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Original Article

Activity of a Novel 2-Phenoxy Nicotinic Acid Hydrazide sulfonamide against *Leishmania tropica*

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Abstract

Introduction: Cutaneous Leishmaniasis is an endemic health problem worldwide. The absence of an alternative chemotherapeutic approach to treating Leishmania infection requires urgent attention. So, the laboratory-trial study aimed to determine the activity of a novel 2-Phenoxy Nicotinic Acid Hydrazide sulfonamide against *Leishmania tropica*.

Methods: The different densities (0.2, 1, 5, 25, and 125 µg /ml) of 2-PNAHS and Glucantime (0.2, 1, 5, 25, and 125 µg /ml) were provided and added to parasite cultures. Then, the anti-Leishmania activity of the 2-PNAHS against Glucantime was determined using an in-cell proliferation ELISA, BRDU (Chemiluminescent).

Results: According to the results, a dose-dependent decrease was observed in the viability of the *Leishmania (L) tropica* promastigotes using Glucantime ($P<0.05$). Administration of different levels of the 2-PNAHS (0.2, 1, 5, 25, and 125 µg /ml) significantly decreased the *Leishmania (L) tropica* promastigotes ($P<0.05$). Additionally, a difference was detected in the anti-Leishmania activity of the 2-PNAHS compared to Glucantime and the control group ($P<0.05$).

Conclusion: These results suggest that 2-PNAHS had anti-Leishmania activity better than Glucantime.

Keywords: Leishmania tropica [MHOM/IR/NADIM3], Sulfonamide derivative, Hydrazide, Promastigotes

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Introduction

Leishmaniasis is a significant parasitic disease that affects approximately two million persons annually, with approximately 350 million at risk of infection (Desjeux, 2004). Cutaneous Leishmaniasis (CL) is an endemic health problem worldwide. It causes ulcers on the body, leaving lifelong scars and even severe disability. Based on the reports, the Americas, the Mediterranean basin, the Middle East, and central Asia are the principal foci of the CL. Over 70% of the CL new cases were identified in Afghanistan, Algeria, Brazil, Colombia, Iran, and Syria (1). For years, chemotherapy for Leishmaniasis has relied on administering pentavalent antimonial compounds such as sodium stibogluconate and Meglumine antimoniate (Glucantime) as first-line agents (2). Amphotericin B, Pentamidine, and nonparenteral Miltefosine are alternative chemotherapies introduced in recent decades (3). However, these drugs also show complications such as side effects and high costs. As well, medications used for *Leishmaniasis* treatment have clinical side effects such as nephrotoxicity, hepatotoxicity, and cardiac arrhythmia (4-6). There is still no progress in the production of newer medications. Thus, there is an urgency for the development of affordable and less-toxic alternative drugs (7). Despite research on producing an effective vaccine against Leishmaniasis, no vaccine exists for any form of Leishmaniasis (8).

Researchers focused on displacing synthetic drugs with new medicines to develop an efficient and cheap parallel synthesis approach (9). Sulfonamides are prominent biologically active compounds with many biological activities, such as anti-microbial, anti-bacterial, and antioxidant (10). However, side effects such as liver, kidney, skin, lung, and heart abnormalities were reported for long-term administration of the sulfonamides (11). In the past decade, investigated the Leishmanicidal activities of Sulfonamides for the first time. Based on the report, 1-(4-X-phenyl)-N′-[(4-Y-phenyl) methylene]-1H-pyrazole-4-carbohydrazides are less toxic than Pentamidine and Ketokonazol and offer new perspectives on the development of drugs with activities against the Leishmania parasite (12). In a study, Bilbao-Ramos et al studied the effect of the N-benzene sulfonamides of amine-substituted aromatic rings, sulfonamides 1-6, against *Trypanosome cruzi* and *Leishmania spp*. Based on their observations, 4-nitro-N-pyrimidin-2-ylbenzenesulfonamide and 4-chloro-N-5-methyl-thiazol-2-yl-benzenesulfonamide had *anti-leishmanial* activity

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(13). The present study carried out the activity of a novel 2-Phenoxy Nicotinic Acid Hydrazide sulfonamide against *Leishmania tropica*. This research investigated the cytotoxic effects of the activity of a novel 2-Phenoxy Nicotinic Acid Hydrazide sulfonamide against *Leishmania tropica*.

Materials and Methods

Phenoxy Nicotinic Acid Hydrazide Sulfonamide Formulation

The formulation of the 2-PNAHS is shown in Figure 1. The 2-PNAHS is a vital intermediate for producing compounds. Using the reported methods, the 2-phenoxynicotinic acids were prepared using the fusion of sodium 2-chloronicotinate and sodium phenoxide derivatives. Etherification of the 2-phenoxy nicotinic acids by a standard acid-catalyzed method afforded the ethyl esters. Then the ethyl esters were treated with hydrazine hydrate in absolute ethanol, which required the product to have hydrazides (14).

