

PARASITS IN- VITRO

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Summary

Extracts of the plants (*Astragalus hamosus* , *Nerium oleander*, *Ficus carica*, *Peganum harmala*, *Punica granatum* & *Glycyrrhiza gloabra*) were investigated for their antileishmanial activities against the intracellular amastigotes of *Leishmania donovani* and *Leishmania major* .

The procedure adopted was the tissue culture for peritoneal macrophage of BALB/C mice, which were infected and investigated in- vitro.

The results showed that the extracts of the plants *A. hamosus* & *N. oleander* exhibited good activity against intracellular amastigotes, whereas the extracts of *F. carica* & *p. hamosus* requires higher concentrations to destroy the parasites.

Introduction:

Leishmaniasis are important diseases in terms of morbidity and some types are severe in terms of mortality. The diseases occurs in four major forms, which are Visceral, Cutaneous, Mucocutaneous & Diffuse Cutaneous Leishmaniasis [1] . In Iraq both visceral & cutaneous Leishmaniasis were reported to occur [2 - 4] .In the field of treatment, medical plants either in the form of crude drugs or their isolated medicinally active substances will be important among the choices available to physicians [5] .

From the Iraq flora 12 plants were screened for their antileishmanial activity against promastigotes & axenic amastigotes of *Leishmania donovani* & *Leishmania major* (under publication) . Among these six plants were chosen to be investigated for their activity against the intracellular form of the same strain using tissue culture technique for mice peritoneal macrophages, which are infected in- vitro. The chosen plants are *A. hamosus* , *N. oleander*, *F. carica*, *P. harmala*, *P. granatum* & *G. globra* .

Materials and Methods:

1. Parasites and animals

Two strains of leishmania were obtained from the medical research center in Al - Nahrain College of Medicine. These are *L. donovan* (WHOM,IQ/1982/BRC1) and *L. major* (WHOM,II/67/Jericho/11), both are human isolates, they are maintained by monthly passage of the amastigotes to BALB/C mice which were also obtained from the same center above.

2. Liquid media that was modified by Al-Bashir [6] was used.

3. Plants.

A. hamosus , *N. oleander*, *F. carica*, *P. harmala*, *P. granatum* & *G. globra* were collected from different regions of Al-Anbar governorate in summer . After drying powdering and garbling , two methods of extraction were used [7,8] .

4. Antileishmanial activity assay:

Mice peritoneal macrophages were elicited infected in-vitro and treated as described by Neal and Croft [9] .

5. Statistical analyses of the results were done according to Daniel[10].

Results and Discussion:

The multiplication index of the intracellular amastigotes of *L. donovani* (fig. 1) and *L. major* (fig. 2) after seven days of treatment with seven different concentrations of each plant extract showed that *A. hamosus* exhibited potent activity on *L. donovani* with LD50 (166.3 µg/ml) and with LD50 (675 µg/ml) . All the parasites of both strains were eliminated at the highest concentration (2000 µg/ml) while 91.4 % of *L. donovani* and 98 % of *L. major* were eliminated at the lowest concentration(31.25 µg/ml).

There are no information in the literature about the antileishmanial activity of this plant may be related to tragacanth, a substances that consists of 60 – 70 % of bassorin (a complex of polymathoxelated acid) which was reported to be found abundantly in it [11] .

Aqueous extract of *N. oleander* exhibited good activity with LD50^s 291.5 and 855 µg/ml on *L. donovani* and *L. major* respectively. Again, there are no information in the literature about the antileishmanial activity of this plant to compare our results.

Its activity in the present study may be related to a complex of mixture of glycosides derived from six different genins that are closely related chemically and physiologically to the digitalis glycosides which are found in it [11] . This plant can be applied locally to the skin, but it is very toxic if taken orally [12]

Aqueous extract of *F. carica* showed relatively weak activity against the intracellular form of both strains, the LD50^s were 1780 & 1192 µg/ml on *L. donovani* & *L. major* respectively. Therefore this plant is less important than the above plants in the field of the present study.

