Sc. J. Az. Med. Fac. (Girls). Vol. 26 , No. 1, Jan, 2005: 1'915-1921.

Preliminary Study On the Effect of *Ziziphus spina* Christi. On Selected *Leishmania species*

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ABSTRACT

The variable sensitivity and resistance of *Leishmania* parasites against different chemotherapeutic agents has become a serious problem thus necessitating the discovery of new line of drugs. Herbal derivatives are the alternative option at the moment. Ethanolic and aqueous extracts of *Ziziphus spina Christi* leaves used in folk medicine in Saudi Arabia was investigated for the first time as anti-*Leishmania* agent.

The extracts were tested in vitro on two *Leishmania* strains, *L.major*(FV1), and *L.donovani*(LV9). The most active was ethanolic extract giving 77.3% inhibition of the FV1growth at 250ug/ml with highly significant value in compare to other extract concentrations P <0.001, while aqueous extract inhibit 57.33% of LV9 growth at 250ug/ml with highly significant value in compare to the other concentrations P <0.001.

INTRODUCTION

Leishmaniasis is a group of diseases that afflict people throughout the tropical and sub-tropical regions of the world (WHO, 1990). The World Health Organization has estimated the global prevalence of leishmaniasis as 12 million people and 350 million at risk. The annual incidence of cutaneous leishmaniasis is 1-1.5 million cases while 500 000 cases for visceral leishmaniasis. Epidemics of cutaneous leishmaniasis occurred in some Arab countries as Jordan, Saudi Arabia and Egypt (Morsy,1989, 1996). The diseases are caused by protozoan haemoflagellates of the genus *Leishmania* and are transmitted to humans, mainly by the bites of infected female sand-flies. The clinical spectrum of leishmaniasis has a wide range from localized self-healing infections producing a simple sore, through destructive mucocutaneous ulcer to disseminated infection of the entire reticuloendothelial system, which may become a major cause of morbidity and mortality (Ashford & Bates, 1998). Each disease has its own epidemiology and ranges of clinical manifestation. The *Leishmania* parasites exist in two morphologically and biochemically distinct forms: a motile extracellular promastigotes in the alimentary tract of its sand-fly vector and an intracellular amastigotes within phagolysosomes of mammalian macrophages. Five species of *Leishmania* are the agents of Old World leishmaniasis *L. major, L. tropica, L. aethiopica* , which are mainly agents of cutaneous leishmaniasis, and *L. donovani and L. infantum* the predominant agents for visceral Leishmaniasis (Ashford & Bates, 1998).

A concentration of 5x 10^6 promastigote / ml counted by Haemocytometer was applied and maintained in each flask.

Subculture of the promastigote was carried on the 7th day of the culture.

2. Plant origin and extract:

Leaves of *Ziziphus spina Christi* was collected from Al Taef district Saudi Arabia in July.

They left to dry in oven at 40°C for 24 h and grounded into powder material by sterile grinder.

2.1 Preparation of the extract:

2.1.1 Aqueous extract

Modified technique of (Ali-Shtayeh, 1998) has been applied for extraction of both aqueous and ethanolic.

10 mg of the plant powder was infused in water until complete exhaustion, and then filtered through Celite no. 545 with 20-40 ul pores.

The filtrate then evaporated in a rotary evaporator at 80°C and kept dry in sterile dark vials in the freezer.

2.1.2 Ethanolic extracts:

10 gm of the dry powdered plant were soaked in 80% ethanol, until complete exhaustion. The extract was filtered using Celite no: 545 with 20-40 ul pores.

The filtrate then evaporated in a rotary evaporator at 80°C and kept dry in sterile dark vials in the freezer.

2.2 preparations of extract concentrations:

The previous extracts for both plants were dissolving in water to get the stock solution 5mg/ml.

3.1 Anti parasitic activity:

The effect of the extracts of *Ziziphus* plant were evaluated in 24-well microtitre plates. Cultures of promastigotes in the logarithmic phase (106cells/ml) were incubated in M199 medium in the presence of different concentrations of the extracts. Three different concentrations of both extracts of *Ziziphus* plant (250ug/l, 125ug/l, 50ug/l) were calculated and added in the wells.

