bodyache and prostration were noted for their presence and absence in the total number of patients on days mentioned (vide supra). The area under the decline for each symptom was calculated for the sequence of response. The means and standard error (SE) were calculated for the basal and follow up parasite counts/mm^3. These were subjected to Student's paired 't' test for the calculation of statistical significance at $p < 0.05$. The slopes of reduction in the parasite count of the response groups were plotted up to day 7, to discriminate the magnitude of change in between the groups. No special slope statistics was applied as the numbers of failure were small.

Results

Total 125 patients were included in the study with 5 dropouts initially. The reason for dropouts was unwillingness to stay as inpatient and subject to blood sampling. The age of patients ranged from 15-65 yrs. There were 101 males and 24 females. Among the 35 Ayurvedic *lakshanas* (features) described for *visham jwara*, 32 were present. The descending frequency of the 10 *lakshanas* present in more than 50% patients at base line is shown in Table 1. As mentioned earlier, fever (100%), chills (100%), bodyache (88%) and prostration (77.6%) were the target features for clinical response to *Nyctanthes arbor-tristis* Linn.

Table 1—Frequency of base-line *Lakshanas* (Symptoms and signs)

Lakshanas	No. of Patients	%
Saitya (Chills)	125	100
Bahyatapa (Temperature)	125	100
Amgamarda (Body ache)	110	88
Daurbalya (Prostration)	97	77.6
Loss of taste (*Arochaka*)	92	73.6
Excess Sweating	89	71.2
Anorexia	87	69.6
Heaviness of body	76	60.8
Muscular pain	73	58.4
Nausea	63	50.4

The decline in the number of patients having four major features is shown in Fig. 2. The area under the decline on day 3 for chills was 95 unit^2 for fever, bodyache and prostration was 225, 282 and 248 unit^3, respectively. On 3rd day though the symptoms of bodyache and prostration were present along with fever in around 50% of patients, chills were present only in 1 patient, which persisted up to 10 days. Bodyache was absent in all patients on 7th day. However, prostration continued in 18 patients until 10th day. The decline in bodyache was slower as compared to the symptoms of chills and prostration. It is interesting to note that in 15 patients fever and prostration continued without chills and bodyache on 7th day. The malarial parasites identified in 120 patients were: P*v* 98 Pf 16, both P*f* and P*v* 6. Mean parasite count in these patients at the time of admission was 4024 ±151/mm^3 (P*v*), 9867 ± 538/mm^3 (P*v*) and 10180 ± 617/ mm^3 (P*f* / P*v*). The therapeutic response was based on the disappearance of parasite as well as absence of the major clinical features. Table 2 summarizes the distribution of parasites as to the groups of responses.

The therapeutic response categories both early and late complete (Group A, C) as well as early partial (group B) showed parasite clearance either on day 3 and 7 with clinical cure within 3 days in the first two

