Antileishmania Effect of Extracts of Some Medicinal Herbs on the Growth of Leishmania Parasites

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Abstract
The present investigation was revealed that Citrulus colocynthis, Hypericum peratum and Dianthus caryophilus aqueous extracts were inhibitory on the growth of Leishmania tropica and L.donovani promastigotes. The C.colocynthis and D.caryophilus extracts were caused a significant decrease in total nucleic acid contents of leishmanial species, thus considering to be an excellent agents for antileishmania chemotherapy.
Keywords : medicinal herb extracts, antileishmanial effect.

1. Introduction

Leishmaniasis is a collection of human diseases that ranges from localized cutaneous lesion to disseminated systemic infections [1]. These diseases are caused by several different hemoflagellate protozoa species of the genus Leishmania [2]. The frequent resistance of lesions to standard therapy with pentavalent antimony and their occasional resistance to subsequent therapy with pentamidine or with amphotericin B [3]. have prompted a search for new more active and less toxic alternative therapies for the treatment of these diseases. Medicinal plants appears to offer chemotheraputically exploitable oppurtunities because of their natural products are considered to be constitute best sources of chemical compounds for the treating of important tropical diseases caused by portozoans [4]. The current investigation was undertaken to use the effect of the extracts of Citrullus colocynthis, Hypericum peratum and Dianthus caryophllus on the growth of promastigotes of Leishmania tropica and L.donovani.

2. Materials and Methods

Parasite growth

The promastigote forms of Leishmania tropica and L. donovani were growth at 26 C˚ in Novy-Neal-Nicolle (NNN) medium as described by Chang et al. [5] and in the Pepton Yeast extract (PY) medium as described by Mehnaz et al. [6].

Preparation of Plant extracts

The aerial part of each plant parts were cleaned with water to remove dust particle and were dried in the incubater at a temperature not exceeding 35C˚, The parts in mixer. The aqueous extraction of each plant parts were prepared as described by Rios et al. [7]. Astock solution (1) mg/ml from aqueous extraction of each plant was used to prepared the final concentration (1000, 100, 10) mg/cm3 in The growth medium.The solution of extracts were sterilized using filtration through (0.45)µm membrane filter.

Estimation of total protein and nucleic acid

Protein content was determined using Lowery method [8]. whereas estimation of nucleic acid DNA and RNA were carried out using method of (Giles and Mayer, 1965).

3. Result and Discussion

As pesented in Tables 1,2, and 3 the number of L.tropica promastigotes decreased gradually by using 10, 100 and mg/ml concentration of C.colocynthis, H.peratum and D.caryophilus aqeous extracts at 1000 mg/ml after 96 hr inhibited the parasite growth by 90%, 80% and 69% respectively. On the other hand the growth of L.donovani promastigotes were inhibited after 96 hr by 80%, 65% and 58% of C.colocynthis, H.peratum and D.caryophilus extracts, respectively (Tables 4, 5 and 6).

As indicated in Tables 7 and 8, the protein content in promastigotes of L.tropica and L.donovani that were treated with IC50 of the tested aqueous extract of C.colocynthis were found to be reduced by 24% and 10% respectively. On the other hand, the IC50 of the tested aqeous extracts of H.peratum and D.caryophilus were found without effect on protein conent in L.tropica and L.donovani promastigotes.

The amount of DNA, RNA and total nucleic acid of promastigotes of L.tropica were found to be reduced to 15%, 11% and 12% respectively when treated with IC50 of the tested aqueous extract of C.colocynthis and to 4%, 4% and 4% respectively with aqueous extract of H.peratum (Table 9). On the other hand, the content of DNA, RNA and total nucleic acid of L.donovani promastigotes were reduced to 14% and 11% respectively when treated with IC50 of the tested aqueous extract of C.colocynthis and to 9%, 4% and 6% respectively with aqueous extract of H.peratum (Table 10). The aqueous extract of D.caryophilus was without effect on content of DNA, RNA and total nucleic acid in L.tropica and L.donovani promastigotes.

The data presented here suggest that C.colocynthis, and H.peratum inhibit the growth of leishmanial promastigotes by inhibition of nucleic acid content by mechanisms related to decrease in the net amount of DNA and RNA per cell and its loss of function or many in part due to the breaking the covalently bonds between. The use of C.colocynthis and H.peratum extracts would be an excellent candidate agent for antileishmanial chemotherapy.