Control wells carried only *Leishmania* with the dissolving solution.

Average number of the parasites in the first three days were counted using a haemocytometer with a light microscope, and the results were compared with those from the controls.

3.2 Screening the anti parasitic activity:

Trypan blue stain was used to differentiate dead from living promastigote hence the count reduction.

Motility and appearance of *Leishmania* was recorded.

4. Data were analyzed by package EPI-INFO 2000 Software (CDC, Atlanta, GA, USA).

RESULTS

The Extracts of the *Z. spina Christi* showed a variable growth inhibition of the promastigotes forms against strains of *Leishmania* (*L. major* (FV1) and *L. donovani* (LV9)) after 72 hours of incubation in vitro (Table 1)(fig 1,2). Relatively, the ethanolic *Z. spina Christi* extracts at 250ug/ml inhibited 77.3% of the FV1 strain whereas only 40% against LV9 strain. The aqueous *Z. spina Christi* on the other hand inhibited 57.33% of LV9 and only 20% of FV1 promastigotes (Tab.2).

Table 1. shows the effect of aqueous and ethanolic extract of *Z. spina Christi* on *leishmania* strains after 72 hrs of incubation (number of parasites x10^6)

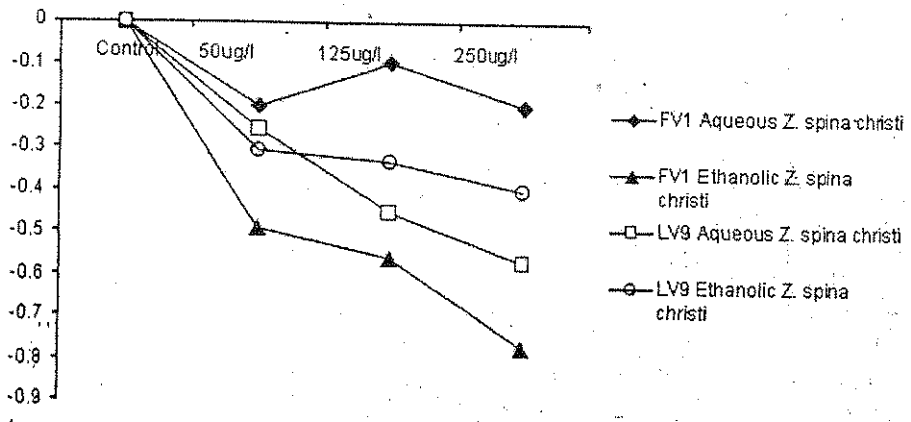

Fig 2.Reduction effect of the different extracts on the *Leishmania sp.*.

Tab.2 Reduction effect of the different extracts on the *Leishmania sp.*.

Leishmania strain			promastigote reduction %		
			50ug/l	125ug/l	250ug/l
L. major	**FV1**	**Aqueous Z. spina christi**	20	10	20
	FV1	**Ethanolic Z. spina christi**	49.3	56	77.3
L. donovani	**LV9**	**Aqueous Z. spina christi**	25.3	45.3	57.3
	LV9	**Ethanolic Z. spina christi**	30.6	33.3	40

The motility of living promastigotes were diminished with both extracts at 48- 72h of administration at concentration 250ug/l.

The promastigote rounded up at concentration 250ug/l. after 48h of incubation with both extracts of *Z.spina Christi.*

DISCUSSION

The presented study is a preliminary evaluation of the aqueous and ethanolic extracts of *z. spina Christi* against the promastigote forms of *Leishmania* species. The results obtained showed that extracts of *z. spina Christi* have antileishmanial activity. A progressive increase in the antileishmanial effect was observed. The best antileishmanial activity was demonstrated using 250ug/l ethanolic *Z. spina Christi* extracts against the L. major stain (FV1) where the parasites count were reduced 77.3%.

The water extract showed 57.3% reduction of *L. donovani* at concentration 250ug/l this result coincide with that recorded by Awadh et al.,(2001) who showed that water extract of *Z.spina Christi* leaves exhibited obvious antibacterial effect against Gram positive strains with no cytotxic effect.

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