Parasite Culture

Leishmania (L) tropica strain [MHOM/IR/NADIM3] promastigotes (PM) were provided from the medical Parasitology laboratory of the Shahid Sadoughi University of Medical Sciences. The Amastigote of *Leishmania (L)* tropica strain [MHOM/IR/NADIM3] was isolated from mice spleens and transformed into PMs in Novy-Nicolle-MacNeal (NNN). After 3 times passages of PMs, NNN medium was progressively adapted to RPMI1640 media (Gibco), including antibiotics, glutamine, and FCS supplemented with penicillin (100 U/ml), streptomycin (100 µg /ml), and 20% heat-inactivated fetal bovine serum (FCS) at 25°C (15).

Study Groups

To determine the anti-Leishmania activity of the 2-Phenoxy Nicotinic Acid Hydrazide, 11 experimental groups were designed as follows:

- Group 1 as negative control group: 200 µl of $RPMI_{1640} + 2 \times 10^5$ cells/ml PMs
- Groups 2- 6 as cases: 200 µl of $RPMI_{1640} + 2 \times 10^5$ cells/ml PMs + 0.2, 1, 5, 25, 125 µg 2-PNAHS
- Groups 7- 11 as cases positive control: 200 µl of $RPMI_{1640} + 2 \times 10^5$ cells/ml PMs + 1 µg 2-PNAHS

Anti-Leishmania Activity Assay

The anti-Leishmania activity was done using C-Cell proliferation enzyme-linked immunosorbent assay (ELISA), BrdU (Chemiluminescent) method as described by Diagnostics GmbH, Roche Applied Science, 68298 Mannheim, Germany (Version March 2015, Cat. No 11 669 915 001). This technique is used to determine DNA synthesis in cell cultures. Briefly, in this system, a fixed initial density of the parasites was transferred to screw-capped vials of liquid medium (5 ml), and different concentrations of 2-PNAHS or Glucantime (0.02, 1, 5, 25, and 125 µg /ml) were added. Then, the solution was stimulated with acetone, and Dioxy bromoorydin was added and incubated at 37°C for 8 hours. Then, the supernatant was removed, and Fixator was added to the permeable membrane. In the next step, the anti-oxibromouridin conjugated with POD was added and

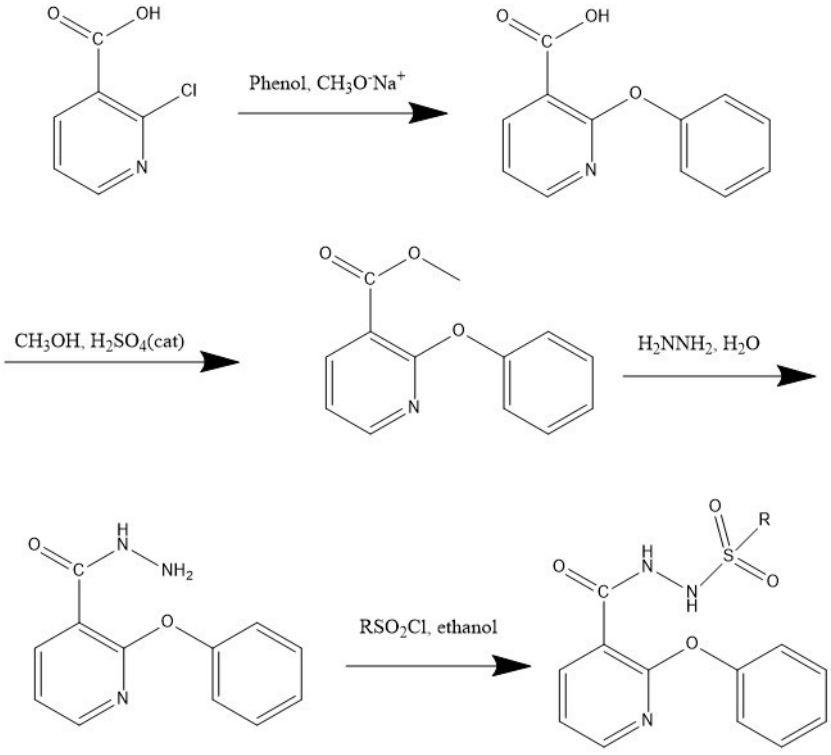

Figure 1. the formulation of the2- *Phenoxy Nicotinic Acid Hydrazide Sulfonamide*

incubated for 3 hours. Finally, the absorbance of the culture is determined using a spectrophotometer at 450 nm.

Statistical Analysis

All data are reported as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed, followed by post-Tukey LSD. Comparisons among the experimental groups were done by one-way ANOVA. The $P<0.05$ level assessed significant protection in treatment groups.

