



How can we avoid lymphoedema in addition to elephantiasis in Lymphatic Flariasis become indelible An update of treatment options in reference to a case report not treated for 3 decade- Review of treatment with historical perspectives

BY

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Abstract

Despite the launching of the National Filariasis Control Program over greater than 50% of the century till 2024 Lymphatic Flariasis(LF) continues to be prevalent worldwide in the tropical along with sub-tropical areas adding considerable hurdles in the aim of global eradication. Apart from affecting the human beings along with the influence over expenditure correlated with livestock cost works as a key in aiding in morbidities as well as disabilities. Presently the situation has got out of control with the urgent requirement of generation of robust therapeutic approaches possessing effectiveness against the adults stage of the nematodes. Pertaining to this evaluation of a target that guarantees death/ demise of microfilariae in addition to macrofilariae is the initial aim. Apoptosis has been plausible for all the 3 stages of the filarial nematode /parasite- for instance-ooocyte, microfilariae(mf) along with adults leading to filarial demise subsequent to the receipt of the signaling from filarial reactive oxygen species (ROS) whose execution utilizes extrinsic as well as intrinsic pathways. Recent advancements have illustrated the effectiveness of natural and synthetic substances(nanoformulations) possess the capacity of stimulating apoptosis with practically negligible inimical sequelae for body of host & having apoptosis induction capacity causing micro along with macrofilaricidal action in micro & macrofilariae.The basic aim of updating this historical knowledge on LF treatment after no therapy given to the patient presented in Delhi.Till efficacious nanoformulations formed atleast a combination of rifampicin plus doxycycline or doxycycline with DEC can be tried if not planning pregnancy in females where all azoles like albendazoles need avoidance due to teratogenicity.Lot of plant extracts don't have inimical sequelae.Hopefully, in future we will have different targeted approaches as detailed here

Keywords: Lymphatic Flariasis(LF); reactive oxygen species (ROS); Oxidative stress(OS)

1. Introduction

Greater than 45 million people are afflicted by filarial lymphoedema in addition to elephantiasis inclusive of hydrocoele[rev in1]. An equivalent percentage are asymptomatic however have infestation with filariae. These patients usually possess injured lymphatic system in addition to have the probability of generation of lymphoedema in addition to elephantiasis[rev in1]. Currently there is availability of efficacious treatments, however, patients that belong to developing countries find them cost prohibitive along with there is reluctance of clinical practitioners in

prescribing them. Amongst these, the initial preference is proper physical treatment. This comprises of the noninvasive provision of large, pancy in addition to diminishing the swelling lasting for substantially long time period; however, the compression garments are necessary. Nevertheless, this is practically not feasible in case of hot, wet, or dirty situations for instance paddy fields. The benzopyrone group of agents possess a myriad of effects in the diminishing of all kinds of acute as well as chronic high protein oedema in animals in addition to humans. The agents that have been evaluated possess the mechanistic modes of escalating macrophages in numbers along with their normal proteolysis/cell [1]. These



agents therefore yield provision of alternative pathway, through which proteins in addition to its osmotically trapped water might be elimination from the tissues. elimination escalated proteins further diminishes chronic inflammation that would be leading to simple chronic accrual of extra plasma proteins as well as sequentially accumulating escalated fibrosis[rev in1]. All the benzopyrones possess akin actions over the high protein oedema, with the consumption of maximum is feasible orally, all possess minimal toxicity with the continuation of their effects for variety of days[1]. The maximum economical is 5-6 benzo- α -pyrone(coumarin). It has good availability in large quantities in addition to possess considerable purity as well as has been evaluated in maximum clinical trials in contrast to any other agents. Whereas its biochemical name is coumarin in addition to represents the parent molecule of the dicoumerol anticoagulant, it does not possess any anticoagulant actions on its own[1] These benzopyrones do not possess any association with benzopyrenes along with possess a separate chemical structure.

Different benzopyrones have been demonstrated to diminish human post mastectomy, primary as well as other lymphoedemas[rev in1], in addition to improvement of outcomes obtained with the utilization of physical treatment [rev in ref 1 20]. Furthermore, reduction in the incidence of these secondary acute inflammation by eliminating the "incubation medium" in reference to bacteria[rev in1]. Nevertheless, till 1993, the actions of these agents over the extensive lymphoedemas along with elephantiasis resulting from filariasis had not been evaluated. therefore Casley Smith et al.[1], performed a study in 1993 corroborating its efficacy, however has not been used further.

The reason this review was decided was my mother suffering from elephantiasis for the last 3 decades and being admitted in the best hospitals in Delhi had not received any prescription for the same although received therapy for Diabetes mellitus(DM), hypertension, pulmonary edema till she sustained a fractured femur which led to her demise yet never got any therapy. Despite my asking to try coumarin nobody was willing to start in Delhi hospitals even though having facilities & personnels to use interdisciplinary team approach. They kept on asking my sisters if she was taking any treatment for Lymphatic Filariasis(LF) in each admission yet nobody prescribed any agent let alone rifampicin & doxycycline combo/Ivm & Alb despite no issue of teratogenicity. Despite my asking my sisters to ask them to add coumarin nobody bothered so that the aid my mother never got -helps rest of patients of LF to benefit from. Thus here we decided to explore the problem which has assumed Worldwide importance for the reasons of no sustainable therapy, other than anecdotal reports using certain ayurvedic treatments or surgical drainage[2].



Figure 1 revealing Lymphatic Filariasis in patient referred to never given therapy

Recently the group of Mukherjee et al who have been working in veterinary science over this issue detailed how worldwide it was becoming difficult to tackle this condition in animals & humans with best method being targeting apoptosis for escalating lost efficacy of earlier agents. Please refer to detailed epidemiological problem in reference no 3.

In view of our reviewing apoptosis and autophagy pathways in detail in role of Bile Acids in Cancer & in Diabetic cardiomyopathy (DbCM) here we decided to further explore their role in LF [4,5].

The detailed historical generation of these drugs is given henceforth.

2. State of Antifilarial Agents along with Drug Targets for Treatment of Lymphatic Filariasis(LF)

Different agents have been generated over the decades for treatment of lymphatic filariasis however their actions kinetics are variable yielding separate targets for assessment

2.1 Cytoskeleton Disturbing Agents

Albendazole(Alb), mebendazole, flubendazole, oxibendazole as well as flubendazole, all of which portray the active constituents of benzimidazole (derived from benzene along with imidazole) have been well acknowledged for their anthelmintic effects; however possess a history of slow absorption via the gastrointestinal tract(GIT), in addition to teratogenicity[6]. Mebendazole, as well as flubendazole possess a robust hampering part in the generation of microtubules which worked in the form of a stimulator to the elimination of cytoplasmic microtubules of the tegumental in addition to intestinal cells of the nematode with subsequent diminished glucose uptake[7], has yielded validation how anthelmintic benzimidazole competes with the colchicine binding area for generating bonding with the nematode β tubulin.

