

A Prospective Open Labelled Clinical Study to Evaluate the Efficacy and Safety of Novel Herbal Formula DENPAP® in Patients with Dengue Fever.

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Abstract - To assess the efficacy and safety of DENPAP®, a polyherbal formulation in the treatment of Dengue fever in patients. 15 Dengue patients within the age group of 18-60 years with a low platelet count (Not less than 70,000 lakhs/cumm) were given 15 ml dosage of DENPAP® twice a day. The study was conducted at ICBio Clinical Research, Bangalore, Karnataka, India. The data analyzed from 15 Dengue patients after all study visits was found to be safe and showed significant increase in platelet count, relief from disease related symptoms and almost all the subjects were cured at the time of the final visit with better quality of life. It can be concluded from the data collected and analysed for the investigational product DENPAP® showed significant percentage rise in platelet count and no adverse effects throughout the study duration with almost all the subjects cured of Dengue symptoms exhibiting an improved quality of life which was considered as an important parameter in this study.

Keywords: *Aedes aegypti* mosquito, Dengue fever, IgG antibodies, IgM antibodies, Low platelet count.

Introduction

Dengue is a vector-borne disease and its classical symptoms include fever, headache, joint pain and skin rash. In 2009, the World Health Organization (WHO) classified Dengue fever into two groups, uncomplicated and severe type [1]. Dengue viruses cause symptomatic infections or asymptomatic sero conversion. Symptomatic Dengue infection is a systemic and dynamic disease with wide range of clinical spectrum that includes both severe and non-severe clinical manifestations. After the incubation period of four to ten days, moderate to severe illness begins abruptly in patients followed by three phases which are febrile, critical and recovery [2]. *Aedes aegypti* is the principal mosquito vector of Dengue which shelters indoors and bites at daytime [3]. The secondary vector for Dengue virus is *A. albopictus*, which contributes significantly to disease transmission in Asia and spreading across Latin American countries. Dengue outbreaks have also been attributed to *A. polynesiensis* and *A. Scutellaris*, but to a lesser extent [4].

Dengue surveillance in India is conducted through a network of more than 600 sentinel hospitals under the National Vector Borne Disease Control Program 2018 (NVBDCP) and Integrated Disease Surveillance Program 2018 (IDSP) [5]. There are three billion people living in risk areas with 50–100 million cases occurring each year [6, 7]. It is believed that the increase in number of cases of Dengue fever is caused by a few factors, which are urbanization, population growth, increasing international travel and global warming. Between 1960 and 2010, the number of Dengue fever cases increased by 30-fold. The increase in Dengue fever is affecting countries around the globe. Presently, Dengue is endemic in more than 100 countries across the globe including India; prior to 1970, only nine countries were reported to have epidemics of severe Dengue [1,8]. Epidemic Dengue is a major public health problem in urban and rural areas of Indonesia, Myanmar, Sri Lanka, Thailand and Timor-Leste which are in the tropical monsoon and equatorial zone where *Aedes aegypti* is widespread with multiple Dengue virus serotypes circulating among adults and children. Between 2001 and 2008, 1,020,333 cases and 4798 deaths were reported in Cambodia, Malaysia, Philippines and Vietnam, the four countries in the Western Pacific Region with the highest numbers of cases and deaths [2]. Between 2001 and 2008, Dengue spread throughout the Pacific Island countries and areas like French Polynesia (35 869 cases), New Caledonia (6836 cases), Cook Islands (3735 cases), American Samoa (1816 cases), Palau (1108 cases) and the Federal States of Micronesia (664 cases) [6]. From 2001 to 2007, more than 30 countries of America notified a total of 43,32,731 cases of Dengue. The number of cases of Dengue hemorrhagic fever (DHF) in the same period was 10,6037 and total number of deaths were 1299, with a DHF case fatality rate of 1.2%. Dengue outbreaks are increasing in size and frequency in the African Region but poor surveillance data is available [2]. In 2009–2010, the Department of Health Research (DHR), Government of India and Indian Council of Medical Research (ICMR) initiated the process of establishing a network of virology diagnostic laboratories with an aim of

strengthening laboratory capacity in the country for timely identification of viral diseases and other agents causing significant morbidity. During 2014–2017, the network of 52 virology laboratories tested more than 2,11,432 suspected Dengue fever patients and confirmed 60,096 cases. Such information proved to be very critical in planning appropriate Dengue prevention and control strategies and also decision-making process regarding suitable sites to undertake clinical trials for Dengue vaccine [5].

Dengue epidemic has become a major public health issue since there is currently no specific treatment and limited disease control in tackling the vector [1]. Intravenous rehydration is the therapy of choice that reduces the case fatality rate to less than one percent in severe cases [2]. Attempts to develop an antiviral agent effective against Dengue have met several hurdles because Dengue is caused by different serotypes which often undergo mutations. Several plants species have been reported with anti-viral activity against Dengue. Recently, the use of alternative medicine and consumption of plant materials have increased in many countries in the world, mostly because plant-derived drugs and herbal formulation are commonly considered to have lesser side effects and toxicity than the synthetic ones [9]. The purpose of the present study was to clinically evaluate the novel polyherbal liquid syrup formulation DENPAP® for its safety and efficacy in patients with Dengue fever in terms of increasing platelet count, improving mental and physical well being, in addition to alleviating disease related symptoms.

