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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).

The treatment of leishmaniasis has always been fraught with difficulties (Berman, 1997). The variable sensitivity of *Leishmania* parasites against different chemotherapeutic agents are mainly due to strain or species differences and patient's response. In some cases as in diffuse cutaneous leishmaniasis the respond to chemotherapy is very poor. The first-line of drug for leishmaniasis since 1920s has been based on pentavalent antimonial compounds, mostly sodium stibogluconate and meglumine antimoniate (Goodwin, 1995). The aromatic diamidines mainly pentamidine have been used as a second line compounds in 1952 (Thakur et al., 1991). Amphotericine B (Fungizone), largely in its liposomal form, have demonstrated a strong activity against *Leishmania* parasites and was increasingly used as the incidence of visceral leishmaniasis cases in immunocompromised patients has increased along with the acquired resistance to antimonials (Thakur et al., 1999). Despite the diverse therapeutic agents studied and tested; the treatment of leishmaniasis is to some extent still empirical. Most drugs available are toxic, costly and have unpleasant side effect. The long treatment regimes are becoming progressively more ineffective, thus necessitating the discovery of new line of drugs (Thakur et al., 1991).

Nowadays many people headed to use folk medicine in a way to overcome the side effects and the expense of manufactured drugs. In the same way scientists started new researches in folk medicine in a trial to overcome the microbial resistance and seeking natural immune provoke remedy.

Plants belonging to *Ziziphus* species are used for many medicinal purposes in folk medicines all over the world. The plant has also been used for its soothing properties (Adzu et al, 2002). It is a recognized genus with potential pharmacological action. In India and China, *Ziziphus* species in particular have been used to treat different diseases. In Saudi Arabia it is used for the treatment of ulcers, wounds, eye diseases and bronchitis. The Bedouin use it for treatment of wounds, skin diseases and anti inflammatory. The broad variety of medicinal properties of *Ziziphus* plants is remarkable, with uses against skin diseases, diarrhea, fever and insomnia. The biological antibiotic and fungicidal activity of extracts of *Z. jujube, Z. spina Christi Z. mauritiana and Z. nummularia* leaves, stems and roots is well demonstrated (Kayser et al., 20001). Some of the chemical groups recognized in *Z. spina Christi* are phenolic compounds (flavonoids and phenolic acids) highest flavonoid content was found in the leaves(Mahran et al., 1996), glycosides, alkaloids, saponin (Nickayar and Mojab, 2003).

The aim of the study is to investigate the effect of the whole extract of *Ziziphus spina Christi* (The Arabs call it Nabak) on some *Leishmania* strains in vitro.

MATERIAL AND METHODS

1. Leishmania strains and culture:

Two different strains were used in this study; both were kindly supplied by Dr. Chance Liverpool School of Tropical Medicine

MHOM/IL/80/Friedlin FV1 *(L. major).*

MHOM/67/HU3: LV9 *(L.donovani).*

25 ml flasks contain axenic cultured media M199 enriched with BEM, HEBES, L.glutamic, 10% inactive FCS and penicillin/ stryptomycin were used.

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 x106)

Leishmania strain			Promastigote count ($x10^6$)			
			Control	50ug/l	125ug/l	250ug/l
L. major	FV1	Aqueous Z. spina christi	75	60	68	60
	FV1	Ethanolic Z. spina christi	75	38	33	17**
L. donovani	LV9	Aqueous Z. spina christi	75	56	41*	32**
	LV9	Ethanolic Z. spina christi	75	52	50	45

*Significant p<0.01 **highly significant p<0.001

Inhibition of more than 50% of FV1 was occured by ethanolic extract of *Z.spina Christi* at 125ug/l and 250ug/l with highly significant effect of 250ug/l in compare to 125ug/l.

Inhibition of more than 50% of LV9 was noticed with aqueous extract of *Z.spina Christi* at 250ug/l with high significant effect P<0.001 over use of 125ug/l, which show significant effect P<0.01 in comparison to using 50ug/l.