2.1.2 Ion channels blocking Agents

Glutamate gated chloride channels (GluCl_s) are limited just to the invertebrates, thereby it might be a probable target for working against filariasis[8]. Ivermectin (Ivm) is presently utilized, that represents a naturally generated avermectin B1 that is a macrocyclic lactone (ML) displaying action against a broader spectrum of parasitic nematodes subsequent to oral/ parenteral delivery. This results in opening of GluCl_s channels in maximum quantities leading to induction of paralysis of pharyngeal pumping. In case of *Caenorhabditis elegans*, the *glc1* gene partially encoding GluCl_α subunit works in the form of the main target for treatment of Ivm[9]. Sequentially, the existence of *avr-14* from the uterine wall of female *B. malayi* in addition to the embryonic stage *B. malayi* microfilariae (mf) provide an explanation for the mechanistic mode for the repression of mf generation by female worm subsequent to avermectin treatment success[10]. Dent et al.[11], reported that mutation of three genes from *C. elegans*, *glc1*, *avr-15* as well as *avr-14* concurrently resulted in resistance to Ivm, while none or minimal resistance ensued once 2/3 of those got mutated. Moxidectin in addition to milbemycin are from the milbemycin oxime which is cognate to avermectin along with has broadened the area of influence in the form of ion channel blockers[12]. Piperazine portrays a GABA agonist working over the ligand gated Cl⁻ channels over the synaptic along with extra synaptic membrane of nematode muscles resulting in hyperpolarization of membrane potential, whereas ensuring the opening of Cl⁻ channels causing dysfunctional sinusoidal motion of nematode referred to as flaccid paralysis [13]. Nicotinic agonist substance imidathiazole (levamisole, betamisolol), tetrahydropyrimidone (pyrantel, morantel, oxantel) quaternary ammonium salts (bephenium, thenium), pyrimidines (methylidene), tribendimidine in addition to spiroindoles result in depolarization of the membrane potential with escalated spike frequency for the induction of a contraction over the nematode muscles[13]. Despite, the study by Colquhoun & Sakmann in 1985 described in considerable details levamisole, pyrantel, morantel as well as oxantel portrayed organic cations of remarkably large sized attempting passing via the nicotinic ion channel from the outside possessed the capacity of blockade of selective filter as well as generation of spastic paralysis over the nematode[14]. Aminoacetonitrile derivatives (AAD) for instance monepantel possessed the capacity of blockade of nicotinic acetyl choline receptors[15], whereas cyclic depsipeptides are involved in targeting potassium (K_{ATP}) channels along with latrophilin receptors[15].

2.2. Metabolic Hampering Agents

Lactate generated from the carbohydrate metabolism works in the form of main energy source in reference to filarial parasite, where diethyl carbamazepine (DEC), antimonial, suramin, benzimidazole as well as levamisole target variable enzymes over the separate steps of the carbohydrate metabolism in addition to diminish the metabolism leading to minimized energy provision. Hampering of the phosphoenol pyruvate carboxykinase

(PEPCK1), fumarate dehydrogenase, as well as succinate dehydrogenase by DEC change the rate of the carbohydrate metabolism[16], whereas suramin inactivates lactate dehydrogenase, malate dehydrogenase [17] along with malic enzymes[18] in *Onchocerca volvulus* which halts tricarboxylic acid cycle (TCA alias Krebs's cycle). As per Saz & Dunbar[19], a substantially greater dosage of the antimonial stibophen is the requirement for the blockade of actions of phosphofructo kinase (PFK) in case of filariae *Litomosoides carinii*, *Acanthocheilonema vitae* as well as *Brugia pahangi* by targeting glycolysis. DEC, benzimidazole as well as levamisole further corroborated their part in the Omit m form of glucose metabolism hampering agents by changing glucose uptake in addition to reduction of glucose use by switching towards homolactate fermentation respectively[20]. Intertransformation of the folate derivatives in case of nematodes blockade takes place by the actions of the DEC along with suramin over the enzymes catalyzed by the complete folate metabolic events, inimical to the nematode in view of hampered purine generation. Specifically, suramin, an agent extensively catalyzing the hampering of dihydrofolate reductase (DHFR) in *O. volvulus* in addition to nicotinamide adenine nucleotide phosphate (NADPH) based 10-formyl tetrahydrofolate (FH₄) dehydrogenase in *Brugia pahangi*[20,21], however DEC modulates variety of hampering separate enzymes inclusive of serine hydroxy methyltransferase, 5,10 methenyl FH₄ reductase, methenyl FH₄ dehydrogenase, 5-formyls FH₄ cycloligase, 10-formyl FH₄ dehydrogenase as well as glutathione formimino transferase in folate metabolism pathways[20,22]. Arsenical melarsen oxide possessing its toxic in addition to idiosyncratic characteristics were involved in the elimination of GSH quantities by hampering enzyme glutathione reductase (GR) in case of cattle filariae *Serratia digitata*, *Onchocerca gutturosa*[20,23], along with *Litomosoides carinii* filariid in cotton rat, while further displayed minimal proneness in human erythrocytes in GR[24]. One more metabolic hampering agent isothiocyanate amoscanate possess effectiveness against *L. carinii* as well as *B. pahangi* in the form of micro in addition to macrocidal in hampering glucose uptake as well as integrating in glycogen[20-24]. Additionally, mevinolin, a hampering agent of 3-hydroxy-3-methyl glutaryl-coenzyme (HMG-CoA) reductase significantly works in blockade of the generation of the geranyl geraniol, ubiquinone along with dolichol in filariid *B. pahangi*[20,25].

2.3 Arachidonic acid (AA) Metabolism Blocking Agents

Anti-filarial agents however possess the capacity of targeting AA metabolism have been evaluated. Noticeably, DEC has been observed to possess the capability of working as a nematocide in view of it repressing the metabolism of Arachidonic acid (AA). This gets attained by blockade of the cyclooxygenase (COX) generation, that hampers the generation of the prostaglandins along with prostacyclins - which portray robust inflammatory reactions[13,26]. Furthermore DEC causes blockade of the Leukotriene synthase, a significant modulator of immune reactions,

transforming it to Leukotriene [13,27]. Razin et al.[28], demonstrated that DEC did not hamper 5' lipoxygenase.