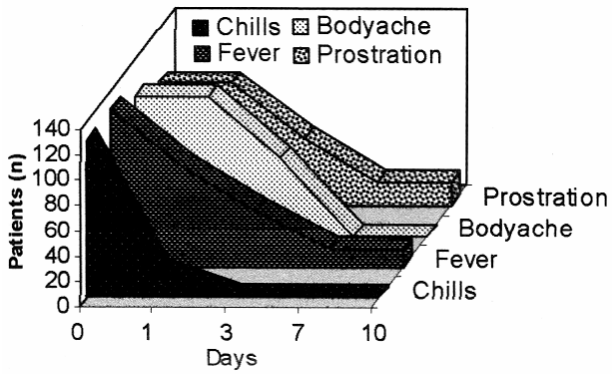

Fig. 2—Symptoms analysis

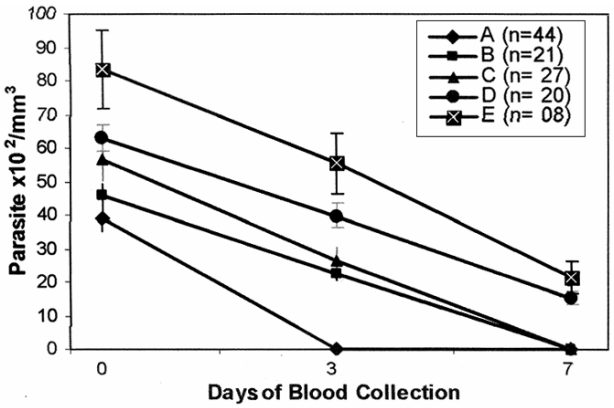

Fig. 3—Reduction in parasite count

Table 2—Therapeutic response and parasite type

Group	Response	Response criteria		Patients (n)	P*f* (n)	P*v* (n)	P*f*./P*v* (n)	%
		Clinical cure	Parasite clearance					
A	Early complete	3rd day	3rd day	44	07	37	Nil	36.7
B	Early partial	3rd day	7th day	21	01	20	Nil	17.5
C	Late complete	7th day	7th day	27	05	20	02	22.5
D	Late partial	7th day	10th day	20	02	17	01	16.7
E	Failure	No	No	08	01	04	03	6.7

groups. Hence, the therapeutic response occurred in 76.7% (92/120) patients. The late partial response group (Group n=20) had persistent parasite beyond 7 days, despite a clinical response on this fixed dose of *Nyctanthes arbor-tristis* Linn. Further continuation of the same dose of *Nyctanthes arbor-tristis* Linn. led to disappearance of the parasite on 10th day (data not shown). The failure was in 6.7% of patients, who responded to chloroquine.

The reduction in parasitemia in all the 5 groups is shown in Fig. 3. In 44 patients (Group A), the parasitemia disappeared on the 3rd day, along with clinical cure. It took 7 days for parasite clearance in 48 patients (Group B C). However, the slope of decline was similar in these patients to that in the groups with late partial response and failure. The highest baseline parasitemia (83.4×10^2/ mm^3 ± 11.8×10^2/ mm^3) was observed in the failure group. The lowest baseline parasite count (38.97×10^2/ mm^3 ± 3.85×10^2/ mm^3) was observed in the group with early complete response. This difference in the baseline parasitemia was statistically significant ($p<0.0005$). The means ± SE of the response in groups were subjected to student's't' test. The analysis shows a delay in temporal relationship of the response with the initial high level of parasitemia. As compared with group A, the initial parasitemia was significantly higher in group B ($p<0.05$). The early complete response was seen in group A. However, in both these groups, clinical response occurred by the third day. Similar pattern of the therapeutic response was seen, despite persistent parasitemia in group C and D. The therapeutic failure Group E had significantly higher initial parasitemia, which did not show response up to 10 days, had very high initial parasitemia. Despite a steady decline of parasite count, the clinical failure necessitated the administration of chloroquine, in standard dosage. All the 8 patients responded to chloroquine.

The tolerability of the paste was good except for the complaints of bitter taste and the nature of the formulation. Thirty-seven patients had bitter taste in the mouth at baseline itself. This is known to be classic feature of *Pitta jwara* in Ayurveda. Despite the bitterness of the medication, 24 patients reported a relief of bitter taste in the mouth by 2nd day. The rest were relieved on subsequent days of treatment. There was baseline symptom of heartburn in 28 patients. By fourth day, all of them reported a relief, suggesting that *Nyctanthes arbor-tristis* Linn. did not aggravate heartburn. Similarly, no aggravation was observed in anorexia, nausea and vomiting noted at base line. Two out of 7 patients with base line diarrhoea continued to have loose stools. There was no skin rash or allergic reactions. As no repeat blood counts and organ functions tests were carried out, it is not possible to comment on other systemic safety.

Discussion

Nyctanthes arbor-tristis Linn., a traditional Ayurvedic plant, administered as the paste of 5 fresh leaves, thrice in a day for 7 days led to parasitic clearance within 7 days in 76.7%. The early complete response at three days and the failure even at 7 days were related to the degree of basal parasitemia level. This suggests that the conventional dose of *Nyctanthes arbor-tristis* Linn. may not be always adequate at higher baseline parasitemia. In the reverse pharmacology path, the experiential documentation is carried out with a conventional dosages form and quantum/frequency. The future studies need a standardised formulation as well as the path of fixed-flexible dosage regimen to permit correlating the therapeutic response with the magnitude of parasitemia. There was no overt difference observed with the type of malaria- Pf or *Pv*- in terms of parasitic response. The number of patients showing mixed infections being small; it would be premature to interpret the presence of 3 mixed infections out of 8 failures. A larger sample size infection is needed to

pursue the finding. In a future study, the blood samples can be preserved for polymerase chain reaction-based identification and persistence of the specified parasite DNA.

