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Review Article

Evaluation of Anti Leishmanial Activities of Withania Somnifera and Solanum Nigrum: A Preliminary Study

Shagufta Taqvi, Samra Jamal

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Corresponding Author:

***Shagufta Taqvi,**
Department of Biotechnology, Faculty of Science, JamiaHamdard, New Delhi.

*Email Id-
taqvi.shagufta@gmail.com

ABSTRACT

This work deals with the study of anti leishmanial activities of plant abstracts that have been evaluated for possible anti leishmanial activities using MTT Assay. In vitro inhibition was observed with chloroform growth and a similar inhibition was obtained with aqueous extract of s.nigrum and all other extracts exhibiting significant toxicity. Promastigotes of L. donovani strain AG83 were routinely cultured at 24^{0}C in M199 medium supplemented with 10% fetal calf serum, 100 U penicillin/ml and 100 µg streptomycin/ml. The IC50 of Pentamidine was found to be 38g/ml while that for w.somnifera and s.nigrum found to be 50g/ml and 58g/ml respectively. This paper records one of the effective measures of cure of the deadly disease by medicinal plants solanum nigrum and withania somnifera against the recent drug pentamidine .

Key-words: L. donovani, anti- leishmaniasis, pentamidine, s. nigrum, w. somnifera, medicinal plants, in-vitro/in-vivo, mitochondrial dehydrogenase, formazan,

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Faculty of Science, Jamia Hamdard, New Delhi.

INTRODUCTION :

Leishmaniasis is a severe, widespread zoonotic disease which is caused by leishmania a genus of trypanosome protozoa the parasite responsible for the disease. This disease is also known as leichmaniosis, leishmaniose and formally orient boils, Baghdad boil, kala azar, black fever , sandfly disease, dumdum fever or espundia.
It occurs in the middle and far east, the Mediterranean basin, south America and in some states in United States of America. Leishmaniasis is primarily a parasite rodents, carnivores, marsupials, edentates, insectivorous and secondary of dogs and humans .severe disease develops in the man and the dog which is characterized either by skin lesions or a general visceral involvement. The dog is reservoir for visceral leishmaniasis in South America and the Mediterranean region. The disease is transmitted by the blood sucking sandfly (genus phleobotomous in the old world and genus lutzomya are caused by these include the L. donovani complex with three main species L.donovani and infantum /L.chagasi in the new world.

Human infection is caused by about 21 of 30 species that infect mammal (L.donovani, L.chagasi ,l.infantum); the L.mexicana complex with three main species (L.mexicana, L.amazonensis and L.venezuelesis); L.tropica; L.major; Llaethiopica and the subgenus viannia with four main species L.brazilienensis, Lguyanensis, Lpanamensis and V.peruviana.
The flagellated form of the parasite, the promastigote is introduced into the human host by the blood sucking sandfly, phlebotomus. These parasitic protozoans are digenetic and have two distinct stages in their life cycles. The motile flagellated promastigote stage lives in the alimentary tract of the sand fly vector, while the non motile amastigote stage resides inside the macrophages of mammalian hosts.
Promastigotes attach to the mononuclear phagocytes via a receptor-mediated mechanism are taken up by phagocytosis into a phagosome which fuses to lysosomes to form the phagolysosome. once inside the macrophage the promastigote, the promastigotes undergo significant biochemical and metabolic changes, which result in the obligatory intracellular form of the parasite, the amastigote.

MATERIALS AND METHOD:

Promastigotes of L. donovani strain AG83 were routinely cultured at 24^{0}C in M199 medium supplemented with 10% fetal calf serum, 100 U penicillin/ml and 100 µg streptomycin/ml.
Plant material: W.somnifera leaves and S.nigrum leaves were collected from herbal garden Jamia Hamdard, New delhi.
Chemicals: RPMI 1640 (without phenol red), ethyl acetate, n- hexane., methanol, SDS, tris, DMSO.
Instruments: laminar air flow hood, CO2incubator, B.O.D incubator, inverted microscope, ELISA plate reader, U.V transilluminator.

METHODS:

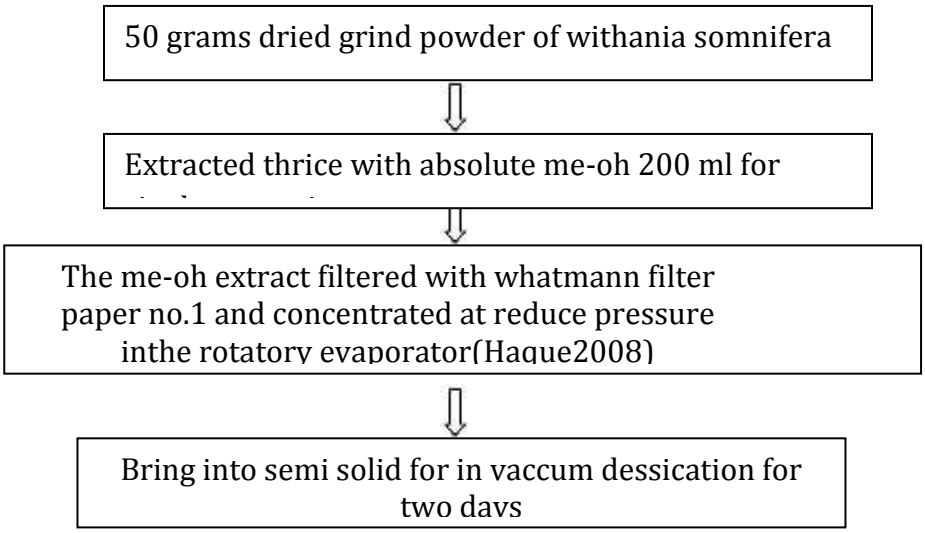

Figure 2.1. Preparation of crude extracts of solanum nigrum and withania somnifera leaves.

PASSAGING OF LEISHMANIA DONOVANI CULTURE:

The growth of the cells in culture usually follows a standard pattern- a lag phase is followed by a period of log phase which is the period of exponential growth which in turn is followed by stationary phase (where the cell division ceases),after which cells enter into death phase (where cell begin to die). When the cell density (cell per cm^2substrate) reaches a level such that all of the available substrate is occupied or when the cell concentration (cell per ml medium exceeds the capacity of the medium, growth ceases or is greatly reduced, then the culture must be divided.

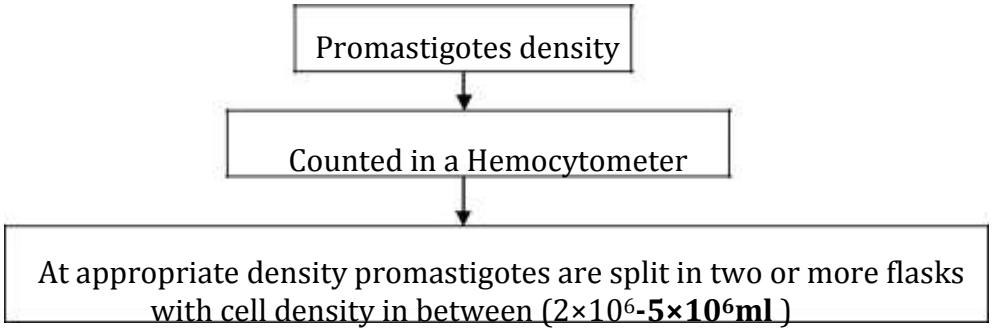

Figure 2.2 Culturing of leishmania donovani

MTT ASSAY: First described by Mosmann (1983), MTT is a laboratory test and a standardcolorimetric assay (an assay which measures changes in colour) for measuring cellular growth. It can also be used to determine cytotoxicity of potential medicinal agents and other toxic material.

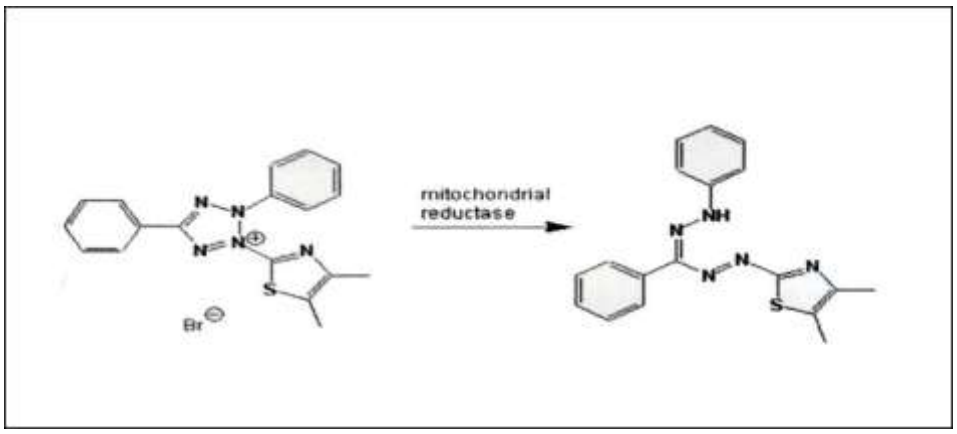

Figure.2.3 Formation of formazan

Yellow MTT [3-(4,5-DIMETHYTHIAZOL-2-YL)-2,5-DIPHENLTETRAZOLIUM BROMIDE] is reduced to purple formazan in the mitochondria of living cells (MTT in particular is reduced at the ubiquinone and cytochrome b and c sites of the mitochondrial electron transport system and is the result of succinate dehydrogenase activity). A solubilization solution (usually either dimethyl sulfoxide or an acidified ethanol solution or a solution of detergent sodium dodecyl sulphate in dilute hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution which can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption maximum is dependent on the solvent employed. MTT assay is rapid, versatile, quantitative and highly reproducible with a low interest variation between data points. Moreover the test can also be used for floating cells, such as leukemias and small cell lung carcinoma and always allows sufficient time for cell replication, drug induced cell death and the loss of enzymatic activity, which generates the formazan product from the MTT substrate (Dutta et.al.2007)