Results

Antileishmanial activity of 2-Phenoxy Nicotinic Acid Hydrazide Sulfonamide against *Leishmania (L)* tropica [MHOM/IR/NADIM3] PMs is presented in Figures 2-4. According to the results, a dose-dependent decrease in the viability of the Leishmania (L) tropica promastigotes was observed using Glucantime ($P<0.05$) (Figure 2).

Administration of different levels of the 2-PNAHS (0.2, 1, 5, 25, and 125 µg /ml) significantly decreased the viability of the Leishmania (L) tropica promastigotes ($P<0.05$) (Figure 3).

Additionally, a significant difference was detected in the anti-Leishmania activity of the 2-PNAHS compared to Glucantime and the control group ($P<0.05$) (Figure 4). The 2-PNAHS has better anti-Leishmania activity in each dose than the same Glucantime dose. Overall, 2-PNAHS had anti-Leishmania activity better than Glucantime.

Discussion

To our knowledge, no previous study has been conducted on the effect of levels of 2-PNAHS compared to Glucantime against *Leishmania (L)* tropica [MHOM/IR/NADIM3] PMs. These results suggest that 2-PNAHS

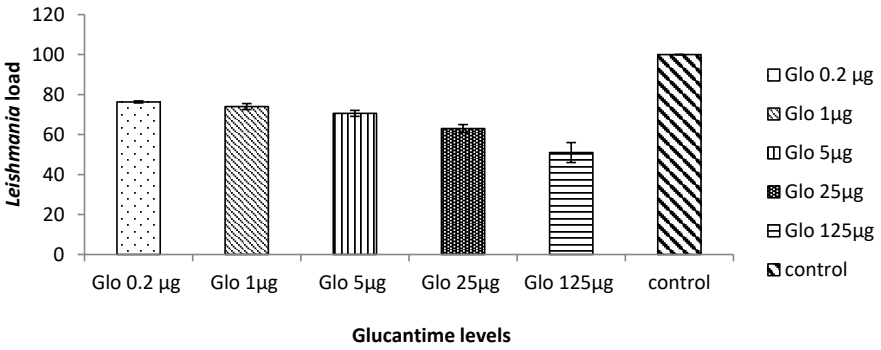

Figure 2. Effect of different levels of Glucantime against *Leishmania (L) tropica* in the stationary phase

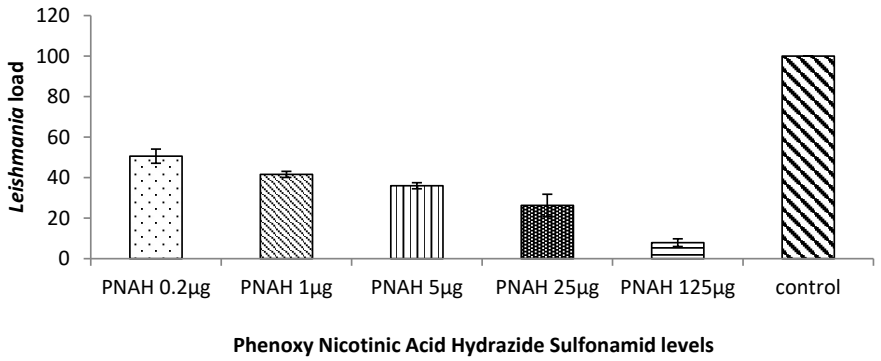

Figure 3. Effect of 2-Phenoxy Nicotinic Acid Hydrazide levels against *Leishmania (L) tropica* in the stationary phase

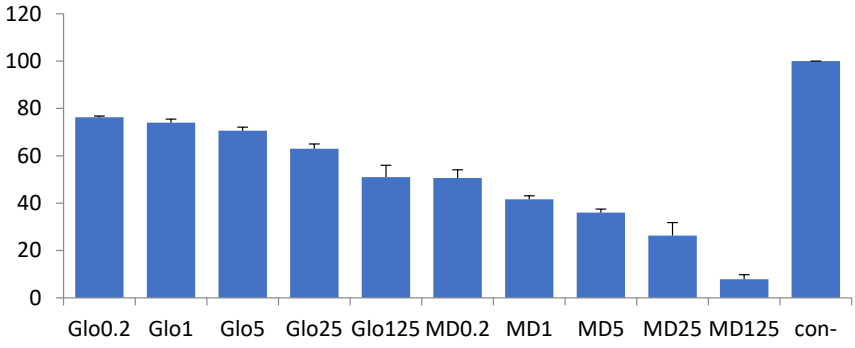

Figure 4. Effect of different levels of Glucantime vs. 2-Phenoxy Nicotinic Acid Hydrazide Sulfonamide against Leishmania (L) tropica in the stationary phase

