Journal of Drug Design and Medicinal Chemistry
2024; 10(1): 8-10
http://www.sciencepublishinggroup.com/j/jddmc
doi: 10.11648/j.jddmc.20241001.12
ISSN: 2472-355X (Print); ISSN: 2472-3576 (Online)

Resveratrol: A Cell Growth Inhibitor Against *Leishmania Tropica*

Kashif Iqbal*, Qaiser Aziz, Ayesha Sana

Department of Pharmacy, IBADAT International University, Islamabad, Pakistan

Email address:
kashifiqbal321@gmail.com (Kashif Iqbal)

*Corresponding author

To cite this article:
Kashif Iqbal, Qaiser Aziz, Ayesha Sana. (2024). Resveratrol: A Cell Growth Inhibitor Against *Leishmania Tropica*. *Journal of Drug Design and Medicinal Chemistry*, *10*(1), 8-10. https://doi.org/10.11648/j.jddmc.20241001.12

Received: December 8, 2023; **Accepted**: January 6, 2024; **Published**: January 23, 2024

Abstract: This study describes cell growth inhibition through *invitro, invivo* antileishmanial and cytotoxic effect of Resveratrol. Resveratrol was tested at range of concentration of 1 - 10 µg/ml for *invitro* analysis on *L-tropica* strain for 24 – 72 h, whereas Sodium Stibogluconate was reference drug. BALB/c mice were subjected to *invivo* test relative to negative control, while mammalian cells (Lymphocytes) were used in cytotoxic test. After 72 h, tested compound showed significant results against promastigotes at concentration of 1 µg/ml, 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml and 10 µg/ml which were 90.5 ± 0.02, 95.2 ± 0.07, 96.5 ± 0.05, 97.8 ± 0.03 and 98.2 ± 0.04 respectively. After 6 weeks, it showed promising results against intracellular amastigotes in mean lesion size which decrease from 0.9 ± 0.2 mm to 0.25 ± 0.2 mm ($p < 0.02$) where as 10 mg/kg (dose) was subjected to BALB/c mice which exhibited decrease in percentage cure rate up to 94.01 (95 % C.I = 92.01 – 96.21). IC_{50} values of lymphocytes showed 14.23 µg/ml (95 % C.I = 10.05 – 17.56) against Resveratrol. After analysis, it was observed that Resveratrol possesses anti-leishmanial activities against the cell growth of *L. tropica* and has no effect on lymphocytes.

Keywords: Resveratrol, *Leishmania tropica*, Promastigotes, Amastigotes, Mammalian Cell

1. Introduction

Leishmaniasis is an infectious disease, which is prevalent all over the world. It belongs to genus *Leishmania*. It affects millions of people annually around the globe [5]. Existing remedies have high cost, drug resistance, toxicity and high dose regimen. Natural entities exhibit safety and efficacy in terms of drug potential against infectious diseases [6]. *Morus Nigra* have number of pharmacological properties like Anti-inflammatory, antioxidant, Anti-leishmanial etc, which are due to presence of natural entities i.e., Flavonoids, Coumarins, Carotenoids, and Anthocyanins [4]. Resveratrol ($C_{14}H_{12}O_3$) was isolated from *Morus Nigra* which possess anti-inflammatory and antioxidant activities [1, 9].

In current work, Resveratrol was subjected to check the inhibition of *Leishmania* cell lines through *invitro*, *in vivo* and cytotoxic test.

2. Experimental

2.1. Chemicals

Resveratrol, Fetal Bovine serum (FBS), Antibiotics (Streptomycin, Penicillin), RPMI-1640 medium, formic acid, DMSO (Dimethyl Sulfoxide) were purchased from Sigma Aldrich, USA.

2.2. In-Vitro Test

Effect of sample was checked on *Leishmania tropica* KWH23 strains having 1.6 x 10^6 cells/ml at 24 – 72 h. Sample solution was prepared with different concentrations i.e., 1, 2.5, 7.5, and 10 µg/ml dissolved in DMSO, as explained by Iqbal et al., 2017 [7].

2.3. In-Vivo Test

BALB/c mice were supplied by AMSON vaccines and

Pharma (Animal house), Islamabad. 03 groups of BALB/c mice were designed which comprised of first group of drug control, second group of positive control (Standard drug) and negative control in third group. 08 samples were prepared in which four were standard drug and rest of four were compound (sample) material as described by Iqbal K et al., 2017a.

