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# Exploring apposite therapeutic target for apoptosis in filarial parasite: A plausible hypothesis ☆

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ABSTRACT

Human lymphatic filariasis is a parasitic disease with profound socioeconomic encumbrance owing to its associated disability, affecting predominantly but not limited to the developing nations of tropics and subtropics. There are several technical issues like poor therapeutic and preventive repertoire as well as administrative and infrastructural limitations which jeopardize the salvage measures and further complicate the plight. Therefore, considering the gravity of the problem, WHO has mandated (under tropical disease research scheme) for placing emphasis on validation of novel therapeutic targets against this disease with the unfortunate tag of 'neglected tropical disease'. However, dearth of knowledge of parasite biology viciously coupled with difficulty of access to parasitic material from suitable animal model along with growing cost burden of high end research poses formidable challenge.

Based on the recent research evidences, here we propose a premise with targeted apoptotic impact as a novel rationale to be exploited towards anti-parasitic drug development. The new era of bioinformatics ushers in new optimism with a wide range of genomic and proteomic database in public domain. Such platform might offer wonders for drug research, but needs highly selective criterion specificity. In order to test our hypothesis presumptively, we deployed a scheme for identification of target proteins from filarial parasitic origin through wide database search with precise criteria of non-homology against the host along with functional essentiality for the parasite. Further screening for proteins with growth potential from such list of essential non-homologous proteins was undertaken to mine out suitable representative target for ensuing apoptotic impact though effective inhibitors. A unique protein enzyme, RNA dependent RNA polymerase, which besides its vital role in RNA virus is believed to have regulatory role in gene expression, emerged as a plausible target. This protein is rather unknown in human host and present in related nematode parasites including the pathogen of human lymphatic parasite. Further exploitation of bioinformatics approach with a proven inhibitor of this enzyme by molecular docking technique revealed the feasibility as valid antifilarial candidate. This strategy also underscored the significance of bioinformatics tools in circumventing the resource intensive research for drug development. This virtually verified paradigm need to be tested in real lab setting not only for therapeutic authentication of this novel rationale but also for development of insight into parasitic biology that may open up new outlook in host parasite relationship. If successful, this might ensure effective measure against this menace of such 'neglected tropical parasitic diseases'.



## Introduction

### *Defining the problem*

Human lymphatic filariasis has been labeled with the tag of so-called 'neglected tropical disease'. However, World Health Organization (WHO) has justifiably appreciated the physical disability suffered from this condition as an equally important concern that might commensurate the fatality of any other disease. Significance of socioeconomic burden associated with such disability cannot be overemphasized; particularly for the tropical countries which are mostly constituted of the developing nations of the world and also unfortunately are the hubs of such parasitic ailments. Worldwide, 40 million people suffering from chronic lymphatic filariasis (LF), makes it the second leading cause of physical disability in the world; it affects 120 millions of people from tropical and sub-tropical regions and another 751 million people are at "risk" of infection. Morbidity is estimated to be 5.5 million disability adjusted life-years (DALYs) [1]. Most common chronic

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manifestations associated with LF are severe lymphedema of limbs, scrotal hydrocele, genitalia and breast which in most cases are irreversible [2]. The vicious cycle of low socioeconomic status, poor hygienic practices, limited health facility and the parasitic disease associated disability burden is obvious omen of a global calamity.

*Control strategy; the current scenario*

Consequently, sensing the gravity, WHO implemented Global Programme to Eliminate Lymphatic Filariasis (GPELF) for transmission and disease control with motivation of eliminating the disease by year 2020 [3]. Under this scheme, 'Mass Drug Administration' (MDA) programs involving mainly Diethyl Carbamazine (DEC) along with Albendazole in combination has been made the mainstay [4]. In the filarial life cycle, the microfilaria serves as the link between the host and the mosquito vector. Therefore MDA program mainly aims at the transmission control mediated by anti-microfilarial impact of the almost sole antifilarial drug, DEC. Although effective on microfilarial stage, however DEC is fairly ineffective for killing adult worms and therefore understandably, MDA program is not totally infallible [5]. Besides, it has several limitations at the level of implementation, including possible emergence of resistance [6]. The vector control is another recognized component of effective anti-filarial campaign; however, there are several challenges those lead it towards inadequacy [7]. Till date, there is no prophylactic treatment available against filariasis and the repertoire of effective drugs is also very limited [8]. Therefore, WHO has not limited its endeavor by implementation of MDA only but also extended its plan of Tropical Disease Research (TDR) highlighting the need of novel antifilarial drug development.