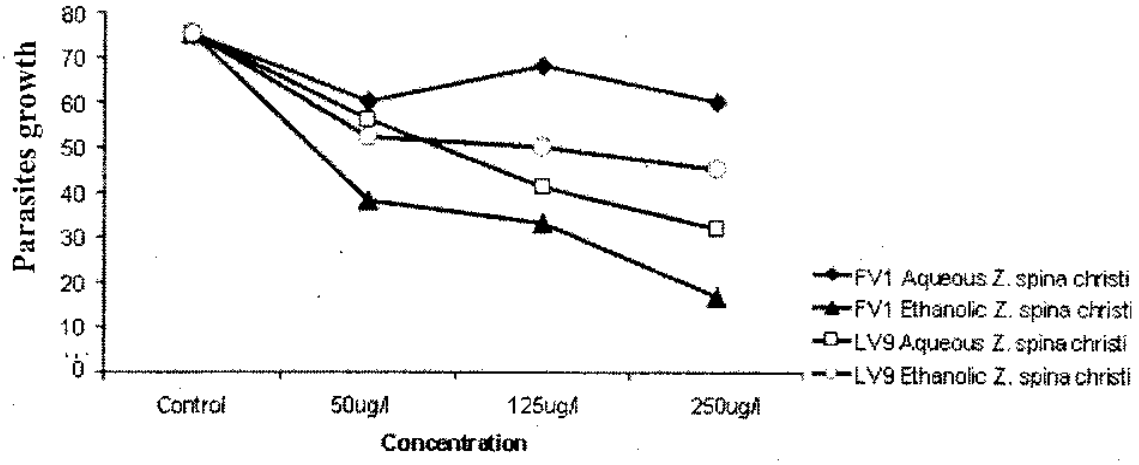

Fig 1: The effect of different concentrations of *Z. spina Christi* extracts against the promastigote cultures of the two *Leishmania* strains FV1 & LV9 incubated for 72 hrs.

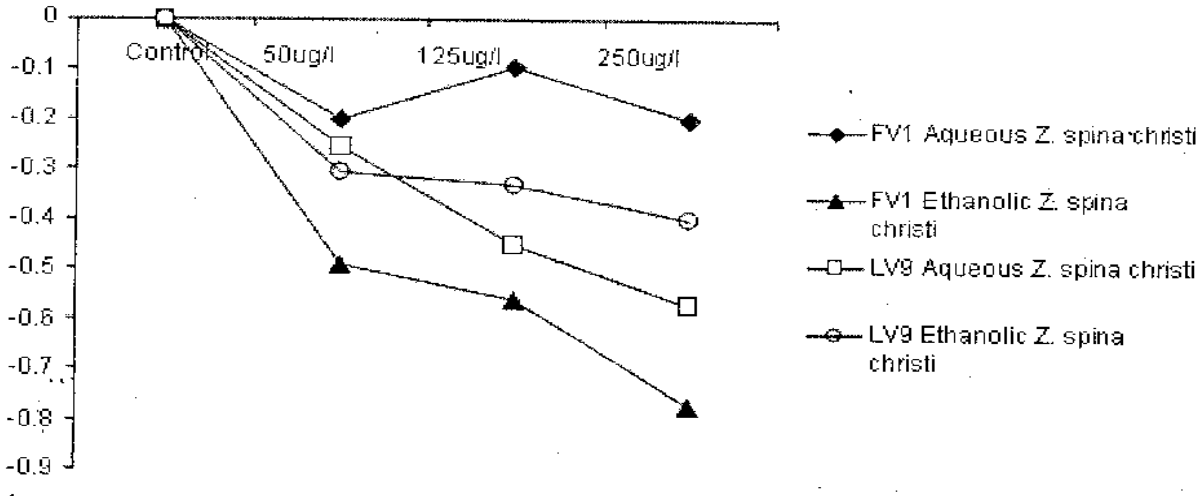

Fig 2.Reduction effect of the different extracts on the *Leishmania sp.*.

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.

Ethanolic extract of *Z.spina Christi* had significant effect on L.major and this result was against what recorded by Ali -Shatayeh et. Al.,(1998)who recorded that the least antimicrobial effect was from *Z.spina Christi* extract either aqueous or ethanolic.

In our study no fractionated component was used separately that why we cannot precisely determine which chemical group has the role as antileishmania. Mittra et al., (2000) reported that flavonoids presents in many herbal treatments are potent antileishmanial agents and have great promise for acting as chemotherapy of leishmaniasis and one of the chemical component of *Z.spina Christi* is flavenoids (Mahran et al., 1996) hence this component may have a role in reduce the growth of *Leishmania spp.* in our study.

Nils et al.,(2004) recorded that saponin from the leaves of Maesa balansae has antileishmania effect, it is one of the chemical groups in *Z.spina Christi* which may play role in elimination of *leishmania*.

Micro-organisms invasion of the skin may lead to peroxidation and generate skin problems. An extract of *Z.spina Christi* leaves found contain phenolic group, which consider an efficient scavenger of peroxyl radicals so this may help in regeneration of infected skin lesion, this may need further investigation to detect the role of *Z.spina Christi* extract as antioxidant specially in case of cutaneous leishmaniasis.

Islam et.al.,(2001) recorded that *Z.spina Christi* has no teratogenic effect and oral LD50 values was >6400 mg/kg and this dose not exceeded in our study.

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