2.4 Oxidative stress(OS) Stimulators

Anti filarial agents like DEC possess the capability of blockade of AA metabolism, while aspirin -a nonsteroidal anti inflammatory drug(NSAIDS) hampers prostaglandin H synthase. As per Singh et al.[29], combination of both DEC as well as aspirin escalated the quantities of nitric oxide (NO), in addition to diminished the GSH along with peroxidase quantities leading to Oxidative stress(OS) .

2.5 Anti Wolbachia treatment

The invention of Wolbachia endosymbiosis with various filarial nematode yielded provision of generating innovative favourable approaches pertaining to the arena of anthelmintic treatments[30]. Doxycycline possesses the capability of resulting in reduction in plasma quantities of vascular endothelial growth factor-A(VEGFA), that is a key controller of generation of hydrocoele in case of patients with lymphatic filariasis[31]. As per Sanprasert et al.[32], combination of doxycycline with DEC initially eliminated *Wolbachia* followed by the parasite with the capability of DEC getting potentiated. Pertaining to the avoidance of generation of resistance to antibiotics for *Wolbachia*, a combination of rifampicin plus doxycycline applicability was diminished at threshold quantities[32], however these antibiotics possess certain restrictions pertaining to the children safety as well as mothers who are pregnant [33]. Despite clinical trials of antibiotics for instance rifampicin as well as doxycycline in the form of single or combined utilization attained considerable successful results[34], there are existent further reports regarding *W. bancrofti* resurging subsequent to 24 mths of rifampicin plus doxycycline treatment[35]. Publications from Thailand have revealed reviving of mf in addition to adult Bancroft filarial infection 6mth subsequent to doxycycline as well as DEC[32].

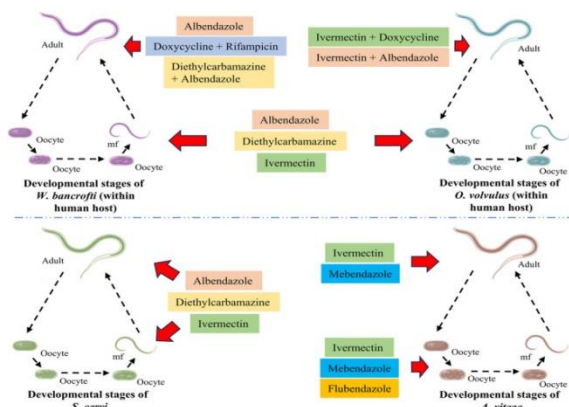
2.6 Combinational Chemotherapy

Alb, Ivm as well as DEC are the maximum favourable therapeutic strategies from the 20th century as per recommendations by Food and Drug Administration(FDA)and Centre For disease control and prevention(CDC). Based on the complicated nature of the disease, utilization of these agents is done alone at certain times in addition to a group of 2-3 agents in variable combinations; for instance combinations of DEC with aspirin in addition to gather the advantageous part of aspirin in inducing OS. Whereas combinational group for instance target DEC+ Alb as well as Ivm +Alb offered a best chance for attaining variable targets actions at the time of single exposure in addition to with the objective of rapid action replay with no anticipation of generation of any newer resistance. Treatment of LFin the coinfection with *Wolbachia* doxycycline in combination with DEC resulted in improvement of success that corroborated the significance of cooperativity using combined drug treatment as well as their targets. One more triple drug based treatment is inclusive of indoleamine 2,3-dioxygenase (IDO), the manner

advocated by World Health Organization(WHO) ,by combination of Alb, Ivm in addition to DEC, in contrast to 2 agent regimens, the IDO trial against *Wuchereria bancrofti* infection in PapuaNew Guinea illustrated a lesser success in sterilizing adult parasite for the duration of just 3yrs[36] in contrast to Alb as well as DEC in combination yielded greater provision of microfilaricidal actions[37]. Other studies conducted with the utilization of DEC at dosages 6mg/kg lone or combined with Alb 400mg illustrated significant diminishing of adult *W. bancrofti* [38]. Following studies performed in American Samoa initiated from 2001 in addition to encompassing time period of 6 yrs implicating combination mass drug administration (MDA) of Alb as well as DEC illustrated significant diminishing of prevalence *W. bancrofti* antigenemia . Thereby reduction of prevalence occurred from 11.5% in 2001 to 0.95% in 2006 [39]. By itself Alb was substantially inferior in contrast to Ivm , however in combination with Ivm ,it displayed a greater mf diminishing rate in addition to significant mf clearance capacity from the peripheral blood [40]. Makunde et al.[41], illustrated safety ,tolerability as well as on long use of Alb, as well as Ivm treatment of bancroftian filariasis on coinfection with Onchocerciasis. Pertaining to a display from 9 villages in south India *W. bancrofti* re-emerged subsequent to successful MDA with the utilization of annual Ivm 400µg /kg as well as DEC at dosages 6mg/kg disproved the newer posit of combinational chemotherapy for the treatment of filariasis, however sustenance of outcomes on proper vectors regulating approaches by all nightlanding catch(ALNC)was used for accessibility for *C. quiquifasciatus*[42]. Intriguingly various clinical trials regarding communities which have been infected by *W. bancrofti* further demonstrated that there were no variations in effectiveness amongst Ivm solely as well as combinational treatment with DEC[43], in addition to amongst Ivm solely as well as combinational treatment with Alb[44].

2.7. Life cycle Hampering Agents

Targeting in addition to blockade of the life cycle of the filarial parasites portray key approaches in the reduction of the transmission as well as infection load . Evaluation of a plethora of epidemiological along with clinical trials studies have illustrated that the constituents of mass drug administration (MDA) possess restricted effectiveness in microfilariae (mf) as well as adult filarial parasites. Moreover the efficacy of these constituents differs amongst, separate species(spp) leading to filariasis(see Figure 2).



Legend for Figure 2

Courtesy ref no-4- Schematic representation of the potential efficacy of different chemotherapeutics across the developmental stages of filarial parasites reside in the human host (*Wuchereria bancrofti* and *Onchocerca volvulus*), bovine host (*Setaria cervi*) and rodent host (*Acanthocheilonema viteae*).

