Published Online on 9 July 2018

Proc Indian Natn Sci Acad **84** No. 3 September 2018 pp. 731-780

DOI: 10.16943/ptinsa/2018/49411

Research Paper

Perspectives of the *Artemisia annua* Dry Leaf Therapy (ALT) for Malaria and of its Re-Purposement as An Affordable Cure for Artemisinin-Treatable Illnesses

RICHA GOEL[1], RAJ KUMARI[2], VIJENDER SINGH[3], RICHA PANDEY[4], RENU KUMARI[5], SUCHI SRIVASTAVA[6] and SUSHIL KUMAR[5,7,*]

[1]*Department of Pharmacognosy, KIET School of Pharmacy, Ghaziabad 201 206, India*

[2]*Department of Pharmacognosy, ITS College of Pharmacy, Ghaziabad 201 206, India*

[3]*School of Pharmacy, Sharda University, Greater Noida, Ghaziabad 201 306, India*

[4]*Division of Natural Product Chemistry, CSIR-Indian Institute of Chemical Technology (IICT), Uppal Road, Tarnaka, Hyderabad 500 001, India*

[5]*National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110 067, India*

[6]*Division of Plant Microbe Interactions, CSIR-National Botanical Research Institute (NBRI), Lucknow 226 001, India*

[7]*SKA Institution for Research, Education and Development, 4/11 Sarv Priya Vihar, New Delhi 110 016, India*

(Received on 2 January 2018; Revised on 05 May 2018; Accepted on 06 May 2018)

Malarial diseases continue to risk the lives of more than 3 billion people in 97 countries in the world, causing sickness in several million people and death to half a million patients. The preponderate malaria causing apicomplexan protozoan parasite species *Plasmodium falciparum* and *Plasmodium vivax* have become genetically resistant to most of the approved antimalarial drugs, including the artemisinin-based combination therapies (ACTs). At this time, there is a vigorous need to make enough efforts to meet the challenge of combating multi-drug resistant malaria by (a) speeding up the trials in progress on relatively more effective, new and mechanistically different antimalarial pharmaceuticals, (b) production of effective vaccines against *falciparum* and *vivax* malaria, (c) devising of new ways to use the presently available anti-malarials such as by using three-drugs ACTs and by using the different two-drug and three-drug ACTs sequentially, and (d) induction of *Artemisia annua dry leaf therapy* (ALT) of recent origin, but of ancient precedent, as an effective treatment for acute and complicated malaria. Here, a perspective type review is presented of the: pre-ALT antimalarial drugs, methodology of their usage and consequences of resistance development; safety, efficacy, affordability, quality maintenance and resilience to resistance development aspects of ALT; and possibilities of ALT re-purposement for treating many infectious-metabolic disorder related- and cancerous-diseases. In conclusion, an urgent need is emphasized for pilot studies and clinical trials on ALT to attest its deployment as anti-malarial and cure for diseases beyond malaria.

Keywords: Antimalarial Drug-Resistance; Antimalarial Pharmaceuticals; Auto-Immune Diseases; Cancers; Infectious Diseases; Non-Artemisinic Secondary Metabolites.

Introduction

Malaria, which results from the transmission of malarial parasite infection to humans by the bites of infected mosquitoes, is the deadliest infectious disease of tropical and sub-tropical climates. Malaria Eradication Scientific Alliance (MESA) has recently updated the research agenda for malaria elimination and eradication (malERA.2017). In recent years (such

*Author for Correspondence: E-mail: sushil2000_01@yahoo.co.in

Table 4: Summarized evidence which shows that Artemisia annua leaf powder and/or leaf extract, artemisinin and/or artemisinin derivatives are inhibitory and putatively curative against a variety of infectious diseases in humans and livestock animals

