ISSN : 0974 - 7508 Volume 10 Issue 5

Natural Products

An Indian Journal

Full Paper

NPAIJ, 10(5), 2014 [149-152]

In vitro and *in vivo* antiamoebic potential of the *Sinularia elongata* tixier-durivault

Vijai Lakshmi[1]*, Sheela Ghosal[2]

[1]Medicinal and Process chemistry, Central Drug Research Institute, Lucknow-226001, (INDIA)

[2]Divisions of Microbiology Central Drug Research Institute, Lucknow-226001, (INDIA)

E-mail : vijlakshmius@yahoo.com

ABSTRACT

Aim : Human amoebiasis due to *Entamoeba histolytica* infection is mainly associated with morbidity thus affecting the quality of life and pace of developmental activities of countries with warm climatic conditions. So far, available drugs have been found to be too toxic or providing only symptomatic relief that leads to obtain novel molecules with antiamoebic activity from natural products. **Material and methods** : The methanol extract of *Sinularia elongate* Tixier-Durivault was prepared which showed antiamoebic activity. Therefore the methanol extract was fractionated into four fractions (hexane, chloroform, n-butanol soluble and n-butanol insoluble fractions). **Results and discussion** : Methanol extract showed MIC 125 µg/ml in our *in-vitro* studies, but when it was tested *in vivo* in rats, it showed 80% inhibition of trophozoites at the dose of 900 mg/kg body weight against *Entamoeba histolytica*. Out of this only chloroform soluble fraction showed 80% inhibition of trophozoites at 900 mg/kg dose. **Conclusion** : Further work is in progress for the isolation and characterization of active molecules.

KEYWORDS

Anti-amoebic activity;
Sinularia elongata against
Entamoeba histolytica.

INTRODUCTION

Human amoebiasis due to *Entamoeba histolytica* infection is mainly associated with morbidity thus affecting the quality of life and pace of developmental activities of countries with warm climatic conditions. A consistently high global incidence of this disease has been reported from surveys carried out at different intervals of time[1,2]. This disease also poses a challenge to our national health programme. A number of therapeutic agents possessing potent *in vitro* action against trophozoites of *E. histolytica* have been used to combat this disease. So far, these have been found to be too toxic or providing only symptomatic relief that leads to obtain novel molecules with antiamoebic activity from natural products, either terrestrial plants or marine organisms. The scope of natural products have widened with the inclusion of marine biota. Sponges are known as a rich source of sesquiterpenes, diterpenes and alkaloids.

Drug from marine resources is an area which offers an unprecedented opportunity for their pharmacological exploration and hence has received great attention during recent years for natural product chemistry, a promising new area of study. Secondary metabolites produced in marine organisms could be the source of bioactive substances and useful in modeling compounds

Full Paper

for drugs[3-7]. Marine microorganisms, whose immense genetic and biochemical diversity is only beginning, likely to become a rich source of novel chemical entities for the discovery of more effective drugs. In our ongoing programme to search for natural products with unique structural features and pronounced biological activities. In order to find new bioactive ingredients from marine organisms, *S. elongata* was collected from south Andaman coast of India and carried out the chemical composition and antiamoebic activity.

MATERIALAND METHODS

Collection of the *S.elongata*

The *S.elongata* a soft coral was collected from South Andaman coast of India. Specimen sample (voucher specimen number 337) has been preserved in the Herbarium of the Botany Division, Central Drug Research Institute, Lucknow, India.

Extraction and fractionation procedure

Freshly collected soft coral (1.0kg) was cut into small pieces and extracted with methanol (4x4lit) at room temperature. The combined extract was filtered, concentrated under reduced pressure below 45^{0}C in a rotavapour to a viscous mass (25.0g). The residual animal was rejected. The crude extract was bio assayed which showed potent antiamoebic activity *in vitro* and *in vivo* system. The methanol extract (20.0 g) was fractionated in to hexane (1.6g), chloroform (2.0g), n-butanol soluble (4.6g) and n-butanol insoluble fractions (11.8g). All these fractions were bioassayed for antiamoebic activity. Out of these four fractions, only chloroform fraction showed antiamoebic activity. It was purified but was found to be a mixture of diterpenoids. Due to paucity of material we could not isolate the pure constituents.

Test models and methodology for antiamoebic activity

(A) In-vitro model

Axenic culture of *E. histoyitica* (200: NIH) maintained TYI-S-33 medium[8] has been used for *in-vitro* screening. Xenic culture 2771 isolated from an acute case and maintained in Robinson's medium[9] was used to produce experimental caecal amoebiasis in rats.

