

Leishmanicidal effects of resveratrol and its derivatives: a systematic review and meta-Analysis

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Research article

Keywords: Resveratrol, Stilbenes derivatives, Leishmania, Leishmaniasis, Meta-analysis

Posted Date: May 5th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-25274/v1>

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Abstract

Background

Leishmaniasis is one of the most important health problems worldwide. The evidence has suggested that resveratrol and its derivatives have anti-leishmanial effects; however, the results are inconsistent and inconclusive. The aim of this study was to assess the effect of resveratrol and its derivatives on the *Leishmania* viability through a systematic review and meta-analysis of available relevant studies.

Methods

The electronic databases PubMed, ScienceDirect, Embase, Web of Science and Scopus were queried between October 2000 and April 2020 using a comprehensive search strategy. The eligible articles selected and data extraction conducted by two reviewers. Mean differences of IC₅₀ (concentration leading to reduction of 50% of *Leishmania*) for each outcome was calculated using random-effects models. Sensitivity analyses and prespecified subgroup were conducted to evaluate potential heterogeneity and the stability of the pooled results. Publication bias was evaluated using the Egger's and Begg's tests. We also followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines for this review.

Results

Ten studies were included in the meta-analysis. We observed that RSV and its derivatives had significant reducing effects on *Leishmania* viability in promastigote [24.02 µg/ml; (95% CI 17.1, 30.8); $P < 0.05$; $I^2 = 99.8\%$; $P_{\text{heterogeneity}} = 0.00$] and amastigote [18.3 µg/ml; (95% CI 13.5, 23.2); $P < 0.05$; $I^2 = 99.6\%$; $P_{\text{heterogeneity}} = 0.00$] stages of *Leishmania*. A significant publication bias was observed in the meta-analysis. Sensitivity analyses showed a similar effect size while reducing the heterogeneity. Subgroup analysis indicated that the pooled effects of leishmanicidal of resveratrol and its derivatives were affected by type of stilbenes and *Leishmania* species.

Conclusions

Our findings clearly suggest that the strategies for the treatment of leishmaniasis should be focused on natural products such as RSV and its derivatives. Further study is needed to identify the mechanisms mediating this protective effects of RSV and its derivatives in leishmaniasis.

Background

Leishmaniasis is a global health problem worldwide which refers to a broad range of disease including cutaneous lesion, muco-cutaneous and visceral forms. This disease is endemic in 98 countries with approximately 12 million infected cases and 350 million people in regions with catching risk of leishmaniasis [1]. The annually estimated incidence of cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) is 1- 1.5 million and 500,000 cases, respectively [2]. CL caused by *L. major*, *L. amazonensis*, *L. panamensis* and *L. tropica* species and *L. donovani*, *L. chagasi* and *L. infantum* are associated with visceral leishmaniasis (VL). *Leishmania* parasite has digenetic life cycle which includes the extracellular promastigote in sand-fly and the intracellular amastigote into the mammalian hosts. Despite various studies such as genomic [3], proteomics [4, 5], metabolomics [6] and protein network [7] analysis to identify proteins as new drug and vaccine targets for leishmaniasis treatment, no vaccine is still available for the disease. Currently, chemotherapy using pentavalent antimonials such as sodium stibogluconate (pentostam) and meglumine antimoniate (glucantime) is main and first-line treatment