The clinical response to *Nyctanthes arbor-tristis* Linn. was observed within 7 days in 93.3% of patients (112/120). However, 20 patients (Group D) still had persistent parasitemia despite the clinical cure. With the standard antimalarial drugs, the clinical and parasitic response usually coincides. For the features of the clinical response, chills responded in a large number of patients than fever. The magnitude of temperature did decline. This suggest that *Nyctanthes arbor-tristis* Linn. may be influencing the mediators of chills, viz. diverse cytokines. An exploratory study with early profiling of cytokines and correlating with clinical features need to be carried out to study this aspect. The tolerability of leaf paste of *Nyctanthes arbor-tristis* Linn. was adjudged to be good based on the record of baseline symptoms and their relief or aggravation. A palatable formulation will be needed for acceptability. However, there is a need to first demonstrate safety with the current paste formulation, utilising standard organ-function tests and adverse event records. This is essential to correlate activity, amply demonstrated by the study, with the safety profile. Congestion of eyes and elevation of blood pressure have been reported with the use of bark of *Nyctanthes arbor-tristis* Linn. used for patients with sciatica[10]. Such an effect may be related to anti-inflammatory and immunomodulatory activity of *Nyctanthes arbor-tristis* Linn. shown in animals[11-13]. But there could be significant phytochemical variations between the bark and leaves of *Nyctanthes arbor-tristis* Linn. For robust reverse pharmacology correlates as well as for standardisation, it is necessary to investigate the polar, mid-polar and non-polar phytoconstituents of different plant parts.

In the past, the antimalarial plants of traditional system took a long time-centuries and millennia-to enter the global mainstream use. This time lag was costly in terms of morbidity and mortality in malaria. This loss of opportunities seen with *Cinchona officinalis* and *Artemisia ammua* should not recur with *Nyctanthes arbor-tristis* Linn. Hence, it is vital to pursue this experimental hit by an effective and trans-disciplinary research path.

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References

1 Patwardhan B, Vaidya ADB & Chorghade M, Ayurveda and Natural Products drug discovery, *Curr Sci*, 86(6) (2004), 789-99.

2 Vaidya AB, *The Medical Aspects of Bhrigu-Samhita*, (MD Dissertation, University of Mumbai, Mumbai), 1963.

3 Garcia da Orta Colloquies on the Simples and Drugs of India, Trust Portuguese Edition 1563, AD, Goa, English Translation 1913, London Reprint, 1987, (Sri Satguru Publication, Delhi) (European translation) 508.

4 Boxer CR, Two Pioneers of Tropical Medicine: Garcia da Orta and Nicholas Monardes, (Wellcome Historical Medical Library, London), 25 Lectures Series No 1 1963.

5 *Vagbhatta Nidan*, 2, 69; *Astang Sanghrah Nidan*, 2, 71.

6 Vaidya Mayaram (1874-1927), Personal notes.

7 Karnik SR, A Study of *Nycatenthes carbor-tristis* Linn. *in the patients of Visham jwara with special reference to malaria*, (MD dissertation, University of Mumbai, Mumbai), 1995.

8 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.

11 Gyanchandani A, Khan ZK & Maitra SC, Arbrtristosides modulate murine peritoneal macrophages for phagocytosis and intracellular killing of *Candida albicans*, *Pharmaceut Biol*, 38(5) (2000) 340-352.

12 Paul BN & Saxena AK, Depletion of tumor necrosis factor-α in mice by *Nyctanthes arbor-tristis* Linn. *J Ethnopharmacol*, 56 (1997) 153-158.

13 Khan ZK, Manglam A, Shukla PK, Puri A, Saxena RP & Tandon JS, Immunomodulatory effect of plant and iridoid glycosides from *Nyctanthes arbor-tristis* Linn. against systemic candidiasis in mice, *Int J Pharmacog*, 33(4) (1995) 297-304.