S.No.	Disease(s)	Causal infectious organism/ agent	Host	Nature of study	Therapeutic test material(s)/ compound(s) used	Observation(s)	Reference(s)
(A) Virus caused:							
1	Hepatitis B	Hepatitis B virus (HBV)	Human derived Hep.G2/2.2.15 cell line	*In vitro*	Artemisinin and artesunate	Both the drugs strongly inhibited viral replication without causing cytotoxicity	Romero *et al*. (2005)
2	Hepatitis C	Hepatitis C virus	Human derived HuH-2 cell line (HCV)	*In vitro*	Artemisinin	Viral replication was inhibited; this effect was potentiated by iron donated by hemin, without cytotoxicity	Paeshuyse *et al*. (2006)
3	Cytome-galovirus	Human cytome-galovirus	Human fibroblast cells	*In vitro*	Artesunate	Viral replication was inhibited, very strongly in the presence	Kaptein *et al*. (2006)
			Human	*In vivo*	As above	Viral load was reduced of iron, without toxicity	Shapiro *et al*. (2008)
4	Herpes virus	Human herpes virus 6A (HHC-6a)	Cultured human cells	*In vitro*	As above	Viral early and late gene expression and replication was inhibited and thereby viral multiplication was arrested	Milbrandt *et al*. (2009)
(B) Bacterial caused:							
5	Tubercu-losis	*Mycobac-terium tuberculo-sis* H37Rv	MGI 960 system, and Ogawa slant medium assay	*In vitro*	As above	A single dose strongly inhibited bacterial growth measured 21 days after treatment	Choi (2017)
6		As above	Sprague Dawley rats	*In vivo*	As above	3.5mg/kg dose a day for 4 weeks cured the rats of infection without causing toxicity	As above
(C) Fungus caused:							
7	Aspergi-llosis	*Aspergillus fumigatus*	Fungus	*In vitro*	Artemisinin	The drug killed the fungus by targetting fungal oxidative phosphorylation and cell wall and ergosterol synthesis pathways	Gautam *et al*. (2011)
(D) Protozoan caused:							
8	a) Acantha-moebiasis	*Acantha-moeba castellani* (free living amoeba)	Cultured trophozoites	*In vitro*	Artemether	Amoebae were killed	Deng *et al*. (2015)
9		Acantha-moeba castellani 309 and Ac32	As above	*In vitro*	Artemisia annua leaves- water, alcohol or chloroform extract	Amoebae were killed	Derda *et al*. (2016)
10		As above	BALB/C mice (Mus musculus)	*In vivo*	As above	As compared to untreated infected mice, the treated infected mice survived for 2 to 4 fold longer time period	As above

11	b) Coccidiosis	*Eimeria tenella*	Domesticated chicken (*Gallus domesticus*)	*In vivo*	*Artemisia annua* dry leaves	Addition of dried leaves @ 20g/kg to feed was coccidostatic as well as growth promoter	Brisbe *et al*. (2008); Bosselman and Gylling (2013)
12		As above	As above	*In vivo*	As above	Addition of dried leaves @ 1.5% eliminated coccidiosis	Dragan *et al*. (2014)
13	c) Leishmaniasis	*Leishmania donovani* (Visceral infection)	Infected human macrophages	*In vitro*	Artemisinin	Parasite load was reduced; the anti-leishmanial activity was via apoptosis of parasites	Sen *et al*. (2007 & 2010)
14		As above	BALB/C mice	*In vivo*	Leaves and seeds of Artemisia annua or their hexane extract	Parasite load decreased on account of apoptosis of promastigotes	Mutiso *et al*. (2011); Islamuddin *et al*. (2012)
15		As above	As above	*In vivo*	Essential oil of leaves of *Artemisia annua*	Intra-peritonial administration of the essential oil reduced the parasite burden in spleen and liver by 90% without toxicity to test animals	Islamuddin *et al*. (2014)
16		As above	As above	*In vivo*	Artemisinin	Administration of the drug loaded nanoparticles reduced the parasite burden and spleen- and hepato-megaly	Want et al. (2015)
17		*Leishmania major* (cutaneous infection)	As above	*In vivo*	As above	Lesion size was reduced via induction of apoptosis in promastigotes	Ghaffarifar *et al*. (2015)
18		As above	As above	*In vivo*	Artemisinin ointment	Ulcers were healed	As above
19		*Leishmania panamensis* (cutaneous infection)	Infected human U-937 macrophage cell line	*In vitro*	*Artemisia annua* leaf powder	Amastigotes were inhibited without toxicity to macrophages and genotoxicity to lymphocytes	Mesa *et al*. (2017)
20		As above	Hamster (*Mesocricatus auratus*)	*In vivo*	As above	Intracellular amastigotes present in the ulcers were killed and 5 out of 6 treated hamsters were cured after 30 days treatment with 500mg/kg/day	As above
21		As above	Humans	*In vivo*	As above	Two patients were cured after 45 days of treatment with 30g of leaf powder (666mg/day)	As above
22	d) Toxiplasmosis	*Toxoplasma gondii* (Obligate apicomplexan parasite)	Human forskin fibroblast (HFF) cells	*In vitro*	Artesunate	Infected cells killed much like intra-erythrocytic malarial parasites	Gomes *et al*. (2012)
23		As above Kunming *Mus musculus*	CDI, OFI,	*In vivo*	Artesunate, dihydroartemisinin and their combination	All three treatments reduced the infection and improved survival time period of the diseased animals	Sarciron *et al*. (2000)
24	e) Trypanosomiasis	*Trypanosoma brucei* (African sleeping sickness)	*Mus musculus*	*In vivo*	Artemether	5 days of treatment eliminated the parasite	Akande and Fagbemi (2011); Yimar and Sahu 2016