(a) Evaluation of in-vitro amoebicidal activity

The stock solution of the test agent is prepared by adding small quantity of DMSO and required amount of water. Further serial double dilutions were prepared using triple glass distilled water. Amoebic inoculum 0.1 ml containing approximately 2000 trophozoites was added to the cavities of shallow cavity slides to which the test sample (0.1 ml) in its required dilution is added. Each cavity was then sealed with cover slip. The slides were kept in the moist chamber at 37^{o}C. Observations were taken at 24 and 48 hrs intervals. The activity of the test agent at the particular dilution was related with cent percent mortality. Metronidazole was the standard compound used. Duplicate sets were kept for each dilution[10].

(B) Antiamoebic activity in-vivo test model

(a) Experimental production of caecal amoebiasis of rats

Rats were fed on autoclaved rice diet for seven days prior infection. The caecal contents of these rats attain a pH of 5.5 to 7.0 without the occurrence of free ammonia which is toxic to these amoebae[11,12] thus aiding in the consistent production of caecal infection. Rats under ether anaesthesia were inoculated intracaecally with 0.2 to 0.3 ml of amoebic inoculum containing 10 x 104 trophozoites of *E.histolytica* and the abdominal lesion sutured. After 48 hours the infected rats were ready for therapeutic evaluation of test agents as trophozoites of *E.histolytica.* These were visible microscopically in the contents and scrapings of the caecal wall. The animals were divided into two groups. One group was given oral administration of the drug, while the other group served as control group.

(b) Treatment schedule

The test material was suspended in gum acacia suspension in distilled water. The rats were administered orally the test agent at 900 mg/kg with the help of a feeding needle once daily for five consecutive days. The rats were sacrificed 48 hours after the last dose of test material with an overdose of ether anesthesia and the caecum examined for trophozoites of *E. histolytica*. The reported method of Neal[13] was used to evaluate

TABLE 1 : Results of antiamoebic activity of *S.elongata* against *E.histolytica* in *in-vitro* and *in-vivo* models

Name of the exts./frs.	Antiamoebic activity against *E.histolytica*		
	In- vitro MIC µg/ml)	*In-vivo*	
		Dose mg/kg (days)	% inhibition
Methanol extract	250	900 (5)	80
		500 (5)	40
Hexane sol. fraction from ethanol extract.	125	900 (5)	62
Chloroform soluble fraction from the methanol ext.	125	900 (5)	82
n-Butanol soluble fraction of the methanol ext.	250	900 (5)	53
n-Butanol insoluble fraction of the methanol extract.	500	-	-
Metronidazole (Standard)	8	50 (5)	60
		100 (5)	100

the degree of infection.

RESULTS AND DISCUSSION

The effect of the *S.elongata* extract on trophozoites of *E. histolytica in vitro* and against caecal amoebiasis of rats is described in Table I. *In vitro* efficacy was recorded for all the test samples. The *in vivo* therapeutic efficacy of the crude extract showed that the methanol extract when administered at a dose of 900 mg /kg body weight for five days affected 80% cures. Chloroform fraction of the same extract exhibited high efficacy with 82% cures at 900 mg/kg dose. It is not uncommon that marine organisms possess activity against pathogenic bacteria, fungus and protozoa. Bhosale[14] has reported that terpenoids isolated from *Pseudoplenauria wagenaari* possess antiamoebic activity *in vitro*. Lobane diterpene derivatives of this organism were active against phytopathogenic fungus, *Cladosporium cucumerinum*, gram positive bacteria, *Bacillus subtilis*, and yeast, *Saccharomyces cerevisia*[15]. Similar derivatives have also been isolated from other marine organisms[16]. In view of the results presented it is evident that marine organisms can provide leads for antiamoebic agents in future. Thus, the ocean with its innumerable biota offers a challenge to both chemists and biologists alike as it is a large reservoir of novel chemical entities with therapeutic potential for human use.

The results assumed significance when viewed regarding the condition of the caecal wall. The caecum of rats receiving the crude extract and the chloroform fraction appeared normal with thin caecal wall comparable to the rats treated with the standard drug metronidazole (100 mg/kg body weight). However, the caecal contents of the rats treated with the test agents although being normal was slightly less formed as compared to the metronidazole treated rats. The results become still more interesting when the caecum of the treated rats are compared with the untreated rat caecum which is shapeless with ulcers on the walls and with mucous and very little faecal matter as contents. It is apparent from the results that *S. elongata* possesses significant amoebicidal activity against *E. histolytica*. This validates the promise held by the ocean as a source of therapeutic agents against human ailments.