Methods

Ethical clearance

The study was performed after receiving approval from Clinical IEC, Independent Ethics Committee for Ethics in Clinical Research, Bangalore, Karnataka, India (Reference number ICBio/CR/GP/0423/38). All patients were explained in detail regarding the aims, methodology, potential risks and anticipated benefits by the investigator and were then given time to consider the information before signing the informed consent form (ICF) to participate in the clinical study. This trial was also registered on 09/06/2014 in Clinical Trial Registry of India (CTRI) with Ref. No. CTRI/2014/06/004660.

Study design

This was a non-randomized, interventional, open labeled study sponsored by Goan Pharma and carried out under ICBio Clinical Research, Bangalore, Karnataka to evaluate safety and efficacy of polyherbal liquid syrup formulation of DENPAP® on 15 subjects suffering from Dengue. The subjects were given dosage of 15 ml each, twice a day for a total of five days. Upon patient screening for inclusion criteria, safety assessment, platelet counts, symptoms of Dengue, patient satisfaction grading and compliance check with visits conducted on day one, two, three, five and ten. An unscheduled visit was conducted at the end for subject's vitals and general physical examination. Screening form-36 (SF-36) Questionnaire was prepared for individual functions that included physical function (PF), Role-physical (RP), Bodily Pain (BP), General Health (GH), vitality (VT), Social Functioning (SF), Role Emotion (RE) and Mental Health (MH) which was used for data collection.

Subject selection

For the clinical study, subjects were required to be between the age group of 18 – 60 years with a low platelet count (Not less than 70,000 lakhs/cumm) as well as test positive for IgM and IgG antibodies. Main exclusion criteria for the subjects included adequate platelet count, negative test for IgM and IgG antibodies and pregnancy or lactation in women. Also the subjects who had bleeding manifestations or mixed infection (positive test for malaria, leptospirosis, thyroid and mental and heart diseases along with Dengue) or a recent (< 120 days) transfusion of platelets and whole blood were excluded from the present study. A sample size of 15 patients was selected for the study (Table 1).

Table 1. Demographic and baseline characteristics of selected patients at the start of study.

Smoking status	
Never smoked	12 patients
Still smoking	3 patients
Quit smoking	0 patients
Mean height	
Men	158.75 cm
Women	147.50 cm
Mean weight	
Men	57.29 kg
Women	59.23 kg
Medical history	
Hypertensive	5 patients
Diabetic	2 patients
Hypertensive and Diabetic	1 patient
Hypothyroid	1 patient

Expected Outcome

The primary outcome of the study was to determine the safety of the herbal drug DENPAP® and its efficacy in increasing platelet count in Dengue patients. The secondary outcome was to improve the physical, mental and social well being of the patients (better quality of life) and alleviate disease related symptoms in the Dengue patients.

Statistical analysis

The data was analyzed with five percent significance level and 80% power using SAS. The difference in the group was assessed using paired t-test. The difference between the groups was assessed using independent t-test.

Results and Discussion

All 15 enrolled subjects were advised to take the study drug as per protocol described to them and attend site visits. The data collected and analyzed from 15 subjects after all study visits showed significant increase in platelet count irrespective of the group. There was no drop out of subjects during the study.

Safety Assessment

Safety evaluation was done on the basis of adverse events, treatment related adverse events, serious adverse events and other significant adverse events and death which on all accounts was nil. Thus, during the study, the product was found to be safe. This finding is similar to a study conducted in subjects undergoing chemotherapy treatment (Ramesh *et al*) [9].

Platelet count

The overall mean platelet count (lakhs/cumm) of the 15 subjects showed a significant increase with treatment (Fig. 1). This finding is similar to that of previous studies conducted jointly by investigators at Dr. B. R. Ambedkar College, Bangalore; King George Hospital, Visakhapatnam, Andhra Pradesh; Prashanth Hospital, Bangalore; Rajiv Gandhi Institute of Medical Sciences, Srikakulam, Andhra Pradesh; and Sri Venkateshwara Hospital, Bangalore on over 300 Dengue patients demonstrated improvement in the blood platelet count due to *Carica papaya* leaf extract [10]. Also, previous research conducted in Malaysia found that consumption of *Carica papaya* leaf juice increased platelets in Dengue patients. Platelet Activating Factor Receptor (PTAFR) gene which is known to be responsible for platelet production and aggregation was expressed 13.42 folds compared to control group of Dengue patients who did not consume *Carica papaya* leaf juice [11].