method for leishmaniasis [8]. The functional mechanism of these drugs is not fully clear. However, some studies have suggested that these drugs led to reduction of ATP production through inhibition of glycolytic and oxidative activity of fatty acids [9]. Unfortunately, because of high toxicity, high cost and side effects of these drugs, and also, appearance of drug-resistant strains, the use of alternative therapies is essential [10]. From 2002, miltefosine has been used for visceral leishmaniasis in India but efficacy of this drug is also low against cutaneous leishmaniasis [11]. According to the limitation of chemotherapy and lack of effective vaccine against leishmaniasis, furthermore the search for new drugs with better effectiveness and without serious side effect is necessary [7]. The use of herbal medicines has recently gained popularity in the world, and medical capacity of these natural compounds has been repeatedly demonstrated in various *in vitro* and *in vivo* systems [12]. In several studies have been shown that resveratrol (3, 5, 4- trihydroxy-trans-stilbene) contain antibacterial [13], antiviral [14], and antiparasitic [15] properties. Resveratrol is a polyphenol micronutrients compound produced by different plant species including pines, berries, and peanuts which mainly found in the skin of grapes and red wine [16]. Resveratrol represents *Cis* and *Trans* isomeric forms in nature that biological activity of it associated with *Tran's* form [17]. Despite several studies about the potential activity of resveratrol against both promastigotes and amastigotes of *Leishmania* parasites [18, 19], associated studies with anti-leishmanial activity of resveratrol still is in its infancy. Moreover, resveratrol or analogues could also be a novel potential drug in leishmaniasis management and need to be further investigated in future. Various surveys have been carried out to assess anti-leishmanial activity of resveratrol and its derivatives in the worldwide, however, considering the fact that these studies are individuals. One way of achieving a conclusive result is the formulation of a meta-analysis study, which mathematically combines and analyses the results of different studies to achieve a more reliable outcome [20]. However, in spite of previously researches about leishmanicidal effects of resveratrol and other stilbene derivatives, there has been no comprehensive review of published data. In the present study, we conducted the first systematic review and meta-analysis for evaluating the anti-leishmanial activity of resveratrol and its derivatives. The meta-analysis was conducted in accordance with the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [21].

Methods

Search protocol

We performed an exclusive search from October 2000 to April 2020 through Medline, Embase, Scopus, Science Direct, Web of Science, EMBASE, and Cochrane Library. All of eligible studies related to the leishmanicidal effects of resveratrol and its derivatives were selected. Furthermore, Gray literature and reference lists were reviewed to identify relevant studies. We searched databases using Mesh terms and keywords ("*Leishmania*" OR "leishmaniasis" OR "*Leishmania* species") OR ("*L. major*" OR "*L. tropica*" OR "*L. infantum*" OR "*L. amazonensis*" OR "*L. braziliensis*" OR "*L. donovani*" OR "*L. aethiopica*") AND ("Resveratrol" OR "resveratrol derivatives" OR "resveratrol analogs" OR "trans-resveratrol" OR "3, 5, 4'-trihydroxystilbene"). Furthermore, Gray literature, Conference abstracts and reference lists were manually searched to identify potentially relevant studies. Searches were limited to the English language. Two reviewers (NA. D and MK) evaluated each article separately, any disagreement between extracted data were resolved by agreement and discussion with a third party (NA). Ethical approval and informed consent will not be applied for because of the relevant data we extracted which does not involve any individual privacy.

Selection Criteria Of Studies

Articles were selected carefully if they met the following criteria: report outcome (the effect of resveratrol and resveratrol analogues on promastigote and amastigote stages of *Leishmania*, English language, published studies in a number of internationally indexed journals and having sufficient information and repetitive articles, studies without limitations in time and the form of resveratrol usage and also studies that reported the IC50 (The compound concentration causing 50%

reduction in parasite viability) of resveratrol and its analogues and control's IC50. The lack of adequate details of study methodology were excluded.

Quality Assessment

A systematic assessment of bias in the included studies was performed independently by two expert authors using Newcastle-Ottawa Quality Assessment Scale (NOS) [22]. In cases of disagreement, a third author would examine such articles. Authors discussed the results comprehensively until they agreed on the accuracy and usefulness of data. The items used for the assessment of each study were composed of main three section coupled with questions within each section as follows: 1) selection, 2) comparability and 3) exposure. NOS score ≥ 5 were considered high quality.

Extracted Data Of Studies

We extracted data from the final included articles using specially-designed data extraction Form. The form piloted and tested against 3 articles in which all authors will extract data from. The form modified according to this pilot test. Data extraction form initially including information about: the first author, the year of publication, origin country, parasite species, type of stilbenes (resveratrol and its derivatives), dose of resveratrol and its derivatives (mg/ml), IC₅₀ value of resveratrol, IC50 values of resveratrol derivatives and exposure time, control's name, control's IC50 and quality assessment score. All extracted data summarized for promastigote and amastigote stages of *Leishmania* in Table 1 and Table 2, respectively.

