IJBPAS, March, 2019, 8(3): 549-559

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

In- vivo STUDY OF INDIRUBIN IN PROMASTIGOTES OF *L.donovani*

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Received 12th Oct. 2018; Revised 11th Nov. 2018; Accepted 22ndNov. 2018; Available online 1st March 2019

https://doi.org/10.31032/IJBPAS/2019/8.3.4664

ABSTRACT

Leishmaniasis is an important complex of protozoal vector-borne diseases that affect both humans and animals. *Leishmania donovani* (*L. donovani)* complex consist of member such as *L. infantum, L. donovani*and *L. chagasi* and has considered as an etiological agent for fatal visceral leishmaniasis disease. Recently, data shows that anti-parasitic agents for overcoming the limitations of present-day leishmaniasis chemotherapy toxicity. Indirubin is the active component of Danggui Longhui Wan (a traditional Chinese medicine formulation). Indirubin has been used in herbal medicine since ancient times, with various biological applications. Indirubin is a non- toxic, highly promising natural antioxidants compound having a wide spectrum of biological function. Although some research has been done on the possible medicinal uses, no studies for drug- development have been carried out as yet. Although the crude extract has numerous medicinal uses, clinical application can be also made only after extensive research on its bioactivity, mechanism of action, pharmacotherapeutics and toxicity studies. The leishmanicidal

activity progressively lessened with decreasing concentration of drug. In MTT assay, O.D. is decreasing with the increasing concentration of indirubin and 50% growth inhibitory concentration was 30 µM of indirubin. About 50% of parasites were killed at 30 µM (indirubin). *In-vivo* study indirubin against of *L.donovani showed* maximum reduction (50%) in spleen weight of mice. According to the present data, Indirubin showed goodanti- leishmanial activity against promastigotes of *L. donovani*starin AG83. It is expected the indirubin may find application as a novel drug in the near future to control leishmaniasis.

Keyword: Leishmaniasis, Indirubin, *Leishmania donovani, L. macropodum, L. enriettii*

1. INTRODUCTION

Leishmaniasis is vector-borne abandoned tropical disease caused by the bite of female phlebotomine sand flies and Trypanosomatid parasitic protozoa coming under genus *Leishmania* [1]. The *Leishmania* genus contains two subgenera *"Leishmania* and *Viannia."Leishmania* is maincause of Leishmaniasis in human while other organisms Leishmaniasis caused by both subgenera. *Mundinia* is the third subgenera of *Leishmania* have proposed for the *L. enriettii*complex, which epidemiologically differs from other *Leishmania*. The *L. enriettii*complex presently contains *L. macropodum, L. enriettii,* formerly known as *"L. australiensis", L. martiniquensis,* and *"L. siamensis"* formerly proposed names are not yet known and unknown *Leishmania* isolated from Ghana people. In Thailand, distinct species identified *"L. siamensis"* from human as well as animal clinical cases amused as *L. martiniquensis.*

Leishmania promastigote parasites transmitted the mammals as well as humans through bite of female phlebotomine sand flies [2, 3]. Several million people worldwide infected by parasites [4,5], mainly in the Indian Sub-continentals, Sub-saharan Africa region and Brazil, cause a spectrum of disease ranging from simple, self-healing harmless oriental shore to fatal visceral leishmaniasis or commonly known in India as kala-azar or dumdum.

L. donovani complex consists of the member such as *L. donovani*, *L. infantum*, and *L. chagasi* and has considered as anetiological agents for fatal visceral leishmaniasis disease. Sole causative vector for visceral

leishmaniasisis *L. donovani,* especially in Indian sub-continent, and it's fatal if it remains not diagnosed. In mammalian blood stream *Leishmania* promastigotes pass then they are phagocytosed by macrophages and if located in macrophages phagolysosomes, promastigotes converted into non-motile amastigote form with multiple forms [6]. *Leishmania* infected risk is in more than 350 million people and around 1.5-2 million new cases found with resulted that 500,000 deaths every year in the endemic areas [1]. Resistance increases of *Leishmania* parasites whereas current therapy toxicity as well as non-existence of a human vaccine which create urgent requirement to discover effective and targeted drugs for the treatment of leishmaniasis [7, 8]. Natural products research leads to be promising for discovering new targeted structures in ranges of diseases including leishmaniasis [8, 9].