Whereas occasional illustrate efficacious ovicidal as well as microfilaricidal characteristics, their effectiveness against adult parasites is usually reduced or postponed[45]. Antibiotics for instance rifampicin as well as doxycycline have illustrated favourable outcomes in depleting human parasites which nurture *Wolbachia* worked in the form of endosymbiant [32,34]. Nevertheless these antibiotics have been observed to be inefficacious against bovine as well as rodent parasites. Furthermore, studies have displayed examples of mf as well as/or antigenemia resurging mths subsequent to treatment, pointing to the incapacity of chemotherapeutic substances in complete blockade of the life cycle of the filarial parasites. Ineffectiveness of agents for instance -Alb, Ivm as well as DEC are not restricted solely to the filarial parasites in view of akin results have been found with the other parasites spp also. DEC, for example has illustrated no efficaciousness against parasites resulting in schistosomiasis. Conversely utilization of coadministration of albendazole along with praziquantel as well as nitazole which target larval forms of *schistosoma mansoni* along with eggs of *schistosoma japonicum*, *schistosoma haematobium*, *schistosoma mansoni*[46]. Intriguingly, Ivm has illustrated greater efficacy against all the generational stages of schistosomiasis[47].

3.0 Apoptosis in the form of plausible target for the treatment of filarial worms

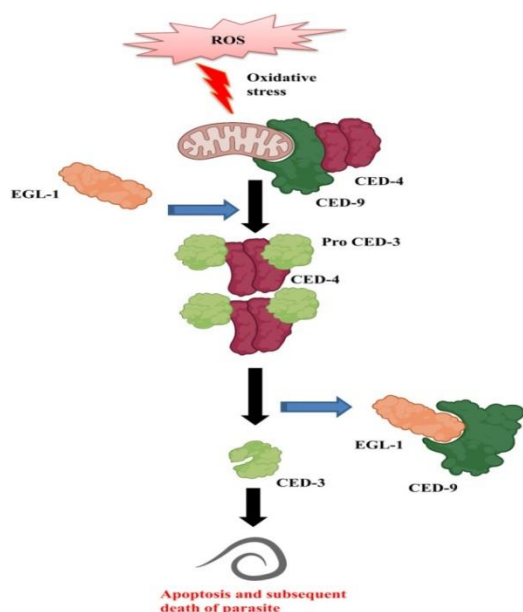
Experiments dependent on the corroboration have posited the assessment of natural events of innate immune reactions thinking of eliminating infective agents. Thus the group of Mukherjee and colleagues got the thought of exploiting natural innate immune reactions for facilitating the defence system for lymphatic filariasis eradication[3]. Mechanistic evidence provision has yielded regarding DEC part in induction of apoptosis *in vitro* finally resulting in demise of the parasites efficaciously. Cells that had been

infected go through programmed cell death alias apoptosis, where the infected cells or demised cells get eliminated by the immune system of the host for the sustenance of the homeostasis balance. At the time of the apoptotic events, modifications of the cytoskeleton takes place, leading to blebbing of the cell membrane, chromatin condensations, DNA breakdown followed by its fragmentation, nuclear fragmentation, resulting in cell shrinking in view of volume reduction. Robert H. Horvith was the person who initially worked over apoptosis towards generating the full designs in reference to programmed cell death subsequent to in depth study over the living cells of *Caenorhabditis elegans*[48]. Apoptotic cells subsequent to the generation of apoptotic bodies get phagocytosed by the macrophages. No substances for instance lytic enzymes /oxidizing molecules get liberated which might result in a local inflammatory reaction.

3.1. Apoptosis in filarial worms: extrinsic as well as intrinsic pathways

The utilization of filaricidal effects have led to lesser of quantities of superoxide dismutase(SOD), catalase(CAT), glutathione peroxidase(GPx), enzymes working in the form of key antioxidant modes[49]. GSH portrays a sulphur possessing protein, carrying reactive electrons from the peroxidase which are impacted leading to reduction of its quantities, in view of filaricidal of induction, while glutathione -S-transferase(GST) results in sustenance of greater quantities of ROS in the mitochondrial, ER, along with peroxisomes quantities, thereby leading to apoptosis[50].

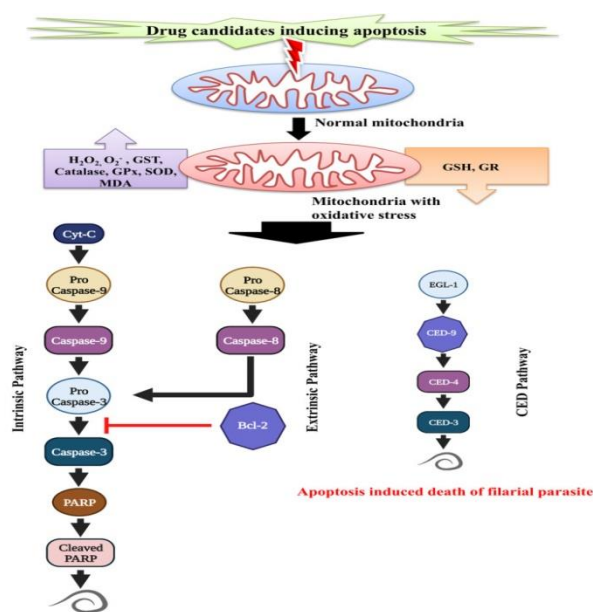
Egg laying defective(EGL-1), cell death abnormal (CED)-3, (CED)-4 as well as (CED)-9 represent 4 necessary proteins along with their respective part in the form of the proapoptotic in addition to antiapoptotic proteins modulating part of apoptosis in filarial parasites[51]. EGL-1 as well as CED-9 proteins portrays Bcl2 family, while CED-4 portrays nematode homologue of mammalian apoptosis protease activating factor(Apaf-1). At the time of apoptosis CED-9 gets negatively controlled by EGL-1, however it does not possess the capacity of displaying superiority over CED-3, as well as CED-4[52]. In case of a normal filarial cell, CED-4 dimers get segregated with CED-9 over the outer mitochondrial surface in addition to hampers apoptosis[53]. Timely stimulation subsequent to apoptotic induction escalated the quantities of EGL-1 in addition to bonded with CED-9 via the BH3 domain which in turn led to interference with CED-4 -CED-9 complex[54]. Subsequent to getting disconnected from CED-9, 2 asymmetric CED-4 dimers oligomerize for generation of tetrameric apoptosome, followed by enrollment of pro CED-3 molecules by this tetrameric structure[55], subsequent CED-3-a cysteine protease activation takes place which leads to apoptosis that is nematode particular[51,53](see Figure3).



Legend for Figure 3

Courtesy ref no-4- Mechanism of apoptosis in filarial parasites. ROS-induced oxidative stress within the filarial parasites activates a major cell death pathway. EGL-1 the apoptosis inducer disrupts the normal CED-4–CED-9 complex form and makes an association with CED-9 to create a pro-apoptotic moiety. Free CED-4 dimers then oligomerize and make tetrameric apoptosomes with proCED-3. Activated and cleaved CED-3 is the ultimate protein responsible for apoptosis in filaria parasites.