Table 1

Baseline Characteristics of included studies the leishmanicidal effects of resveratrol and its derivatives on promastigotes stage of *Leishmania* species in this systematic review and meta-analysis

Athour's name (Ref)	Country	Parasite species	Type of stilbenes	Dose (mg/ml)	Exposure IC50 (mg/ml)	Exposure time(h)	Control IC50 (mg/ml)	Quality assessment
Kedzierski et al. (2007) [23]	Poland	<i>L. major</i>	RSV	10, 50, 100	45	48	-	4
		<i>L. major</i>	Hydroxylated analogs of RSV	5 10	40.1	48	-	4
Lucas et al. (2013) [19]	Germany	<i>L. major</i>	RSV	45	196.9	48	1	5
Fuchino et al. (2013) [15]	Japan	<i>L. major</i>	<i>Lonchocarpus nicou</i> stilbenes (compound1)	50 (mL)	5.5	48	4	6
		<i>L. major</i>	<i>Lonchocarpus nicou</i> stilbenes (compound4)	50 (mL)	3.9	48	4	6
Ferreire et al. (2014) [24]	Brazil	<i>L. amazonensis</i>	RSV	100	27	48	0.108	5
Passos et al. (2015) [25]	Brazil	<i>L. amazonensis</i>	Pterostilbene	-	18	48	0.1	6
		<i>L. amazonensis</i>	Piceatannol	-	65	48	0.1	6
		<i>L. amazonensis</i>	Polydatin	-	95.5	48	0.1	6
		<i>L. amazonensis</i>	oxyresveratrol	-	65	48	0.1	6
Coimbra et al. (2016) [26]	Brazil	<i>L. amazonensis</i>	RSV analoges	1.56-25	3.81	24	8.56	7
		<i>L. braziliensis</i>	RSV analoges	1.56-25	1.60	24	11.44	7
		<i>L. major</i>	RSV analoges	1.56-25	7.84	24	8.15	7
Castelli et al. (2016) [18]	Italy	<i>L. infantum</i>	trans stilbenes derivatives	4	2.1	48	2.1	6

RSV: Resveratrol

Athour's name (Ref)	Country	Parasite species	Type of stilbenes	Dose (mg/ml)	Exposure IC50 (mg/ml)	Exposure time(h)	Control IC50 (mg/ml)	Quality assessment
(2018) [27]	Italy	<i>L. major</i>	trans stilbenes (ST18)	15	14.2	48	14.9	5
		<i>L. aethiopica</i>	trans stilbenes (ST18)	3	2.9	48	2.9	5
		<i>L. donovani</i>	trans stilbenes (ST18)	3	3.2	48	> 50	5
		<i>L. tropica</i>	trans stilbenes (ST18)	3	2.5	48	19.5	5
		<i>L. amazonensis</i>	trans stilbenes (ST18)	15	14.4	48	15.4	5
		<i>L. braziliensis</i>	trans stilbenes (ST18)	6	4.3	48	8.2	5
(2019) [28]	Brazil	<i>L. amazonensis</i>	RSV analoges (AR27)	0.4–30	2.6	72	0.11	6
		<i>L. braziliensis</i>	RSV analoges (AR27)	0.4–30	0.7	72	0.12	6
		<i>L. infantum</i>	RSV analoges (AR26)	0.4–30	3	72	0.05	6
RSV: Resveratrol								

Table 2

Baseline Characteristics of included studies the leishmanicidal effects of resveratrol and its derivatives on amastigote stage of *Leishmania* species in this systematic review and meta-analysis.