Ethanollic extract of *P. harmala* showed moderate activity but require high doses, LD50^s were 1406 & 1319 µg/ml for *L. donovani* & *L. major* respectively. This plants seems to be promising because of it's activity in the present study in addition to it's usage by El-Refaie [13] for the treatment of cutaneous Leishmaniasis with improvement, moreover, Evans & Croft [14]use this plant for obtaining harmaline which showed significant . activity in –vitro against *L. amazonensis* amastigotes using infected mice peritoneal macrophages.

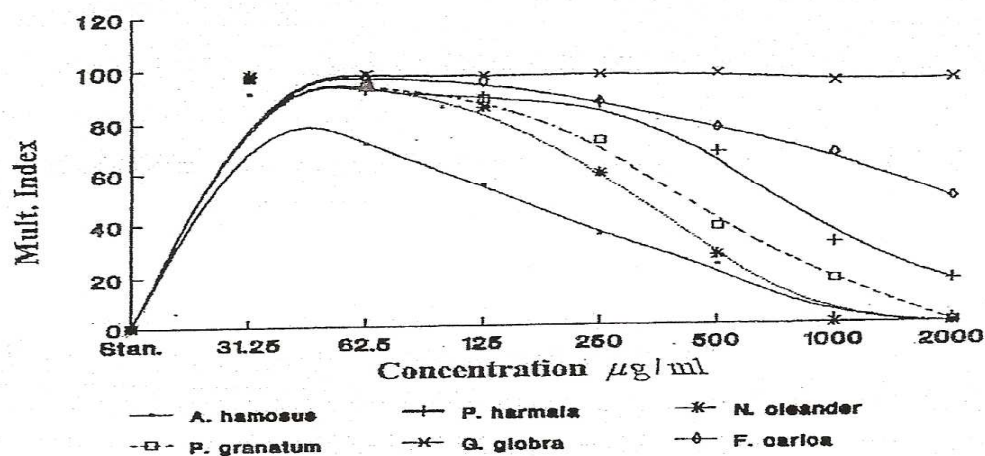


Fig (1) Multiplication index of intercellular form of *L. donovani* in different concentration of six plants

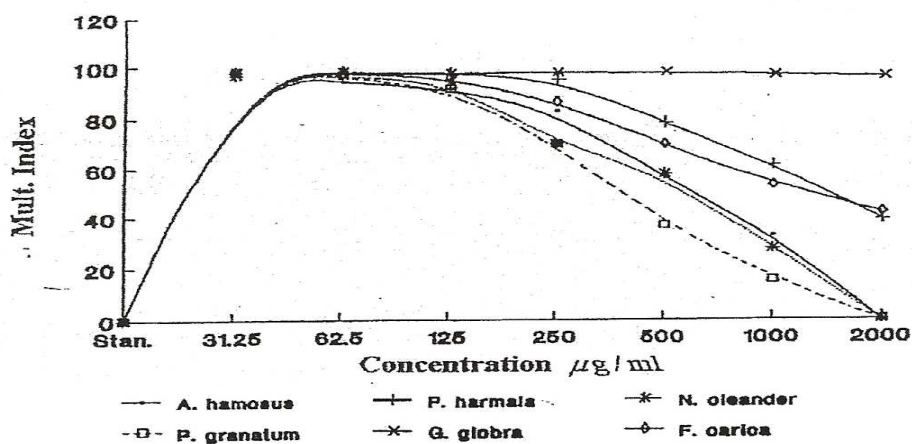


Fig (2) Multiplication index of intercellular form of *L. major* in different concentration of six plants

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الخلاصة:

تمت دراسة تأثير مستخلصات نباتات الدقيس *A. hamosus* و الدفلة *N. oleander* والتين *F. carica* والحرمل *P. harmala* والرمان *P. granatum* و عرق السوس *G. globra* على الشكل اللاسوطي داخل الخلوي للطفيليات اللشمانيا الاحشائية والجلدية وذلك باستخدام الزراعة النسيجية للخلايا البلعمية المأخوذة من بيرتون الفئران المختبرية. أظهرت النتائج وجود فعالية جيدة لمستخلصات نباتي الدفلة والدقيس . أما مستخلصات نباتي التين والحرمل فأنها تحتاج الى تراكيز عالية نسبياً لقتل الطفيليات الموجودة داخل الخلايا البلعمية.