*Drug development today*

The TDR plan mainly focused on the RNA interference based approach for drug development, keeping pace with the advent of the filarial genome database in recent years [9]. However, quite presumably this process demands access to higher technological support and consequent financial sufficiency. Therefore, the practicality of such therapeutic modality in the scenario of developing world may be doubtful. The advancement in this direction is just taking up [10] and yet to undergo the test of time.

Another important area identified, which is probably of more practical significance in context of the tropical region, involves the best use of the traditional medicine based on the rich herbal resources of these regions [11]. The beauty of this system lies in its time tested socio-cultural acceptability and also the cost effectiveness; however on the flipside, such practice is largely empirical and there is hardly any scientific validation in its favor. Owing to age-old references on the natural resource based medicines coupled with relative ease of access to the plant material there is a plethora of studies with several such traditional drugs. Consequently several active principles have come into limelight as candidate for therapeutics.

Further, with the gradual advancement in the knowledge about the metabolic profile of the parasite a parallel research in search of effective drug targets has also opened a new vista. All such approaches mostly referred to as the reverse pharmacologic principle has significant potential. However, despite such serious efforts, unfortunately no novel molecule posed any formidable challenge against the gold standard status of the age-old drug DEC. The bottom line of this apparent triumph of DEC is the lack of the in-depth understanding of the parasite biology and more importantly the intricacy of the host parasite relationship. This factor is not only responsible for failure of understanding the basic mechanism of action of DEC and also prohibiting the development of effective new drug. Moreover another serious shortcoming in the development of an effective new drug is due to the relative paucity of the parasite material; since the access to the animal model for this disease is limited. Several researchers tried to explore the surrogate system of *Seratia digitata* which is a related cattle parasite, for its comparative easy accessibility; however, the results of such work may not always be suitable for extrapolation in human filarial disease. Thus there is definite need for high degree of precision in the selection criterion for the effective target identification so as to confine the use of parasites as well as to ensure effective utilization of the limited resources.

## Hypothesis

In the quest for designing novel drug against the filarial parasite, it appears that an evidence based definitive mechanistic clue should be sought at rather than testing unknown speculative targets. Most ideal for such purpose may be served by looking at the natural process of innate response against such infective pathogens, which mostly involve the macrophage-mediated oxidative attack. Interestingly the most popular rationale contemplated for DEC is the promotion of such innate defense mechanisms of the host [12]. As an indirect proof of this idea, it has been observed that even though DEC has reasonably good activity *in vivo*, it failed to show any satisfactory results *in vitro*, under cell free condition. Off late, evidence of apoptosis had been reported in filarial parasite treated with DEC *in vitro*; albeit such effect was found to be insufficient to kill the parasite [13]. This prompted us to augment this effect of DEC with $H_2O_2$, a well-established apoptosis inducer as well as oxidant against the parasite *in vitro*; not surprisingly we observed a significant synergistic impact which is sufficient enough to inflict fatal damage [14]. The subtle nexus of oxidative stress and apoptosis might be able to explain these observations.

Another striking piece of evidence came from our tryst with antifilarial drug development pursuit with certain herbal extracts namely *Vitex negundo* Linn., *Aegle marmelos* Corr. and *Butea monosperma* Linn which showed promise [15]. Interestingly major active ingredients found in these extracts were polyphenolic compounds [16]. Polyphenol (flavonoid) biosynthesis roots from shikimate pathway, which is also used for the production of folate compounds. Although folate synthesis is mainly restricted in plants only like the polyphenols, however its utilization is of paramount importance in animal kingdom for DNA synthesis [17]. The NADPH-dependent reduction of dihydrofolate (DHF) to tetrahydrofolate deploying the catalytic activity of DHFR enzyme is a key reaction for this purpose [18]. Consequently, inhibition of DHFR, resulting in the disruption of DNA biosynthesis, is the basis of the chemotherapeutic action of a range of DHFR inhibitors, generically known as "anti-folates [19]." Large body of evidence suggests significant role of such agents in cancer therapeutics by hindering the proliferation potential of malignant cells and also for treating microbial infection [20]. Remarkably certain flavonoids isolated from green tea not only share structural analogy with the conventional DHFR inhibitors but also are shown to be inducers of apoptosis through DHFR inhibition [21]. Hence a competitive inhibition based on structural resemblance between polyphenolic compounds and folate derivatives can be envisaged. Earlier study demonstrated the presence of DHFR sequence in numerous closely related nematodes [22]. Although filarial genome has been found to contain DHFR sequence however, definite evidence for the actual DHFR protein of filarial origin is still lacking. Screening of synthetic polyphenolic compounds might provide an alternative evidence for its role as antifilarial agents. In this context, our studies carried out with classical (Trimethoprim, Pyrimethamine) and synthetic DHFR inhibitors (biguanides, dihydrotriazines and