Athour's name (Ref)	Country	Parasite species	Type of stilbenes	Dose (mg/ml)	Exposure IC50 (mg/ml)	Exposure time(h)	Control IC50 (mg/ml)	Quality assessment
Kedzierski et al. (2007)	Poland	<i>L. major</i>	RSV	40 45	20	48	-	4
Lucas et al. (2013)	Germany	<i>L. major</i>	RSV	9.9	43.6	48	1	5
Tolomeo et al. (2013) [29]	Italy	<i>L. infantum</i>	TTAS	-	4.3	48	7	4
Ferreire et al. (2014)	Brazil	<i>L. amazonensis</i>	RSV	200	42	24	0.0088	5
Passos et al. (2015)	Brazil	<i>L. amazonensis</i>	Pterostilbene	-	33.2	24	8.8	6
		<i>L. amazonensis</i>	Piceatannol	-	45	24	8.8	6
		<i>L. amazonensis</i>	Polydatin	-	29	24	8.8	6
		<i>L. amazonensis</i>	oxyresveratrol	-	30.5	24	8.8	6
Coimbra et al. (2016)	Brazil	<i>L. amazonensis</i>	RSV analoges	1.56-25	5.73	24	2.2	7
Castelli et al. (2016)	Brazil	<i>L. infantum</i>	trans stilbenes derivatives	1	0.81	48	2.1	6
Bruno et al. (2018)	Italy	<i>L. major</i>	trans stilbenes (ST18)	1.0-24	16.3	48	10.4	5
		<i>L. aethiopic</i>	trans stilbenes (ST18)	1.0-24	3	48	3.2	5
		<i>L. donovani</i>	trans stilbenes (ST18)	1.0-24	5.8	48	24.8	5
		<i>L. tropica</i>	trans stilbenes (ST18)	1.0-24	12.5	48	16.8	5

Author's name (Ref)	Country	Parasite species	Type of stilbenes	Dose (mg/ml)	Exposure IC50 (mg/ml)	Exposure time(h)	Control IC50 (mg/ml)	Quality assessment
		<i>L. amazonensis</i>	trans stilbenes (ST18)	1.0–24	13.4	48	12.8	5
		<i>L. braziliensis</i>	trans stilbenes (ST18)	1.0–24	2.6	48	4.2	5
Antinarelli et al. (2019)	Italy	<i>L. braziliensis</i>	RSV analoges (AR26)	3.1–50	15.9	72	0.069	6

Statistical analysis

We evaluated the mean and 95% confidence intervals of the half-maximal inhibitory concentration (IC_{50}) values by pooling the results from all the selected articles. Considering the existing heterogeneity among studies, we performed this meta-analysis using the random-effects model. In this meta-analysis we used from both Q-Cochran test ($p < 0.1$ indicate heterogeneity) and I^2 method ($I^2 < 50\%$ no heterogeneity and $I^2 > 50\%$ indicate heterogeneity) for detect heterogeneity. Publication bias was evaluated using Funnel plot and Begg and Egger tests. The trim-and-fill analysis was used to adjust any significant publication bias detected. To establish the possible sources of heterogeneity between the studies on meta-analysis outcome, we performed subgroup analyses based on *Leishmania* species. We also conducted sensitivity analyses to evaluate the influence of individual articles on the pooled results. Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ) [30].

Results

The results of literature search

The search identified 97 records. These included 8 duplicate articles, 5 reviews and 1 editorial, which were removed, leaving 83 unique articles to be screened by title and abstract. Out of the 83 articles screened, 53 were excluded as they did not meet the eligibility criteria: 22 articles were performed on other parasites, 31 articles were not measured the leishmanicidal impact of resveratrol and its derivatives. The full text of 30 articles were then evaluated out of which 21 articles were excluded for the following reasons: i) incomplete reported data, and ii) brief report and review article. Finally, 9 studies met the inclusion criteria for meta-analysis (Fig. 1).