Peixoto et al. [56], were the founder of the molecular mechanistic modes validation for apoptosis in microfilariae of *W. bancrofti* [56]. Additional studies performed by Landmann et al. [51], as well as Roy et al. [57], displayed that the CED pathway works via induction, propagation in addition to orchestrating apoptosis in filariids. The utilization of signaling molecules by the CED pathway are akin to pathway in free living nematode *C. elegans* [58,59]. This CED pathway was **observed** to be present in case of both Wolbachian (*W. bancrofti* as well as *Brugia malayi*) in addition to non-Wolbachian (*Sertaria digitata* along with *Sertaria cervi*) filarial parasites [51,52,57,60], of which indicated that this CED pathway worked in the form of a universal drug target. Studies performed by Mukherjee et al. [50,52,61], in which filaricidal treatment had been employed displayed enrichment of cysteine protease family protein alias caspase yielding provision of a newer trajectory appropriate for the filarial apoptosis via activation of other inactive proforms by generating cleavage at a particular sequence adjacent to aspartate. The implications of caspase protein cas-9 cas-8 cas-3, cytochrome-c in addition to poly(ADP ribose) polymerase (PARP) in the nematode [50,61], suggesting the induction of extrinsic as well as intrinsic pathways in apoptosis. Thereby drawing conclusions that this nematode particular CED pathway in addition to the newer disclosed caspase pathways, both possess significant part in orchestration of apoptosis in filarial parasites (see Figure 4).



Apoptotic death of filarial parasite

Legend for Figure 4

Courtesy ref no-4- Apoptosis as a target for developing antifilarial drugs. Apoptosis-inducing drugs primarily raise oxidative stress inside the parasite body and subsequently activate intrinsic, extrinsic as well as filarial conserved apoptosis pathways, EGL-1/CED-9/CED-4/CED-3 pathway.

4. Part of agents causing - Apoptosis in the form of Antifilarial Drugs

Natural in addition to synthetic substances have been believed to be alternate treatment approaches in the past 3 decades in view of them demonstrating probability of resulting in death/demise of the adult filariids by apoptosis induction. Model portraying apoptotic target killing have been generated in bovine parasites *S. cervi* [62] along with *S. digitata* [63] in view of their biochemical, immunological as well as morphological simulation with *W. bancrofti* as well as *Brugia malayi* & other Brugian parasites.

4.1 Anthelmintic agents

Besides the maximum favourable targets, MDA recommended drugs further possess certain characteristics of apoptosis induction as well in filarial parasites. Amongst such agents, DEC possesses a key part in the form of metabolic hampering agents. As per Peixoto et al. [50], Singh et al. [29], along with Shukya et al. [64], on utilization of DEC in the form of a single agent or DEC combined with aspirin displayed a definite part in modulating apoptosis. Microtubules assembling avoidance by Alb in addition to Wolbachian eliminatory antibiotic therapy for instance of rifampicin as well as doxycycline on delivery induction have demonstrated tunnel positive outcomes in addition to existent chromatin condensations & DNA fragmentation that validated implications of apoptosis in filarial parasites demise [65]. Animal studies as well as in vitro findings of tetracyclins over the *B. malayi* have yielded provision of promising knowledge, Wolbachian elimination is crucial for apoptosis induction in filariids [51].

4.2 Phytochemical Therapies

Real Time- Polymerase Chain reaction (RT-PCR), as well as ROS estimation study displayed that hydroxycinnamic acid: a phenolic agent identified in the form of ferulic acid from *Hibiscus mutabilis* possesses robust anti filarial actions in case of ROS generation as well as significantly diminishing the expression of CED-9 that subsequently led to active CED-3 for the propagation of apoptosis in mf in addition to adults in bovine filariids *S.cervi* [66]. The tunnel positive study by of Nayak et al. [67], revealed the beneficial part of the anticarcinogenic *Curcuma longa* obtained curcuminoid curcumin for the elimination of parasite quantities of GST as well as of improvement of ROS generation resulting in apoptosis of the micro along with macrofilariaids. Other than antimalarial, antileishmanial, antitrypanosomal as well as antinematocidal characteristics, ursolic acid portray a pentacyclic triterpenoid (PT) carboxylic acid isolated from the leaves of *Nyctanthes arbatristis* further possessing efficacious anti filarial actions in inducing the CED modulated apoptosis in mf in addition to adult *S.cervi* [68]. Azadirachtin portrays a tetraterpenoids phyto substance ethanolic extracts from the leaves of *Azadirachta indica*, escalates the quantities of superoxide anions as well as generates OS which ultimately leads to apoptotic demise of *S.cervi* [68]. MTT outcomes from the study by Mukherjee et al. [68], displayed that azadirachtin significantly diminishes variability of worms in mf in addition to adult parasites. Furthermore Hoechst as well as tunnel staining over the *Sertaria digitata* got therapy by oleanolic acid significantly corroborated proof of chromatin condensations in addition to generation of apoptotic bodies in reference to all generational stages of parasite [53].

4.3 Extracts from Plants

Methanolic leaf extracts of *Aegie marmalos* generally referred to beal possessed enrichment of polyphenol that is enough for generating OS at the considerable greater quantities as well as believed to be a prooxidative in addition to proapoptotic substance for microfilariae parasites [69]. Treatment of mf of *B. malayi* the utilization of the greater quantities methanolic root extracts of *Vitex negunda* (nirgundi) results in elimination of their motility by just around 50% whereas combined with the H₂O₂ leading to complete motility elimination [69]. As per Sahare et al. [70], root extracts of *Vitex negunda* possesses saponin that has apoptotic properties. *Butea monosperma* is well acknowledged regarding its medicine properties, utilized for therapy of variable disorders with regards to Ayurveda, generally referred to as flame of forest /palasa possessing antimicrofilariae actions against *B. malayi* [71]. Validation of lipid peroxidation in the *B. monosperma* leave extracts that are utilized for therapy of microfilariae possess the capacity of OS induction in addition to existence of saponin in the extracts has confirmed the association of OS with apoptosis [71]. *Cajanus scarabaeioide* generally referred to as pigeonpea possesses variation of therapeutic implications for instance therapy of anaemia, smallpox, dysentery, cholera, gonorrhoea in addition to rinderpest in the form of