25		As above	Rattus rattus	*In vivo*	As above	Growth of parasite was arrested and life span was extended	Oluyomi *et al*. (2009)
26		As above	Cultured trypomastigotes	*In vitro*	Artemisinin	Parasite growth was arrested and the mechanism of parasitic killing was like that of intra-erythrocytic malarial parasites	Mishina *et al*. (2007)
27		*Trypano-soma cruzi* (Chagas disease)	Cultured epimastigotes	*In vitro*	As above	As above	As above
28		As above	Cultured in Vero cells (kidney epithelial cells of African green monkey)	*In vitro*	Artesunate	All intracellular stages of parasite were inhibited	Akande and Fagbemi (2011); Olivera *et al*. (2015)
(E) Metazoan caused:							
29	a) Schisto-somiasis	*Schistosoma mansoni* (Helminth flatworm; common in Africa and South America)	Humans	*In vivo*	Artemether	The drug prevented as well as reduced the parasite's infection	Utzinger *et al*. (2000); Kaiser and Utzinger (2007)
30		As above	BALB/C mice	*In vivo*	Dihydroar-temisinin	A single dose of 300mg/kg was found on 21st day post infection to have reduced much of parasite burden	Li *et al*. (2012)
31		*Schistosoma japonicum* (common in Southeast Asia)	As above	*In vivo*	Artemether, artesunate and dihydro-artemisinin	All were effective; 200, 300 and 400mg/kg drug administered on 20,21 and 22 day post infection strongly reduced the parasite load	As above

References to review type papers: Efferth (2008); Ho *et al*. (2014); Efferth *et al*. 2016 ; Muthamiselvan *et al*. (2016); Yimer and Sahu (2016); Mesa *et al*. (2017)

Table 4 summarizes results of some studies on the effects of artemisinins and artemisannua on viral-, bacterial-, fungal-, protozoan- and helminth-infections, in cell lines *in vitro* and/or on model animals or humans *in vivo*. The drugs artemisinin and artesunate have been found to inhibit replication/multiplication of hepatitis causing hepatitis B (HBV) and C (HCV) viruses and sore inducing herpes virus and its close relative cytomegalovirus in cultured human cells. The *in vitro* growth of *Mycobacterium tuberculosis* (the bacterium which causes tuberculosis in humans), as well as the tubercular bacterial growth in infected mice, has been found to be arrested by artesunate. Addition of artemisinin to the culture of *Aspergillus fumigatus* (which causes aspergillosis in human) has been observed to stop the growth of fungus. Artemether and extracts of *A. annua* leaves have proved lethal to *in vitro* growing *Acanthamoeba castellani* (a cause of amoebiasis in humans). Treatment of mice infected with *Acanthamoeba* with water-, alcohol- or chloroform- extract of *Artemisia annua* leaves was observed to have increased the life span of diseased animals. Feeding of *A. annua* leaves to the broiler chickens infected with *Eimeria tenella* parasites saved the infected animals from development of coccidiosis disease. Growth of both visceral and cutaneous leishmaniasis causing *Leishmania* parasites, in human macrophage cultures, was found to be attenuated by the treatment of artemisinin. Analogously, the leishmania infections in model animals were also observed to have been arrested by treatment with artemisinin or *A. annua*

Table 5: Curative effect of *Artemisia annua* leaf extracts, artemisinin or its derivatives on the diseases of human /model animal immune and digestive systems and cancers of various organs