Characteristics Of Eligible Studies

We identified 9 studies that reported the IC_{50} of leishmanicidal effects of RSV and other stilbenes along with its derivatives as an outcome. Tables 1 and 2 show the included studies and their characteristics in this meta-analysis on the promastigote and amastigote stages of *Leishmania*, respectively. Most of studies on the promastigotes have been performed on *L. major* and *L. amazonensis*, respectively. Among them, Coimbra et al. three species (*L. amazonensis*, *L. braziliensis* and *L. major*), and Bruno et al. five species (*L. major*, *L. tropica*, *L. donovani*, *L. amazonensis* and *L. aethiopica*) have studied, while other researcher one species. The locations where the studies were conducted on the promastigotes were as follows: Brazil (n = 4), Italy (n = 2), Poland (n = 1), Germany (n = 1) and Japan (n = 1). Among 9 included articles in case of promastigotes, 5 studies reported both resveratrol and its analogs including 2 studies only RSV and 3 studies only RSV analogs. It should be noted

that one of the studies reported both of RSV and analogs results. The remaining studies included other stilbenes. Studies on the amastigotes have included *L. major* (n = 3), *L. infantum* (n = 2) and *L. amazonensis* (n = 4). The Poland (n = 1), Germany (n = 1), Italy (n = 3) and Brazil (n = 4) were the countries that conducted the studies. Of included articles in field of amastigotes, 3 studies reported only RSV and 6 studies reported RSV analogs and other stilbenes derivatives results. Most of included studies in this meta-analysis were used amphotericin B, pentostam and miltefosine as positive control of *Leishmania*.

The quality assessment of studies included in our meta-analysis is summarized in Table 1 and Table 2. All the included studies were confirmed by a low to high risk in each three parts of NOS checklist. Taken together, the quality of included studies was low to high.

Quantitative Data Synthesis

Leishmanicidal effects of resveratrol and its derivatives

Figure 2 presents the results of the pooled mean of IC₅₀ of leishmanicidal effects of resveratrol and its derivatives in both promastigote and amastigote stages of *Leishmania*. Based on random-effects model the pooled mean of IC₅₀ were observed for promastigote stage [24.02 µg/ml (95% CI 17.1, 30.8) p < 0.05] and amastigote stage [18.3 µg/ml (95% CI 13.5, 23.2) p < 0.05] following treatment of resveratrol and resveratrol derivatives. These results showed that resveratrol and its derivatives significantly reduced *Leishmania* viability in both promastigote and amastigote stages of *Leishmania*. There was a statistically significant heterogeneity between studies [(I² = 99.8%, p < 0.05)].

Publication Bias

The Begg's rank correlation tests (Kendall's Tau with continuity correction = 0.52, Z = 3.5, two-tailed p-value < 0.001) and the Egger's linear regression tests (intercept = 29.9, standard error = 9.4; 95% CI = 10.2, 49.5, t = 3.1, df = 22, two-tailed p = 0.004) were statistically significant. Thus, the funnel plot of the study precision (inverse standard error) by effect size (mean IC₅₀) was asymmetric and indicated potential publication bias in reporting the leishmanicidal effects of resveratrol and its derivatives on promastigote and amastigote stages of *Leishmania*. The observed publication bias was imputed using trim-and-fill correction. Trim-and-fill correction imputed 15 potentially missing studies resulted in a corrected effect size of (5.5; 95% CI: -2.8, 13.8) and (2.7; 95% CI: -2.3, 7.8) for promastigote and amastigote stages of *Leishmania* respectively. According to "fail safe N" method, 60969 and 14887 theoretically missing studies were required to bring p-value to > 0.05 in both promastigote and amastigote stages, respectively (Fig. 3).

Sensitivity Analysis

We performed sensitivity analysis, when any study was excluded from this meta-analysis. In the sensitivity analysis, we have found that the outcome (mean of IC₅₀) was not significantly changed by removing one study by means of "leave-one-out method" from the analysis compared to overall effect size for the leishmanicidal effects of resveratrol and other stilbene derivatives in promastigote [22.1 µg/ml (95% CI 15.3, 28.9) p < 0.05] and amastigote [18.2 µg/ml (95% CI 13.3, 23.2) p < 0.05] (Fig. 4). Therefore, this pooled analysis outcome could be regarded with a higher degree of certainty.