ethnomedicine. Out of the chloroform, ethanolic, ethyl acetate, petroleum etheric extraction obtained from the *C. scarabaeioide* possess polyphenol enrichment as well as reveal the maximum plausible actions on treatment against oocyte, mf, adults *S.cervi* in inactivating ROS based CED pathway of apoptosis [72]. Neem tree portrays to be a member of the maliaceae family utilized in the canonical medicine for over last 2 millenia by Sidha along with Ayurvedic drs in the form of anthelmintic, antifungal, anti viral, antidiabetic, contraceptives, sedatives. Terminal-deoxynucleotidyl-transferase dUTP nick end labeling (TUNEL) positive apoptotic nuclei in case of mf, as well as adult *S.cervi* point that the ethanolic extraction from the leaves of *Azadirachta indica* (EEA) displayed antifilarial part in modulating apoptosis in *S.cervi*, *Dirofilaria immitis* [73], with heart worms of dogs is further an anti apoptotic target for the EEA where 100 µg ml⁻¹ dosage of the EEA possesses the capacity of changing the extrinsic morphology of the mf possessing compressed sheaths breakdown as well as holes over the body [59]. Innovative antifilarial substance n-butanol extract (NBE) obtained from the *Diospyros perigrana* stem bark extract efficient in development of *in vitro* effectiveness against mf in addition to adult kinds of *S.cervi* by upregulating proapoptotic proteins followed by leading to downregulation of anti apoptotic proteins [74]. Assessment of tissue injury along with logo properties of apoptosis in the ones who got treatment for *S. digitata* showed that rhizome extracts of *Curcuma zedoaria* was efficient in development of proapoptotic proteins, thereby the extract was robust in the form of microfilaricidal as well as macrofilaricidal [75].

4.4 Synthetic substances

Synthetic cyclic sulfonamide, sultams, sulfones, as well as cyclic sulfonates portray biologically considerably active substances which display antifungal, anti viral, antiparasitic actions [153]. Of these quinolone fused cyclic sulfonamide (4,7, dimethyl, 3,4,7,8 tetrahydro-3λ6-(1,2thiazino(4,3,-f) quinolone -3,3,8 trione) 8L represents a hybrid substance formed by fusion of coumarin in addition to quinolone along with is necessary in reference to accrual of ROS which escalates OS with subsequent signals for apoptosis by stimulating extrinsic as well as intrinsic pathways in reference to all generational stages of *S.cervi*. Additionally, 8L is further advantageous against *Wolbachia* infection [52]. More appropriately, 8L possesses greater effectiveness in contrast to Ivms in addition to it having non toxic characteristics for the mammalian host which targets apoptosis, thereby placing it as maximum favourable drug candidate out of all the filaricidal substances. As per Guchait et al. [77], compound 4a, out of the series of developed carbamo(dithioperoxo)thioate derivatives work in the form of having maximum acceptability in reference to apoptosis induction against oocyte, mf, adult stage of *S.cervi* subsequent to the flow cytometry assessment of oocyte, mf in addition to western blotting of apoptotic proteins of adult. The implications of apoptotic modulator caspase-3, caspase-8, caspase-9, cytochrome-c, along with PARP in addition to filarial particular CED gives corroboration regarding trans-

stilbene derivatives possess effectiveness in stimulating apoptosis in filariids[50]. Furthermore, trans-stilbene derivatives in addition to resveratrol(Rsv) demonstrate considerably greater robustness in contrast to Rsv solely in modulating apoptotic demise in nematode *S.cervi*[50]. Generated substance C-cinnamoyl glycosides possess substantially greater probability the form of an anti filarial along with apoptotic substance resulting in demise of bovine parasites which is permanent along with filarial parasite afflicting humans *W. bancrofti*[78]. Butylated hydroxyanisole is a well acknowledged food additive which gets utilized against the treatment of adult *S.cervi*, displaying adulticidal actions escalating its attractiveness and promise. Nevertheless, in contrast to macrofilaricidal methyl substituted chalone(MC), MC takes over subsequent to the parasites motility along with viability estimation[79]. 2,4 diamino pyrimidines in addition to 2,4 diamino-s-triazine derivatives enzymes portray dihydrofolate reductase(DHFR) hampering agents working in the form of robust antifilarial substances corroborated by apoptosis determination ethidium bromide-acridine orange (EB/AO) validating these derivatives were involved in apoptotic injury in *B. malayi* mf in addition to adult[80]. Following the determination of TrxR activity in 2,4 dinitrochlorobenzene (CDNB) *S.cervi* mf, which got treatment, there was clearance of CDNB targets TrxR, disturbed balance of the cellular redox homeostasis, mitochondrial membrane lipid peroxidation along with protein carbonyl generation which ultimately starts intrinsic pathways of apoptosis[81]. As per Mandivar et al.[82], 1m10 (5benzylidene-2-imino-3(4 phenyl thiazole-2-4) thiazolidine-4-one), a heterocyclic thiazolidine obtained compound possesses significantly greater probability in contrast to DEC on inducing ROS modulated apoptosis in case of treated *B. malayi* mf in addition to adult.

4.5 Nanotechnology use as Nanoparticles

Nanotechnology portrays the maximum innovation where utilization of nanoparticles work in the form of one more treatment methodology for the administration of the drug to their targets. Different filaricidal nanoparticles have been generated by now, hoping that they would be employed regarding treatment of filariasis in future for instance chitosan biopolymer functionalized gold nanoparticles that possess compatibility with mammalian host in addition to minimizing allergy generating actions along with biodegrading actions with negligible cytotoxicity. It possesses the capacity of upregulating the ROS generation amongst filarial parasite leading to DNA fragmentation, thereby having acceptable efficacy in the form of a non therapeutic inducing substance filariasis [83]. Chitosan in addition to *Terminalia chebulla* extract dependent gold nanoparticles get formed by utilizing green methodology possessing considerable antifilarial activity with corroboration of externalization to membrane phosphatidyl serine on cell surface over filarial parasites getting treatment subsequent to double staining with 6 carboxy fluorescein diacetate in addition to annexin-V Cy 3. TUNEL staining positive outcomes further corroborate its apoptotic characteristics in the form of filaricidal[57]. One more constituent of gold

nanoparticles with chitosan along with extract of *Piper nigrum* is substantially active against *S.cervi* regarding OS, disturbed redox balance induction in addition to further led to activation of proapoptotic proteins [84]. As per Roy et al.[85], BCH(chitosan stabilized) worked **substantially as better effectors in contrast to** BEG(ethylene glycol stabilized) in addition to BST(starch stabilized) silver nanoparticles(AgNP) over apoptotic cell demise. Once application is done in substantially greater dosages about 250µL/ml to *S.cervi*, maximum of the mf along with adults had depletion of their viability. Silver nanoparticles constituents are biomolecules tyrosine along with natural polymer starch that are substantially active in induced ROS production in the parasite's larval stage in addition to the vector resulting in filariasis namely *C. quinquefasciatus*[86]. Other silver nanoparticles generated from *Acacia auriculiformis* displayed their antifilarial activity at a substantially lesser dosage, which pointed 50% demise at simply 3.58 µg/ml quantities in contrast to raw extract of the plant along with further significantly resulted in stimulating OS which ultimately modulated apoptosis of the filariids[87]. Yadav et al.[88], suggested that the raw extract of the plant *Andrographis panicula* substantially lesser effectiveness in contrast to silver nanoparticles produced with same plant extract resulting in demise of parasites by stimulating apoptosis in addition to corroborating success in reference to nanoformulation for treatment of filariasis.