has medical potential much better than Glucantime. *Leishmania* spp. cause a broad spectrum of infectious diseases ranging from self-healing cutaneous ulcerations to progressive and lethal visceral infection (16). Currently, the drugs used for *Leishmaniasis* treatment present many disadvantages, including serious clinical side effects such as nephrotoxicity, hepatotoxicity, and cardiac arrhythmia. In contrast, the emerging strain resistance to available drugs has also decreased the treatment options (17). However, there is no report on the role of the 2-PNAHS against *Leishmaniasis*; in this study, we compared our results with previous reports; however, the chemicals' origin might differ. Previous reports imply that sulfonamide functionality can display antiparasitic, antibacterial, and anti-viral HIV activities (18). The addition of the sulfonamide together with the bromide improved the *Antileishmanial* activity of this series compared to our previous data from 5-amino-1-aryl- 4-(4, 5-dihydro-1H-imidazol-2-yl)-1H-pyrazoles derivatives (19). Additionally, Rahavi E et al reported sulfonamide-1, 2, 4-triazoles, 1, 3, 4-thiadiazoles, and 1, 3, 4-oxadiazole as potential antibacterial and antifungal agents. Also, sulfonamide-1, 2, and 4-triazole derivatives have antifungal and antibacterial activity (20), similar to commercial ketoconazole and bifonazole (21).

Minimum inhibitory concentration (MIC) determination was performed by a serial dilution technique using 96-well microtitre plates. As observed in this study, the MIC of 2-PNAHS was determined at 0.02 µg /ml. The interesting finding of the current study was that at the level of 0.02 µg /ml, the 2-PNAHS had better results than Glucantime compared to the control group. While new *anti-leishmanial* compounds are continuously developing, drug combination is currently considered one of the most rational alternatives to improve treatment efficiency, prevent drug resistance, and reduce treatment duration (22). Currently, the recommended drugs for the treatment of *Leishmaniasis* are administered parenterally and are limited, such as old drugs and new components, such as pyrazole carbohydrazides, 2-phenoxy-1, 4-anthraquinone, and Sulfonamide 4-Methoxychalcone derivatives (23). The key goal in pharmaceutical development is a good understanding of in vitro and in vivo performance (24). As observed in the current study, 2-Phenoxy Nicotinic Acid Hydrazide Sulfonamide had a positive effect against *Leishmaniasis*. The proposed mechanism of action for the biological effects of 2-Phenoxy Nicotinic Acid Hydrazide Sulfonamide against *Leishmaniasis* is unclear. In a possible mechanism, it is reported that Sulfonamide derivatives have antioxidant and Antiacetylcholinesterase activities and might exert their effect against Leishmaniasis via a pathway (25). We think the current results can be used as base information, and further research is needed for the direct cellular and molecular action of the Antileishmanial activity of 2-Phenoxy Nicotinic Acid Hydrazide Sulfonamide. It is reported that the sulfonamide group can bind to active, aromatic components and heteroaromatic systems and might impress antiparasitic activity (26-28).

Garedaghi Y et al studied the Antileishmanial activity of the various forms of the Sulfonamide 4-Methoxychalcone derivatives. Based on their report, all Sulfonamide derivatives were able to affect *L. amazonensis* PMs in a concentration-dependent manner and with low cytotoxicity. During the evaluation of new synthetic compounds against experimental Leishmaniasis in the murine model, it is necessary to evaluate possible toxic effects. These data will enable the study and application of the new drugs. The obvious question was to evaluate toxicity in treated animals. This evaluation assessed body weight, leukocyte counts, hepatic enzymes, and urine keratinization (29-31). We think further research is needed to determine the safety of the 2-PNAHS as a potential *Antileishmanial* drug. In conclusion, these results suggest that 2-PNAHS has medical potential better than Glucantime. Further research is needed to determine the cellular and molecular mechanisms of action.

Conclusion

These results showed that 2-phenoxynicotinic acid hydrazide sulfonamide produces better results than Glucantime under the same conditions in terms of time and amount of exposure in the culture medium. However, this comparison should be studied on a larger scale and in different sizes.

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Authors' Contribution

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Project administration: Ali Fattahi Bafghi.
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Supervision: Ali Fattahi bafghi.
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Visualization: Ali Reza Moradi, Arefeh Dehghani.
Writing–original draft: Ali Fattahi Bafghi, Neda Khanizadeh.
Writing–review & editing: Ali Fattahi Bafghi.

Competing Interests

The authors have no conflicts of interest.

Data Reproducibility

The dataset presented in the study is available upon request from the corresponding author during submission or after publication.

Ethical Approval

This paper was distilled from a research project approved by the Committee of Ethics for Human Research, Center for Preventing and Controlling Tropical Infectious Diseases at Shahid Sadoughi Hospital, Yazd, Iran, with the code of ethics IR.SSU.MEDICINE.REC.1394.287.

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