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Table 1 : effect of different concentrations of aqueous extract of Citrullus colocynthis on the number of promastigotes of L.eishmania tropica different growth interavals

Number of Promastigotes						
Time	24hr	48hr	72hr	96hr	%Growth	%Inhibition
Control	4.6x105	1.3x106	4.4x106	9.7x106	100	0
10µg/ml	3x105	9x105	2.5x106	4.5x106	46	54
100µg/ml	2.6x105	6x105	1.4x106	3x106	31	69
1000µg/ml	2.1x105	3.9x105	6.3x105	1x106	10	90

Table 2 : Effect of different concentrations of aqueous extract of Hypericum peratum L. on the number of promastigotes of Leishmania tropica at different growth intravls.

Number of promastigotes						
Time	24hr	48hr	72hr	96hr	%Growth	%%Ihibition
Control	4.6x105	1.3x106	4.4x106	9.7x106	-	-
10µg/ml	3x105	1x106	3x106	5.7x106	59	41
100µg/ml	2.5x105	7.4x105	2.1x106	4.9x106	51	49
1000µg/ml	2x105	5.2x105	1.1x106	1.9x106	20	80

Table 3 : : Effect of different concentrations of aqueous extract of Dianthus caryophllus on the number of promastigotes of Leishmania tropica at different growth intervals.

Number of Promastigotes						
Time	24hr	48hr	72hr	96hr	%Growth	%Inhibition
Control	4.6x105	1.3x106	4.4x106	9.7x106	100	0
10µg/ml	3.4x105	1.3x106	3.3x106	6x106	62	38
100µg/ml	2.8x105	1.1x106	2.7x106	5.2x106	54	46
1000µg/ml	1.4x105	6.6x105	1.7x106	3x106	31	69

Table 4 : Effect of different concentrations of aqueous extract of citrullus colocynthis on the number of promastigotes of Leishmania donovani different growth intervals

Number of Promastigotes						
Time	24hr	48hr	72hr	96hr	%Growth	%Inhibition
Control	5x105	1.4x106	5x106	1x107	100	0
10µg/ml	3.5x105	1x106	3.2x106	5.3x106	53	47
100µg/ml	2.6x105	8x105	2.1x106	4.4x106	44	56
1000µg/ml	1.9x105	3.8x105	9.2x105	2x106	20	80

Table 5 : : Effect of different concentraions of aqueous extract of Hypericum peratum on the number of promastigotes of Leishmania donovani at different growth intravals.

Number of promastigotes						
Time	24hr	48hr	72hr	96hr	%Growth	%Inhibition
Control	5x105	1.4x106	5x106	1x107	100	0
10µg/ml	4x105	1.1x106	3x106	6.4x106	64	36
100µg/ml	3x105	9x105	2.2x106	5.5x106	55	45
1000µg/ml	2x105	6x105	1.4x106	3.5x106	35	65

Table 6 : Effect of different concentrations of aqueous extract of Dianthus caryophllus on the number of promastigotes of Leishmania donovani at different growth intervals.

Number of Promastigotes						
Time	24hr	48hr	72hr	96hr	%Growth	%Inhibition
Control	5x105	1.4x106	5x106	1x107	100	0
10µg/ml	3.8x105	1x106	3x106	6.8x106	68	32
100µg/ml	3.2x105	8.4x105	2.2x106	5.9x106	59	41
1000µg/ml	2.9x105	7.6x105	1.9x106	4.2x106	42	58

Table7 : Effect of IC50 concentration of aqueous extracts of the Citrullus colocynthis and Hypericum peratum L. and Dianthus caryophllus on the protein content of L.tropica promastigote at 96 hours of growth.

Treatment	IC50 µg/ml	Total protein content	% Protein	% Reduction
Control L.tropica	-	10±420	10	0.0
Aqueous extract of C.colocynthis	10	8±320	76	24
Aqueous extract of H.peratum	100	6±420	100	0.0
Aqueous extract of D.caryophilus	100	9±421	100.2	0.0

Table8 : Effect of IC50 concentration of aqueous extracts of the the Citrullus colocynthis and Hypericum peratum L. and Dianthus caryophllus on the protein content of L.donovani Promastigote at 96 hours of growth.

Treatment	IC50 µg/ml	Total protein content	% Protein	% Reduction
Control L.donovani	-	6±480	100	-
Aqueous extract of C.colocynthis	90	8±430	90	10
Aqueous extract of H.peratum	111	10±480	100	-
Aqueous extract of D.caryopilus	85	7±480	100	-

4. Conclusions

1-Possibility of using the aqueous extract of citrullus colocynthis as an alternative treatment for anti-leishmaniasis in evidence of the inhibitory effect on the growth and quantity of protein and nucleic acids of the promastigote of tropical and visceral leishmaniasis.

2-The structural sequential reaction technique proved to be a highly sensitive test for diagnosing the type of parasite, and the results indicated that L.donovani is the main causative parasite of visceral leishmaniasis.

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