Throughout natural product scaffolds, alkaloids display extensivestructure diversity can be exploited for the innovation of novel antileishmanial [8, 10]. In addition, marine indole-based alkaloid scaffolds [10] such as variolin [11], roscovitine [12], leucettines and halogenated indirubins [14], known to target kinases, represent significantly structured compounds for the innovation of new targeted for treatment of antileishmanial [15, 16].

An active ingredient Indirubin (Figure-1) is the Chinese traditional medicine "Danggui Longhui Wan", containing plants like *Indigo feratinctoria L.* and *Isatistinctoria L.* In 1980s China shows interest in clinical use of indirubin, when scientists with medical doctors together started examined it's as clinically use for the treatment of chronic myelocytic leukemia (CML), overproduction of granulocytes characterized the slowly progressive disease [17-20]. Around 50% of the treated CML patients exhibited fractional or complete remission [19-22], comparable to the advanced treatment using the cytostatic agent busulfan [21]. Toxicity of Indirubin was very low whereas side effects experienced by about half of the members suffer from mild abdominal pain, nausea, and diarrhea [20]. Reversible pulmonary arterial hypertension and cardiac insufficiency were reported in three cases [23].

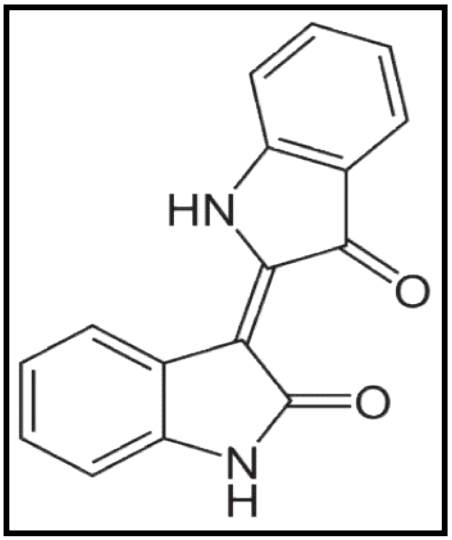

Figure 1: Chemical structure of indirubin

2. METHOD AND MATERIALS

2.1 Parasites:

Leishmania parasites used in this study are *L.donovani* (AG83) Indian strain were cultivated in BALB/c mice. Promastigotes of this strain were cultivated in medium 199 supplemented with 10% heat inactivatedfoetal bovine serum, 100 units of penicillin G/ml and100µg of streptomycin sulphate/ml at 22°C.Parasites viability was determined microscopically. The log phase viable promastigotes were further subcultured in M199 at 22°C.Promastigotes viability wasmonitored throughout the culture period.

2.2 BALB/c mice female

BALB/c female mice around 30 to 35 grams, about 28 to 35 days were used for the study. Animals were maintained at 25° C under suitable parameter.

2.3 Cell Cytotoxicity Assay

MTT assay is a standard colorimetric assay which measures changes in color for measuring cellular proliferation. It is mainly used for the determination of toxic materials and cytotoxicity of potential medicinal agents [21].

Yellow MTT (3-4, 5-dimethylthiazol –2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. A solublization solution (0.4N HCl diluted in isopropanol) is added to dissolve the insoluble purple formazan product into a colored solution. In ELISA reader absorbance of colored solution quantified by measuring at a certain wavelength

(550 to570nm) and plots the graph with the help of graph pad.

2.4 In-vivo antileishmanial Activity of indirubin

Swiss albino mice (28-35 days) were infected *in-vivo* through freshly transformed promastigotes of *L.donovani* ($2x10^7$/mouse) [25]. Doses of drugs indirubin started two months after infection. Indirubin high dose (1.0mg/kg)/bodyweight) was injected to two groups of mice intraperitoneally. Pentamidine used as a positive control, empty served as infected control.