Subgroup Analysis

Subgroup analysis was performed according to *Leishmania* species (*L. major*, *L. amazonensis*, *L. braziliensis*, *L. donovani* and *L. infantum*) and type of stilbenes (resveratrol and its derivatives and stilbenes derivative). In the subgroup analysis,

significantly, increased levels of the leishmanicidal effects of resveratrol and its derivatives were observed in *L. major* [40.08 µg/ml; 95% CI (24.1, 56); p < 0.05], *L. amazonensis* [36.4 µg/ml; 95% CI (13.6, 59.1); p = 0.002] and *L. infantum* [2.3 µg/ml; 95% CI (1.9, 2.8); p < 0.05] of promastigote stage, while, it had no significant leishmanicidal effect in *L. braziliensis* [2.1 µg/ml; 95% CI (-0.31, 4.7); p = 0.08]. Furthermore, in amastigote stage of *Leishmania*, the leishmanicidal effects of resveratrol and its derivatives were significantly revealed in *L. major* [26.2 µg/ml; 95% CI (12.8, 39.7); p < 0.05] and *L. amazonensis* [28.03 µg/ml; 95% CI (17.3, 38.7); p < 0.05], although, no significant increases in *L. infantum* [2.5 µg/ml; 95% CI (-0.91, 5.9); p = 0.15] and *L. braziliensis* [8.5 µg/ml; 95% CI (-4.3, 21.5); p = 0.19] was observed. On the other hand, the results of subgroup analyses showed that the leishmanicidal effects were significantly increased in subgroups of RSV and its derivatives and stilbene derivatives in both promastigote and amastigote stages of *Leishmania* (Table 3). Taken together, subgroup of resveratrol and its derivatives in both stages of *Leishmania* significantly reduced *Leishmania* viability.

Table 3

Evaluation of the leishmanicidal effects of resveratrol and its derivatives on promastigote and amastigote stages of *Leishmania* using subgroup analysis.

Subgroup	Number of comparisons	Mean (µg/ml) (95% CI)	Z-value	p	Test of Heterogeneity	
					I ² (%)	P
Promastigote						
Overall	9	24.02 (17.1, 30.8)	3.1	< 0.05	99.8	< 0.05
<i>Leishmania</i> species						
<i>L. major</i>	7	39.7 (24.04, 55.5)	4.9	< 0.05	99.7	< 0.05
<i>L. amazonensis</i>	6	32.8 (11.61, 54.01)	3.03	0.002	99.9	0.05
<i>L. infantum</i>	2	2.4 (1.5, 3.2)	5.6	< 0.05	44.4	< 0.05
<i>L. braziliensis</i>	3	2.1 (-0.31, 4.7)	1.7	0.08	93.06	< 0.05
Type of stilbenes						
RSV & its derivatives	6	39.3 (24.6, 54.1)	5.2	< 0.05	99.9	< 0.05
Stilbenes derivatives	10	3.8 (2.9, 4.7)	8.5	< 0.05	87.06	< 0.05
Amastigote						
Overall	9	18.3 (13.5, 23.2)	4.5	< 0.05	99.6	< 0.05
<i>Leishmania</i> species						
<i>L. major</i>	3	26.2 (12.8, 39.7)	3.8	< 0.05	93.3	< 0.05
<i>L. amazonensis</i>	7	28.03 (17.3, 38.7)	5.1	< 0.05	99.3	< 0.05
<i>L. infantum</i>	2	2.5 (-0.91, 5.9)	1.4	0.15	96.9	< 0.05
<i>L. braziliensis</i>	2	8.5 (-4.3, 21.5)	1.2	0.19	89.9	0.002
Type of stilbenes						
RSV & its derivatives	6	29.09 (20.2, 37.9)	6.4	< 0.05	99.1	< 0.05
Stilbenes derivatives	8	5.1 (3.3, 6.8)	5.6	< 0.05	96.6	< 0.05

Discussion

Leishmaniasis is one of the health problems worldwide, for reducing this parasitic disease, many strategies are considered including polyphenols such as resveratrol. Previous published studies have reported the conflicting results regarding the leishmanicidal effects of RSV and other stilbenes in promastigote and amastigote stages of *Leishmania*. In total, nine eligible studies were included for promastigote and amastigote. In the current investigation, a combination of relevant studies via a systematic review and meta-analysis demonstrates that *Leishmania* viability significantly decreased after treatment with RSV and other stilbenes, in both promastigote and amastigote stages of *Leishmania*. Interestingly, we observed significant leishmanicidal effects of stilbenes types (RSV and other stilbenes) not only on *Leishmania* species but also in promastigote and amastigote stages of each parasite species.