Additionally, D-glucose as well as hydrazine composite silver nanoparticles further portray a considerably robust filaricid in the formation of oxidative attack at a considerably minimized dosage[89]. Demonstration of nuclear DNA breakdown using propidium iodine staining corroborates apoptotic role of poly(vinyl alcohol) capped silver nanoparticles over treatment against *S.cervi* [90]. As per Zafar et al.[91], copper(II)oxide nanoparticles possess significantly greater efficacy in generating OS in contrast to Alb. Furthermore, this study validated how economic this nanoformulation is which might aid us as future therapeutic approach in the treatment of filariasis.

5. Reasons for Apoptosis

Despite all of the FDA/CDC recommended substances regarding filariasis are still being used, however robustness in attaining filarial demise has diminished significantly. Whatever approaches used, they are not successful with its reduction over time as well as recurrence of the infection resulting in a worldwide problem. Here apoptosis might be possessing a significant part in working in the form of a target for tackling filariasis.

Cytoskeleton disturbing agents for instance Alb leads to microtubules generation blockade with subsequent diminished glucose uptake, however is not efficacious, but its utilization as nanoformulation results in synergistic apoptotic effect. As per Zafar et al.[91], 100µg/ml Alb-CuO nanoparticles was observed against mf to be implicated in 47.3RM outcome in adult *S.cervi*, whereas 87.1 RM outcome was observed subsequent to treatment which utilized sole Alb

in case of dark surroundings, however on use of UV light RM outcome diminished intriguingly. Around 2 fold greater mortality was observed over treatment utilizing Alb-CuO nanoparticles at 100µg/ml quantities in contrast to sole Alb[91]. Furthermore, azoles possess certain teratogenic characteristics thereby their restricted use, whereas maximum of the apoptotic resulting agents possess origin from extracts of plants, therefore no fear of toxic inimical sequelae, in addition to their displaying considerable filaricidal efficacy. Ethanolic extracts of *C. scarabaeioides* is basically not toxic (NT) along with possess maximum effectiveness of all plant extracts targeting apoptosis in view of 50µg/ml is enough for attaining 60% mortality of adult *S.cervi* in addition to possessing ovicidal characteristics[72]. Other plant extract EEA is further not toxic having LC₅₀ outcome of approximately 47.12 µg/ml against adult *S.cervi*, whereas against mf it was 27.42 µg/ml, making this EEA a considerably efficacious in resulting in demise of filarial parasites[73,59].

Ion channel blockers (ICB), blocking agent Ivm targets GluCl channels having different trials revealing success however contrasted to apoptotic targeted agents it has been observed that Ivm is far inferior in robustness as filaricidal. From the MTT assay it was revealed that 90% demise of *W. bancrofti* over the treatment using ursolic acid at quantities of 10µg/ml along with 70% demise utilizing Ivm[87]. Further Ivm got success as microfilaricidal with part efficacy in adults, however maximum apoptotic substances possess plausibility against all generational stages akin to Rsv & trans-stilbene derivatives on therapy with 50% mortality attained at just 8.867±0.82 µg/ml quantities over adult *S.cervi* however Ivm could do at 50 µg/ml [50]. One more study following applications of carbamo(dithioperoxo)thioate derivatives, 100% hampering occurred over adult *S.cervi* in contrast Ivm hampered about 48% at 100µg/ml quantities[77]. Levamisole one more ICB, possesses spp particular microfilaricidal is efficacious just against *B. malayi* & *W. bancrofti*, however not in *O. volvulus*, additionally different anectodes of resistance failing a robust filaricidal[92]. More ICB for instance -piperazine, pyrantel, morantel & oxantel, despite good have restricted to just targets in cattle, sheep, dogs, goat. In humans they are barely used against nematodes, basically hookworm with restrictions, therefore significant need for apoptotic agents formation against human LF.

DEC has pleiotropic actions, believed to have maximum success as filaricidal agents i) causes blockade of the AA metabolism, ii) hampering carbohydrate metabolism, ii) generating OS however in view of inimical sequelae, recurrence along with resistance generation therefore significant need of generating agent with akin efficacy. That way apoptotic induction by quinolone fused cyclic sulfonamide 8L might be utilized in the form of substitute and form future antifilarial in view of it being NT to host ii) its substantially active against all generational stages of *S.cervi*. As per Mukherjee et al.[52], LC₅₀ was 281.4µM for

adult, 166.9 µM for mf & regarding oocyte it was 17.3µM, with this robustness ensures 8L possesses maximum effectiveness of synthetic substance. Metabolic hampering agent suramin portray greater antifilarial effects however it is substantially toxic, therefore can't use commonly in view of inimical sequelae for instance muscle & joint aches, whereas all innovative apoptosis targeting agents possess no inimical sequelae.

Regarding Anti Wolbachia treatment, variable antibiotics have been utilized for substantial time period for eliminating Wolbachia, therefore probability of generating resistance exists in such antibiotics, warranting a replacement, where 8L might be advantageous in efficacy for Wolbachia infection.

Thereby in toto these studies illustrate natural and synthetic substances having apoptosis induction capacity are substantially efficacious for mf & adult LF stages etiological parasites. A comparative study performed to validate displayed maximum non apoptotic filaricidal agents showed lesser efficacy contrasted to apoptotic inducing agents. Furthermore autophagy inducing agents for instance epigallocatechin gallate (EGCG), C-cinnamoyl glycosides illustrate significantly greater LC₅₀, contrasted to ursolic acid, trans-stilbene derivatives & carbamo (dithioperoxo) thioate derivatives [55,58,84,85]. Moreover apoptotic inducing tetracyclins & 8L displayed greater effectiveness against Wolbachia [52].

Overall these studies illustrated host's innate immune reactions would portray great approaches for avoidance of production of resistance, with their no inimical sequelae in host having substantial efficacy against mf & adult filarial parasite in LF and stimulating ROS induced apoptosis.