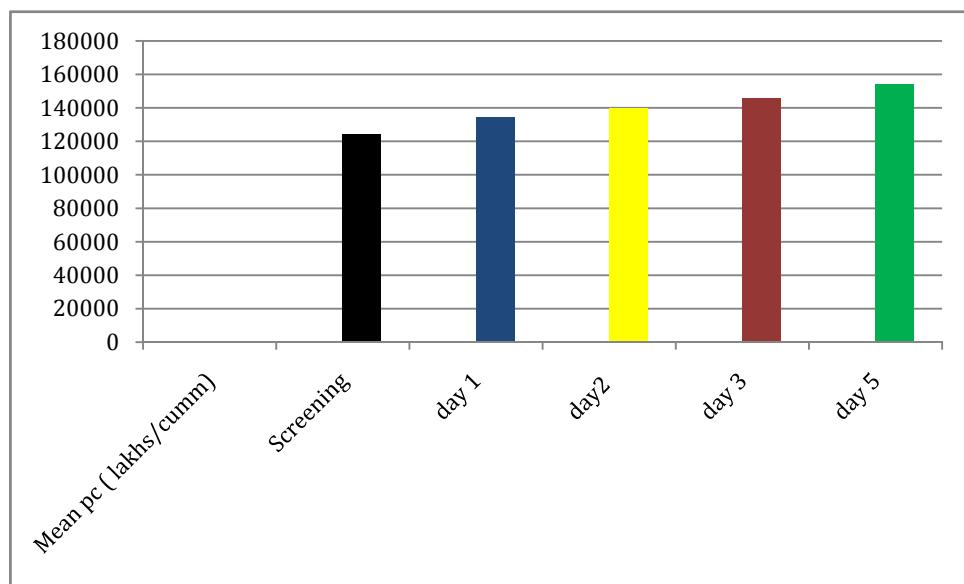


Fig. 1: Mean platelet count (lakhs/cumm) of patients.

SF-36 questionnaire evaluation and Dengue symptoms

The patients showed improvement in functions after treatment when compared from baseline indicating improved quality of life (Fig. 2). The product DENPAP® was analysed for illness symptoms associated with Dengue fever. It was observed that few subjects during screening complained about various symptoms associated with Dengue fever such as body pain, back pain, chills, fever, headache and mood swings. These were noted at the baseline by the investigator and followed up till the final visit to indicate that almost all the subjects were cured of the symptoms. These findings are similar to those studies conducted using *Ocimum sanctum*, *Carica papaya* and other herbs to provide relief from symptoms without any untoward effects and altered hematological, hepatic and renal function were completely reversed with 100% compliance which was attributed to the potent active ingredients of the constituting herbs. *Carica papaya* leaves have been shown to exhibit antipyretic, immune-modulatory, anti-inflammatory and anti-oxidant properties while *Ocimum sanctum* have been found to alleviate fever and bodyache [12].

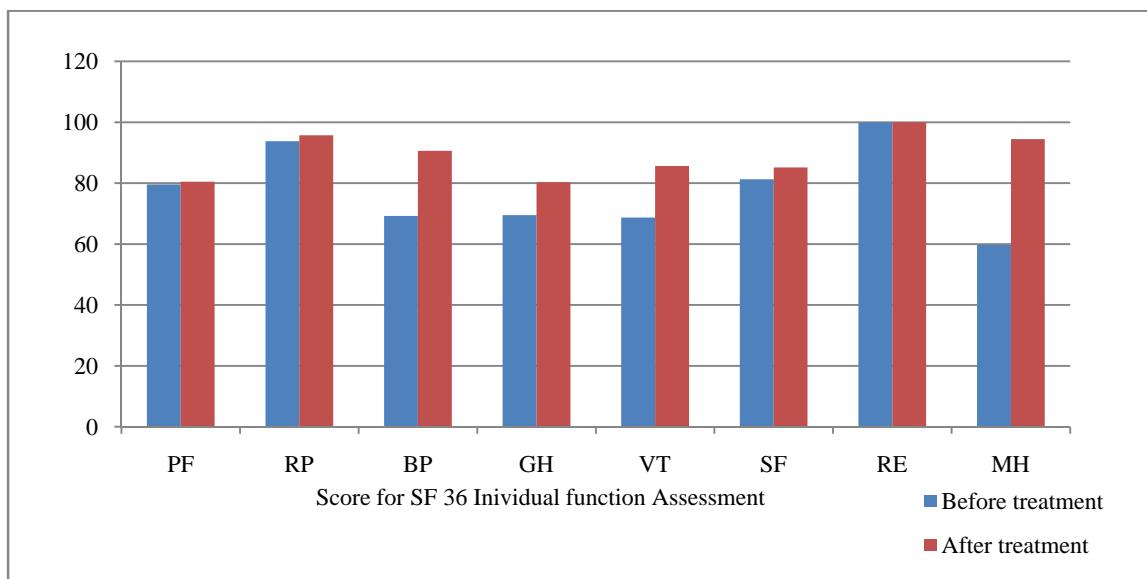


Fig. 2: SF-36 questionnaire evaluation for individual functions.

Conclusion

It can be concluded from the data collected and analysed for the investigational product DENPAP® showed no adverse effects and significant percentage rise in platelet count throughout the study duration when screening platelet levels in all enrolled subjects which was considered as an important parameter in this study with almost all the subjects cured of Dengue symptoms exhibiting an improved quality of life.

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