In the present meta-analysis, the funnel plot, Begg rank correlation and Egger tests showed a potential publication bias among all studies included in this meta-analysis. Moreover, there is a significant heterogeneity in this meta-analysis. Therefore, in order to explore the obvious heterogeneity, subgroup analyses based on *Leishmania* species (*L. major*, *L. amazonensis*, *L. infantum* and *L. braziliensis*) and type of stilbenes (RSV and other stilbenes) were performed. Initially, we analyzed subgroups of *Leishmania* species and type of stilbenes on each stage of *Leishmania* separately. The results showed a significant increase in the leishmanicidal effects of RSV and other stilbenes in species of *L. major*, *L. amazonensis* and *L. infantum* of promastigote stage of *Leishmania*, while, the leishmanicidal effects of RSV and other stilbenes on amastigote stage of *Leishmania* in species of *L. major* and *L. amazonensis* were significant. In addition, subgroups of RSV and other stilbene derivatives in both of stages of *Leishmania* significantly reduced the *Leishmania* viability. Subsequently, these findings confirm the leishmanicidal effects on promastigote and amastigote stages of *Leishmania* following treatment with RSV and other stilbene derivatives.

Resveratrol is a natural polyphenol with potential health benefits. There is increasing evidence showing the beneficial effects of RSV on alleviating the pathological status of metabolic diseases [16]. Apart from that, resveratrol is effective for the treatment of leishmaniasis. As a result, our data in this meta-analysis demonstrated that *Leishmania* viability reduced after resveratrol treatment, which is in accordance with some of studies treated with resveratrol [23, 24]. Thus, the findings of this meta-analysis collectively implicate RSV and its derivatives as a potential agent in the diminishing of *Leishmania* viability.

Promastigotes are extracellular forms and amastigotes are intracellular forms of *Leishmania* parasites that resveratrol and its analogs effects on these two stages of *Leishmania* evaluated in several study. *Leishmania* have a dimorphic life cycle consisting of an extracellular flagellated promastigote stage within the midgut of a sandfly vector, and a morphological distinct intracellular amastigote stage within macrophages of a mammalian host [31]. Several studies have demonstrated that resveratrol and its derivatives have an anti-leishmanial activity [29, 32]. Ferreira *et al.* (2014) reported that resveratrol have an anti-leishmanial activity against *L. amazonensis* in both promastigote and amastigote stages [24]. The study of Coimbra *et al.* (2016) with evaluation of leishmanicidal effects of resveratrol analogs showed that these compounds have different effects against *Leishmania* species [26]. To that end, Kedzierski *et al.* (2007) reported the effects of resveratrol and its hydroxylated analogues against *L. major*. Their results demonstrated the leishmanicidal effects of resveratrol on promastigotes and intracellular amastigotes stages while, its hydroxylated analogues only showed anti-leishmanial activity against promastigotes [23]. Moreover, Study on anti-promastigote activity of trans-stilbenes and terphenyl compounds have shown that one of trans-stilbene derivatives have anti-promastigote effect similar to pentostam as an important drug against leishmaniasis [17]. Tolomeo *et al.* revealed the anti-promastigote and anti-amastigote activities of TTAS (Trans-3, 4, 5-trimethoxy-3'-amino-stilbene) as one of the stilbene derivatives [29]. Likewise, in a study the effect of four resveratrol analogs including pterostilbene, piceatannol, polydatin and oxyresveratrol evaluated against *L. amazonensis*, and its results showed that among four RSV analogues the piceatannol analog could be potential compound in further studies for leishmaniasis treatment [25]. In this regard, our data support the leishmanicidal effects of RSV and other derivatives of stilbenes on promastigote and amastigote stages of *Leishmania*.