S.No.	Type of disease(s)	Organism(s)/ system(s) used for testing	Disease condition: origin/method by which induced	Therapeutic agent tested for its efficacy	Observation(s)	Reference (s)
A. Autoimmune						
1.	Rheumatoid arthritis	Rat	Collagen	Artesunate	The inflammatory symptoms were attenuated by intra-peritoneal treatment with the drug (3-5 mg/kg/d)	Li *et al*. 2013
2.	Crohn's disease (Inflammatory bowel)	Mouse	Variously	As above	The symptoms of disease were ameliorated by administration of the drug @ 150 mg/kg/d	Yang *et al*. 2012
3.	Allergenic asthma	BALB/C mouse	Ovalalbumin	As above	The treatment suppressed both the inflammation and oxidative damage associated with severe asthma	Cheng *et al*. 2011
4.	Lupus	B6D2F1 and nephritis	Pristane DBA/2 mice	Artemisinin	The drug relived the symptoms	Wu *et al*. 2010 of the diseases
5.	Uveitis	Long-Evans rat	Lipopolys-	Artesunate accharide	The drug suppressed the uveitis	Wang *et al*. 2011 induction
B. Digestive system						
6.	Obesity	C57BL/6 mouse	Nutrition rich diet	Boiled water extract of *Artemisia annua* dry leaves	Oral administration of the extract reduced the weight of animals without affecting their appetite	Baek *et al*. 2015
7.	As above	Sprague Dawley rats	As above	*Artemisia annua* leaves	Body weight, adipose tissue mass, adipocyte cell size, total cholesterol level were decreased	Song *et al*. 2017
8.	Fatty liver	CB7BL/6J mouse	High fat diet	Dehydrated water extract of *Artemisia annua* leaves	Twelve weeks treatment prevented hepatic fibrosis, obesity and inflammation of liver by reducing the accumulation of lipids	Kim *et al*. 2011
9.	Diabetes	Albino rat	Alloxan	*Artemisia annua* leaf extract	One month of treatment prevented hyperglycemia and ameliorated diabetes related metabolite abnormalities	Helal *et al*. 2014
10.	As above	Zebrafish, mouse, rat, human pancreatic islet	Type I diabetes impairment	Artemether	The glucagon producing pancreatic α cell got transformed into insulin producing β cells via activation of GABA receptors by loss of Arx function and thereby insulin production got restored	Li *et al*. 2017
E. Cancers						
11.	Squamous cell carcinoma of oral cavity	Human cells	Gingival epithelial cancerous (1HGK) cells	Dihydroarte-misinin	The drug was apoptotically cytotoxic to cancerous cells	Yamachika *et al*. 2004

12.	Pancreatic cancer	Human cells	PANC-1, Bx Pc-8 and CFPAC-1 pancreatic cancer cell lines and HL-7702 normal hepatic cell line	Artesunate	The drug caused oncosis-like cell death multi-fold more on cancer cells than on normal cells	Du *et al*. 2010
13.	As above	Human cancer cell xenograft on mouse	Panc-1tumor xenograft in mouse	As above	Artesunate caused dose dependent tumor regression	As above
14.	Hepatocellular cancer	Human	Hep G-2 and BWTG-3 cells	As above	Artesunate reduced the cancer cell viability in a dose dependent manner	Vandewynckel *et al*. 2012
15.	As above	Mouse	Diethyl nitro-samine induced tumor in liver	As above	Tumor burden was reduced without hepatotoxicity	As above
16.	Gastric cancer	Human cells	SGC-7901, BGC-823 and AG5 gastric cancer and GES-1 normal cell lives	As above	Cancer cells were killed by oncosis like process, but there was no significant effect on non-cancerous cells	Zhou et al. 2013
17.	As above	Human tumor xenograft on mouse	Gastric tumors were xenografted on nude mice	As above	The treatment regressed the tumors without detriment to animals	As above
18.	Colorectal cancer	Human	Patients	As above	Administration of 200 mg drug daily for 14 days cured 8 out of 9 patients	Magalhaes *et al*. 2012 ; Krishna *et al*. 2014
19.	Gall bladder cancer	As above	GBC-SD and NOZ gall bladder cancer cell lines	Artemisinin	The treatment stopped cell prolife-ration and caused cell killing by apoptosis	Jia *et al*. 2016
20.	As above	As above	The xenograft of above cancers on BALB/C mice	As above	The xenograft growth was inhibited by drug treatment	As above
21.	Renal cancer	As above	Renal cell carcinoma cell lines: Caki-1, 786-O and SN12C-GFP-SR Lu 2	Artesunate	Cancer cells were killed by oncosis via ROS generation and ATP depletion	Jeong *et al*. 2015
22.	As above	Human cancer xenograft on mice	Xenograft of 786-o-Luc cells planted subcutaneously	As above	Intra-peritoneal administration of the drug repressed the tumor (growth, metastasis and angiogenesis were all inhibited)	As above
23.	Cervical cancer	Human	HPV-39 inhibi-ted cervical cancer cells	Artemisinin	The cancerous cells stopped proli-ferating and were killed apoptotically by the effect of the drug	Mondal and Chatterji 2015