ACKNOWLEDGEMENTS

We are grateful to the Director CSIR-CDRI for providing excellent research facilities at Central Drug Research Institute Lucknow. Ministry of Earth Sciences, Government of India, New Delhi is acknowledged for financial support. The author (VL) is thankful to the Head-HRDG-CSIR for financial support in the form of Emeritus Scientist ship, which enabled to compile the research work. The authors also wish to thank to Dr.M.N.Srivastava, scientist Central Drug Research Institute, Lucknow, India for collection of the material.

REFERENCES

[1] F.Beltran; Epidemiologica de las infeciones on Entamoeba histolytica, Sect. VIII, Protozoan diseases. Proceedings of the 4th International Congress on Tropical Medicine and Malaria, Washington, **2**,1056 **(1948)**.

[2] S.L.Jr Stanley; Amoebiasis, The Lancet, **361**,1025 **(2003)**.

Full Paper

[3] D.J.Faulkner; Marine natural products. Nat. Prod. Rep.,**18**, 1 **(2001)**.

[4] B.Haefner; Drugs from the Deep Marine Natural Products as Drug Candidates, Drug Discovery Today.,**8**, 536 **(2003)**.

[5] V.Anjaneyulu, P.V.S.Rao, P.Radhika, H.Laatsch, R.N.Asolkar; A new glycolipid and a new monohydroxy sterol from *Cladiella* species of Andaman and Nicobar Islands, Ind. J. Chem., **40(5)**, 405 **(2001)**.

[6] V.Anjaneyulu, P.V.S.Rao, P.Radhika, H.Laatsch, R.N.Asolkar; A new lipid glycoside from two Sinularia species of Andaman and Nicobar Islands, I.nd. J. Chem., **39(2)**, 121 **(2000)**.

[7] P.T.Nguyen, H.N.Nguyen, X.C.Nguyen, H.T.Bui, H.Q.Tran, T.T.N.Nguyen, T.T.L Bui, Y.Y.Seo, H.C.Chun, K.Sohyun, C.Doobyeong, K.Young-Sang, V.K.Phan, V.M.Chau, H.K.Young, Steroidal Constituents from the Soft Coral *Sinularia dissecta* and their InhibitoryEffects on Lipopolysac- charide-Stimulated Production of Pro-inflammatory Cytokines in BoneMarrow-Derived dendritic Cells, Bull. Korean Chem. Soc., **34**, 3949 **(2013)**.

[8] L.S.Diamond, D.R.Harlow, C.C.Cunnick; A new mediun for the axenic cultivation of *E. histolytica* and other *Entamoeba*, Trans. Roy. Soc. Trop. Med. Hyg., **72**, 431 **(1978)**.

[9] G.R.Robinson; Laboratory cultivation of some human parasitic amoeba, J.Gen.Microbiol., **53**, l9 **(1978)**.

[10] S.R.Das; A novel and rapid method for in vitro testing of antiamoebic agents against aerobic and anaerobic amoebae growing axenically or with bacteria, Curr.Sci., **44**, 463 **(1975)**.

[11] B.N.K.Prasad, I.Bansal; Interrelationship between faecal pH and susceptibility to *Entamoeba histolytica* infection of rats, Trans. Roy. Soc. Trop. Med. Hyg.,**77**, 271 **(1983)**.

[12] G.J.Leitch; Intestinal luminal and mucosal microclimate H^- and NH^+ concentration asfactors in the etiology of experimental amoebiasis, Am. J.Trop.Med.Hyg., **38**,480 **(1988)**.

[13] R.A.Neal; Some observations in the variations of virulence and response to chemotherapy of strains of *Entamoeba histolytica*, Trans. Roy. Soc. Trop. Med. Hyg, **44**, 439 **(1951)**.

[14] S.H.Bhosale, T.G.Jagtap, C.G.Naik; Antifungal activity of some marine organisms from India against food spaelage Aspergillus strains, Mycopathologia, **147**,133 **(1991)**.

[15] R.A.Edrada, P.Proksh, V.Wray, L.Wilte, L.V.Ofrogen; Four new bioactive lobane diterpenes of the soft coral Lobophytum pauciflorum from Mindoro, Phillipines, J.Nat. Prod, **61**, 358 **(1998)**.

[16] J.Shin, W.Fenical; Fucoside A-D anti-inflammatory diterpenoid glycoside of new structural classes from the Caribbean gorgonian Eunacea fusca, J.Org. Chem., **56**, 3153 **(1991)**.