Bioethics statement: The study was approved by Bioethics Committee, The University of Lahore (UoL), Islamabad (Approval ref. No. BEC/PHARM/0501) for antileishmanial analysis. The mice were maintained in rely with UoL, Islamabad guidelines on the care and use of laboratory animals [3]. Satndard diet and water ad libitum was given to mice during analysis.

2.4. Cytotoxicity Test

In cytotoxic analysis, given four strenght (1, 2.5, 7.5, and 10 µg/ml) were checked against mammalian cells obtained from healthy volunteers whereas having promastigotes concentration was 1.6 x 10^6 cells/ml at 24 – 48 h under light microscope. Method was adopted as described by Iqbal k et al., 2016b [2].

3. Statistics

% Inhibition of cell growth was expressed as mean± SD of three replicate measurements. Cytotoxic test was expressed as % inhibitory concentration (IC_{50}) and analysed by non-linear regression analysis. For *in vivo* assays, mean lesion size (mm) and percentage cure rate were analysed with GraphPad Prism 5 software (GraphPad software, San Diego, CA). P values <0.05 were taken as significant.

4. Results

4.1. In-Vitro Inhibition of Cell Growth

Invitro cell growth inhibition was checked on KWH23 having concentration 1.6 x 10^6 cells/ml. Resveratrol showed significant inhibition of cell growth 90.5 and 98.2 at 1 µg/ml and 10 µg/ml (Table 1) respectively. When the sample was compared with standard drug (Sodium Stibogluconate), it has 91.0 and 94.5 at 1 µg/ml and 10 µg/ml at 72 h respectively.

Table 1. *Showing inhibition of cell growth by Resveratrol. Data represent mean precent inhibition ± S.D of three replicates.*

Compound (Sample)	Compound (Sample) concentrations (µg/ml)	% inhibition at Time (h)	
		24	72
Resveratrol	1	70.1±0.01	90.5±0.02
	2.5	65.5±0.00	95.2±0.07
	5	64.1±0.02	96.5±0.05
	7.5	60.5±0.05	97.8±0.03
	10	59.1±0.08	98.2±0.04
Sodium Stibogluconate	1	50.2±0.01	91.0±0.02
	2.5	52.4±0.00	92.5±0.05
	5	54.6±0.04	92.2±0.01
	7.5	55.3±0.00	93.1±0.02
	10	56.4±0.00	94.5±0.00
NC	1	0.00±0.00	0.00±0.00
	2.5	0.00±0.00	0.00±0.00
	5	0.00±0.00	0.00±0.00
	7.5	0.00±0.00	0.00±0.00
	10	0.00±0.00	0.00±0.00

4.2. In-Vivo Inhibition of Cell Growth

BALB/c mice were infected via intraperitoneal route with *L. Tropica* strain having concentration of 1.6 × 10^6 cells/ml for 120 days. When treated with Resveratrol, it showed decrease in lesion size from 0.9 to 0.2 mm, having % cure rate of 95.05. Sodium stibogluconate showed 92.01 % cure rate and lesion size decreased from 0.92 to 0.4 mm, in which all the mice survived, when compared with negative control in which lesion size increased from 0.91 to 1.4 mm and all the infected mice killed.

Table 2. *Showing invivo inhibition of cell growth by Resveratrol. Data showed mean lesion size ± S.D.*

Sample	Dosing Regimen (For 05 Days)	Mean Lesion (mm) Pre-treatment	Mean Lesion (mm) After Treatment (After 08 Weeks)	%age Cure Rate (with 95% Confidence intervals)	No: of mice cured/No: of mice Infected	Mean survival time (Days)
Resveratrol	15 mg/kg	0.9±0.00	0.2±0.5	95.05 (93.01-98.05)	6/6	≥60
Sodium Stibogluconate	7.5 mg/kg	0.92±0.02	0.4±0.2	92.01 (90.01-94.05)	6/6	≥60
NC	10 mg/kg	0.91±0.01	1.45±0.01	0.000	0/6	≥30

4.3. Cytotoxic Effects

Resveratrol showed potent cytotoxic effect having IC_{50} value 13.05 at 72 h, when treated with lymphocytes.