6. Estimation of Apoptosis in Filarial Parasites along with Screening of Antifilarial Substances

Efficacy along with apoptosis stimulating characteristics of all antifilarial substances detailed earlier have been invented in the past 1-2 decades experimentally. Correct approaches use for estimation of apoptosis induction has been critical for getting insight regarding effectiveness of natural and synthetic/semi synthetic substance along with nanoparticles against various filarial parasites. Of the methodologies utilized for screening antifilarial substances, cell viability [93]. MTT assay (3,4,5-dimethyl thiazole-2yl)2,5-diphenyl tetrazolium bromide portrays a yellow tetrazolium salt whose colour changed into purple formazan over the effect on cellular mitochondrial reductase /NAD(P)H dependent oxidoreductase enzymes portraying cell viability [85]. Conversely living cells refuse to hold trypan blue dye whereas demised cells accrue dye in view of elimination of membrane permeability turning blue. Changes in nuclear morphology secondary to chromatin condensations, monitoring is easy with Hoechst, DAPI, propidium iodine, ethidium bromide - acridine orange (EB/AO) etc. alteration in the membrane architecture particularly placement of phosphatidyl serine working as signatures for cells getting into apoptotic

demise[94], gets estimated with ease by fluorescence staining by FITC- annexin-V. With all these qualitative, thus flow cytometry has aided in quantitative estimation of all apoptotic paradigm. For instance, oocyte going via apoptotic alterations with the agents might be assessed for quantitative apoptotic induction along with percentage of cells in early/late apoptotic cells, necrotic cells along with living cells can get attained. Elimination of intactness of the genetic material portrays properties of the programmed cell death (PCD). Drug stimulated injury detected efficaciously utilizing DNA fragmentation assay(DFA)-a simple methodology for isolating DNA fragmentation in an agarose gel electropherogram[95]. TUNEL assay determines in situ DNA fragmentation by ease where double DNA having breakdown picked up by ease in histological filarial parasite exposed to antifilarial agents .

7. Further Arenas of Exploration & Conclusions

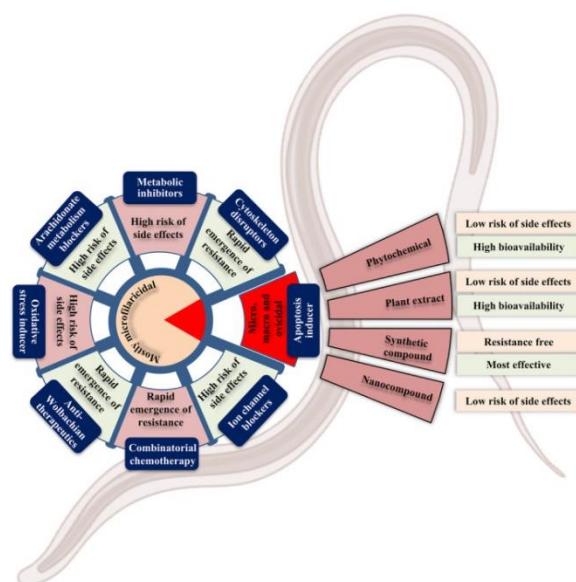
Apoptosis gets targeted by natural and synthetic substances . Therefore newer candidates might be able to hamper prevalence of LF. Since targeting apoptosis might be great strategies, however total genome sequencing of *Sertaria digitata*, a substitute for *W. bancrofti* would aid in obtaining greater insight regarding this strategy as well as in future generating genomic medicine which targets anti apoptotic modulators. Nevertheless, Senananayake et al.[96], have been successful in unravelling the genomic sequence of *S. digitata* by now along with knowledge of the genetics might aid in looking for negatively controlling genes of filarial apoptosis. As has been acknowledged earlier small interfering RNA (siRNA) possesses the capacity of silencing genes, therefore generation of siRNA having complementary sequences might target anti apoptotic RNAs. Utilization of canonical agents chemical get utilized in the binding with the particular target proteins in addition to illustrate their inimical sequelae, however contrasted to RNA therapeutics nucleic acids get targeted, exerting toxicity more particularly. Despite various complications regarding accessibility of off target effectiveness chemical modifications for instance phosphorothiate modification along with nanoparticles encapsulation might be able to get over these hurdles[97]. A part from siRNA, miRNA along with antisense oligonucleotides (ASO) might be fashioned for targeting genes of significance with the idea of hampering their expression in blockade of apoptosis. miRNA possesses the benefit of controlling the expression of plethora of messenger ribonucleic acid (mRNAs), therefore targeting miRNAs might open newer path for antifilarial treatment, while ASO has the capacity of targeting miRNAs safety as well as with effectiveness. We had earlier reviewed how akin therapeutic approach might be generated for eliminating Chronic Hepatitis B virus (HBV) Infection[98] omit .ion

Bioinformatics has come a long way regarding Drug fashioning. A proteomic study on *B. malayi* & *W. bancrofti* might observe innovative proteins that are targeted by apoptosis stimulating substances, whereas metabolomics

might find the chemical pathways utilized till apoptotic demise. In certain instances autophagy aids in apoptosis demise, therefore activation of autophagy by inducing expression of ATG family of genes might stimulate apoptosis- eliminating parasite. Acknowledged the innovative proteins along with/or genes implicated in stimulating apoptosis, use of Bioinformatics is feasible for fashioning apoptotic pathways by Computational means. Substantial scientific researchers have corroborated filarial parasites have immunomodulatory characteristics possessing the capacity of modulating the host immune system[99]. They might show proinflammatory or anti-inflammatory environment amongst the host implicated in immunopathology becoming overt. Inspired from these observations significant propagation has been attained by researchers in newer studies in fashioning antifilarial immunotherapeutic substances to vaccines along with antibodies for depleting filarial infection with the restoration of immune homeostasis[76,100]. Antifilarial probability of these therapeutics for the coming decade needs evaluation in reference to apoptosis with the provision of greater trajectories for deeper understanding.

Thereby a summary is provided how apoptosis generating antifilarial agents are efficacious & what needs to be done at worldwide level for eradicating LF and why it has become indelible & why most of physicians are not conversant with it.

Other than WHO not much efforts put in & although all these thiazoles, ivermectin, DEC are not nobody tries using them with no new articles over case reports with use of say doxycyclin with rifampicin when pregnancy not a fear or safer helminthics used or even tried (Figure 5 sums up all the above details with associated inimical sequelae (low/high risk).



Legend for Figure 5
Apoptosis as an efficacious target in developing antifilarials in comparison to conventional drug targets.

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