2.5 Micewere divided into following four groups which are as follows

1. Infected mice treated with PBS
2. Infected mice treated with Pentamidine control.
3. Infected mice treated with high dose of indirubin (1.0mg/kg body weight).
4. Mice have taken as healthy control in which no drug was administered.

3. RESULTS

3.1 Effect of indirubin in L. donovanipromastigotes

In order to determine the concentration of indirubin at which approximately 50% death of *L. donovani* promastigotes would occur, we had tested the effect of several concentration of the drug via the MTT assay. Data shows of *L. donovani* promastigotes under the *invitro* conditions. The death profile was initially slow when concentration 5-50 µM were used. Subsequently, a very rapid and dose dependent death occurred with indirubin concentration between 30µM shown in Figure 2.

***3.2 In-vivo* assay**

The *in-vivo* leishmanicidal activity of indirubin against promastigotes of *L.donovani* is shown in figure-3.

In case of spleen weight after 21 days post treatment, maximum reduction (50%) in spleen weight was observed in case of indirubin whereas 41% was observed in case of pentamidine respectively.

After 35 days post treatment, maximum reduction in spleen weight (55%) was observed in case of indirubin whereas in case of pentamidine it was 52% while comparing with infected control.

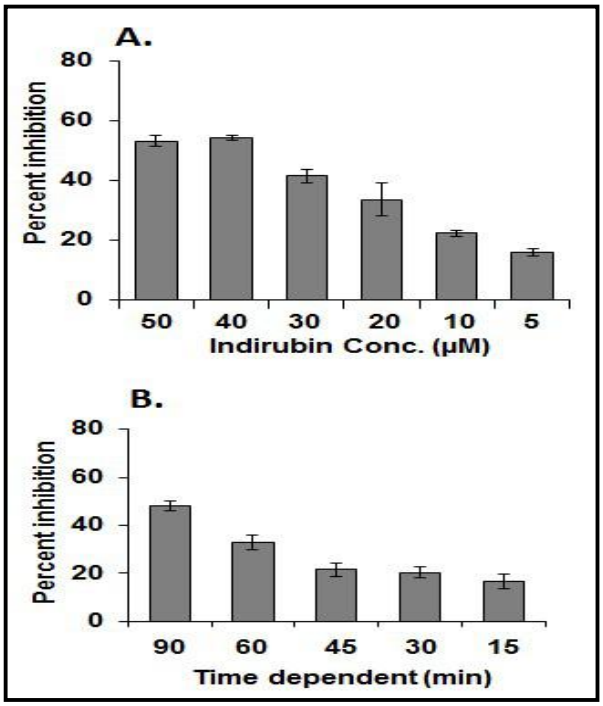

Figure 2: Antileishmanial activity of indirubin (A) Conc. Curve (5-50µM)and (B) Indirubin constant conc. 30µM at different time interval (15-90 min)