Sample	IC_{50} (µg/ml) 95% confidence intervals
Resveratrol	13.05 (10.01-15.05)

5. Discussion

In this study, Resveratrol was selected for *invitro* antileishmanial effect which showed significant results 98.2% (10 µg/ml) at 72 h when compared with standard drug which showed an agreement with reported Antileishmanial research work [10]. BALB/c mice were infected via *L. Tropica* strain in which mean lesion size (mm) decreased to 0.2, having 95.05% cure rate with all mice survived within 120 days. The inhibition of strains in BALB/c mice may be due to inhibition of metabolic pathway in *L. tropica* [6]. Safe profile of compound was observed in cytotoxic test when compared with previous findings [8, 10]. *Morus Nigra* possess a lot of biological and pharmacological properties which are due to present of phytochemical active

moieties. Resveratrol is one of active constituent of *Morus Nigra*. It is the first time when Resveratrol is subjected to *Leishmania* cell lines for *invitro, invivo* and cytotoxic effects for comprehensive analysis.

6. Conclusion

In this study, Resveratrol possesses a potent effect against *Leishmania tropica* strains which is due to metabolic pathways and that would lead to further findings for development of convenient, safe and less resistance antileishmanial drug.

Acknowledgments

We would like to thank AMSON Vaccines and Pharma, Islamabad, Pakistan for providing the Albino mice and necessary technical support.

Contribution of Authors

We declare that this work was done by Mr. Kashif Iqbal and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Mazimba, O., Majinda, R. R., Motihanka, D. (2011). Antioxidant and antibacterial constituents from Morus Nigra. *Afr. J. Pharm. Pharmacol*. 5(6): 751-754.

[2] Iqbal, K., Iqbal, J., Afreen, M. S. (2016b). Comparative Study on Antileishmanial and Cytotoxic activity of *Lawsonia Inermis* bark and *Aloe Vera* leaves. *Int. J. Biol. Pharm. Allied. Sci.* 5(6): 1490-1500.

[3] National Research Council of The National Academy of Sciencies. (2010). Guide for the Care and Use of Laboratory Animals: Eight Edition. Washington, D. C.: The National Academies Press.

[4] Iqbal, K., Iqbal, J., Umair, M., Farooq, U., Iqbal, M. M., Qamar, S., et al. (2016a). Anti-leishmanial and cytotoxic activities of extracts from three Pakistani Plants. *Trop. J. Pharm. Res*. 15, 2113-2119.

[5] World Health Organization (WHO). (2010). Control of Leishmaniases: Report of the Meeting of the WHO Expert Committee on the Control of Leishmaniases. WHO, Geneva, Switzerland.

[6] Iqbal, K., Jamal, Q., Iqbal, J., Afreen, M. S., Sandhu, M. Z. A., Dar, E., et al. (2017a). Luteolin as a potent Antileishmanial agent against intracellular *Leishmania tropica* parasites. *Trop. J. Pharm. Res.* 16(2): 337-342.

[7] Iqbal, K., Iqbal, J., Staerk, D., and Kongstad, K. T. (2017). Characterization of Antileishmanial compounds from *Lawsonia Inermis* L. Leaves using Semi-high Resolution Antileishmanial Profiling combined with HPLC-HRMS-SPE-NMR. *Front. Pharmacol.* 2017b; 8: 337.

[8] Hu, H. B., Liang, H. P., Li, H. M., Yuan, R. N., Sun, J., Zhang, L. L. et al., (2018). Isolation, modification and cytotoxic evaluation of Resveratrols from Acanthopanax leucorrhizus. *Fitoterapia*. 124: 167-176. doi: 10.1016/j.fitote.2017.11.007.

[9] Ghada M. Abbas, Fatma M. Abdel Bar, Hany N. Baraka, Ahmed A. Gohar & Mohammed-Farid Lahloub (2014) A new antioxidant stilbene and other constituents from the stem bark of Morus nigra L., Natural Product Research: Formerly Natural Product Letters, 28: 13, 952-959, DOI: 10.1080/14786419.2014.900770.

[10] Antinarelli, L. M. R., Meinel, R. S., Coelho, E. A. F., Silva, A. D., & Coimbra, E. S. (2019). Resveratrol analogues present effective antileishmanial activity against promastigotes and amastigotes from distinct Leishmania species by multitarget action in the parasites. *Journal of Pharmacy and Pharmacology.* doi: 10.1111/jphp.13177.