2.2. PROCEDURE:

Promastigote number: 2 x 10^5 Promastigotes well

⇩

Highest drug concentration 500µg/ml. serially diluted

⇩

Incubation time: 96 hrs at 22C

⇩

MTT (5mg/ml); 20µl/ml well.incubation: 4-6 hrs

⇩

Formazan solubilized in 10% SDS and Absorbance taken at 570nm (Paris C.et.al.2004)

RESULT AND DISCUSSION:

The promastigotes form of the parasites can be easily maintained in vitro following isolation from patients , therefore allowing rapid ,preliminary screening of antileishmanial compounds. The anti promastigote efficacy of methanolic and crude extracts of withania somnifera leaves and the aqueous was evaluated by MTT assay wherein the conversion of MTT to formazan by mitochondrial dehydrogenase is an indicator of cell viability, accordingly, a decrease in formazan production directly correlates with decreased cell viability.Screening of anti promastigote activity with above mentioned test extracts revealed a dose dependent reduction in promastigote viability. As evident from the graph no appreciable decrease in cell viability is observed. Maximum growth inhibition (47.06 %) was observed with choloform extract and a similar inhibition was obtained with aqueous extract of s.nigrum and all the other extract exhibiting significant cytotoxicity. Pentamidine a known anti leishmanial drug acted as positive control. Control wells in which promastigotes were grown in presence or absence of DMSO (0.25%) showed no decrease in viability.

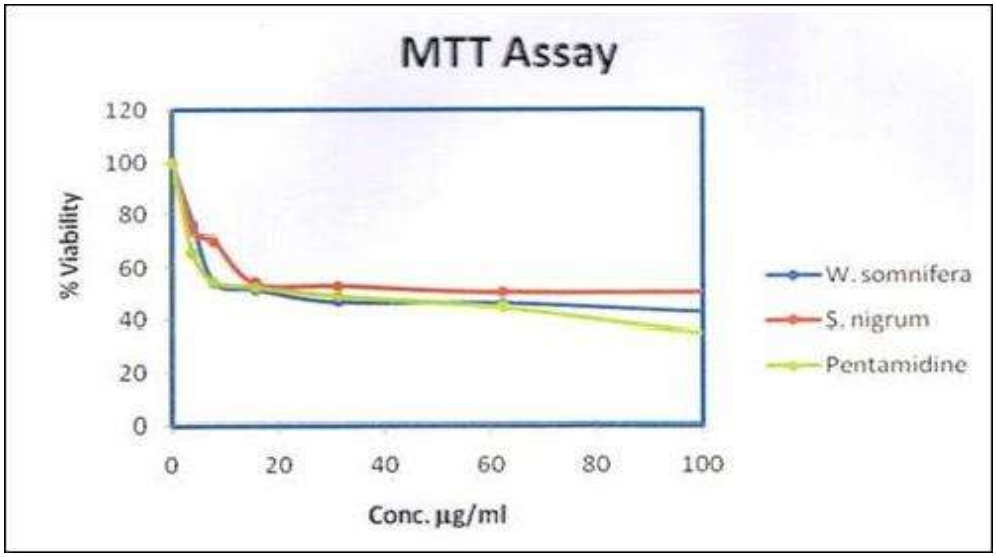

*Figure 2.4.*MTT Assay

CONCLUSION :

A graduate and moderate inhibition of growth of L.donovani promastigotes was obtained with both choloform and aqueous extract of s.nigrum leaves. The extracts can be further checked in vitro at higher concentration for their leishmanicidal activity and their cytotoxicity (at an

equivalent concentration) against macrophages can be studied or the extracts can be studied for their synergistic effect. In the present study, only s. nigrum leaf was fractionated, may be other parts of the plants (such as roots, fruits and stem bark) may provide us with better results.

Ethanolic extraction of withania somnifera did exhibit a well defined cytotoxic behavior against L. donovani promastigotes. In vitro,However ,a compound known as witherin glucoside (present in the leaves of withania somnifera) in its nanocarrier encapsulated form has been already reported by tyagi et.al(2005) for its anti leishmanial activities. However in our studies bio active fraction extraction could be obtained from w.somnifera and solanum nigrum adopting differentexperimental extraction procedures of s.nigrum could provide us with better results. Thus , more experiments need to be carried out to further validate the present data.

FUTURE PROSPECTIVES

i. Extraction of crude extracts of different parts of W. Somnifera and S. Nigrum with different established experimental procedures.
ii. In vitro assessment of leishmanial properties of various extracted crude extracts of W. Somnifera and S.Nigrum
iii. Study of synergistic effects of respective crude extract of W.Somnifera and S.Nigrum.

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