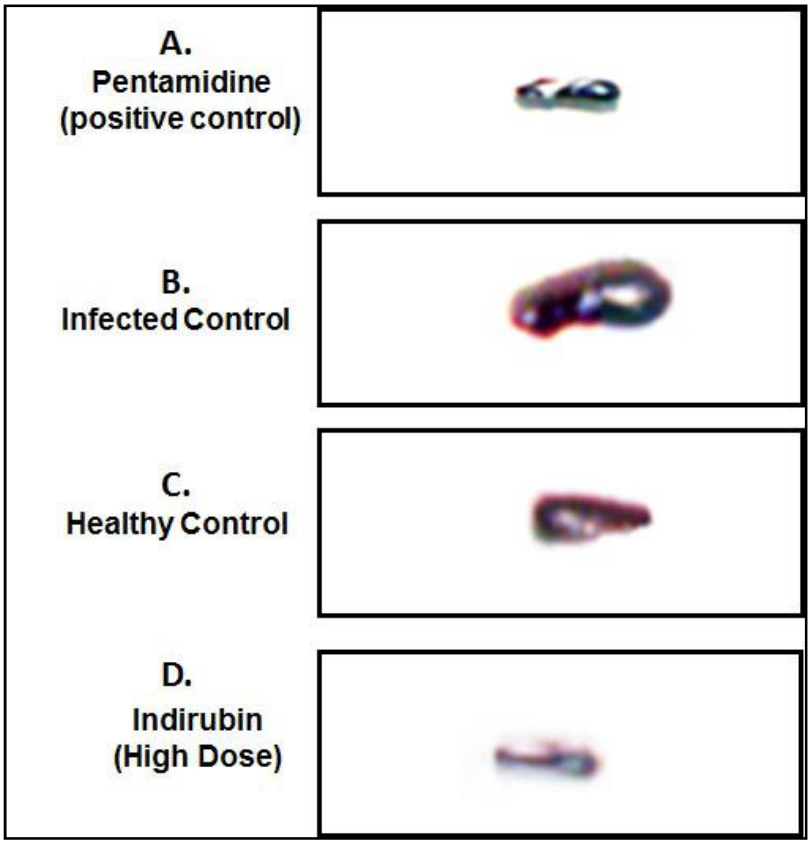

Figure-3: Dramatic reduction in size of the spleen of mice showing significant differences (A) Positive Control, (B) Infected with parasite (C) Healthy control and (D) indirubin treatment

4. DISCUSSION

In the present study, indirubin shows leishmanicidal activity on promastigotes of *L.donovani in vitro*. It is known that natural products have a future in the search for new and selective agents for the treatment of leishmaniasis [26]. A majoradvantage of indirubin screening is the structural diversity of substances that could create natural products a source of novel lead compounds against leishmaniasis. Indirubin showed about 50% inhibition at 40µM concentration on promastigotes of *Leishmania donovani* parasites by MTT assay. All these results encourage furthering investigating the leishmanicidal activity *in-vivo* with indirubin to check whether indirubinhas promising anti-leishmanial activity *in-vivo* and *in-vitro.*

The antileishmanial activity could be seen with the help of MTT assay. In MTT assay concentration of drug was 5µM-50µM. At 50µM concentration, indirubin showed more than 54% at high dose concentration cell proliferation could be seen.

In case of spleen weight after 21 days post treatment, maximum reduction (50%) in spleen weight was observed whereas 41% and 55% were observed in case of pentamidine and indirubin respectively.

These *in-vitro* and *in-vivo* results clearly suggest that indirubin are a better antileishmanial agent than the standard drug which is frequently used in therapy are toxic but also introduce the targeted delivery system for intracellular parasites. The results presented here provide motivation for further exploration of these compounds, particularly as antileishmanial agents. Laboratory synthesis and the possibility of modifying the chemical structure of indirubin are important advantages for the development of new antileishmanial agents using as herbal plants.

5. CONCLUSION

Indirubin used as Herbal medicine since ancient times mainly started used in China, with biological applications. Although in possible medicinal applications some work has been done, but no studies have been carried out yet for development of drug drug-development. On the other hand, the crude extract has numerous medicinal applications, clinical applications confirmed after extensive research on its pharmacological therapeutic, bioactivity, mechanism of action and toxicity studies.

However, availability of indirubin in pure form which indicates a broad spectrum of biological activities and help to make easily work to develop the new drugs from this compound after extensive studies on its mechanism of action and pharmacological effects. Recently few years ago we have seen an increased frequently interest shownfor treating various diseases with natural products. As studied the characteristics of indirubin are non-toxic, highly promising natural antioxidant compound with broad range of biological function. According to the present data indirubin showed good anti-leishmanial activity against promastigotes of *L.donovani* strain (AG83).It is expected that indirubin may find application as a novel drug in the near future to control *Leishmaniasis.*

Conflict of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

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