There are several possible mechanisms for the leishmanicidal effects of RSV and other stilbene derivatives on the promastigote and amastigote stages of *Leishmania* parasite. Of these, apoptosis mediated by phospholipids could be

considered one of the mechanisms by which the *Leishmania* death process is initiated. Phospholipids are major types of lipids in membrane of eukaryotic cells. Among phospholipids, Phosphatidylserine (PS) has low abundance in most biological membranes and in normal condition it is present only in the inner plasma membrane of eukaryotic cells [29]. PS also plays a role in the infectivity of *Leishmania* [33]. A central point in the host-parasite interaction involves the adhesion to and invasion of host macrophages, by the metacyclic promastigotes and amastigotes. Both of the promastigotes and amastigotes use receptor-dependent phagocytosis for invasion. Furthermore, according to studies, exposure of PS on the cell surface of the parasite mimics apoptosis and encourages the macrophages in the host organism to phagocytose the parasite [34]. Investigations on amastigotes of *Leishmania amazonensis* showed that the signaling via exposed PS is a critical mechanism for *Leishmania* establishment in the mammalian host. The surface PS of amastigotes lead to inhibition of macrophage inflammatory activity. This lipid using interacting with macrophages, contributes in parasite internalization and induces an anti-inflammatory response by inhibition of macrophage NO activity, increasing IL-10 message and TGF β -1 secretion [35]. In addition, treatment of infected mice with PS-targeting monoclonal antibody ameliorated parasite loads and lesion development and also enhanced dendritic cell (DC) activation and antigen presentation *in vitro*. Therefore, the recognition of PS exposed on the surface of amastigotes plays a role in down-modulating DC functions, in a matter similar to that of apoptotic cell clearance [36]. Also, it can be concluded that PS can modulate interactions between *Leishmania* organisms and host cells and may be key factor for the result of the clinical course of leishmaniasis.

In a study that conducted on the *L. amazonensis*, the promastigotes were treated or not with piceatannol and incidental death measured using the expression of annexin-V. The results showed that the piceatannol significantly increased the expression level of annexin-V compared to untreated control [25]. Another study on *L. infantum* promastigotes was shown that treatment of parasites with 10 μ g/ml compound 3 (resveratrol derivate) for 48 h induced PS externalization suggesting that apoptosis activation is the cause of parasites death, while this compound has not shown apoptotic effects on macrophage cells [18]. Ferreira *et al.* (2014) reported a 2.9-fold increase in the percentage of annexin V-positive *L. infantum* promastigotes upon resveratrol treatment which suggests leishmanicidal activity of resveratrol [24]. TTAS (Trans-3, 4, 5-trimethoxy-3'-amino-stilbene) as a new stilbene derivate that induced PS externalization which sign apoptosis activation in *L. infantum*. TTAS showed an LD₅₀ on normal CFu-GM of 17.7 μ g/ml more than 6 times higher than that showed on the *L. infantum* strain [37]. Results of several studies in recent years indicate stilbenes and its analogs altered cell cycle of different species of *Leishmania* parasite with increase of parasites in sub G0/G1 phase of cell cycle [15]. Presence of cells in sub G0-G1 phase indicates the occurrence of apoptosis. Other investigation showed that TTAS induced the arrest of *Leishmania* prevalently in G2-M phase of the cell cycle and an increase of the sub-G1 apoptotic peak [29].

The second mechanism mediated by stilbenes is mitochondrial-induced apoptosis. *Leishmania* and mammals apoptosis share similar characteristics, such as internucleosomal DNA fragmentation, phosphatidylserine exposure on the external surface of the plasma membrane, and loss of mitochondrial transmembrane potential [38]. Since maintenance of mitochondrial transmembrane potential is essential for the survival of a single mitochondrion-parasite, studies of the effects of resveratrol on *Leishmania* spp., evaluated mitochondrial integrity of promastigotes testing the mitochondrial membrane potential. Previous research findings showed a reduction of mitochondrial membrane potential in parasites treated with resveratrol or it's derivate in comparison with untreated parasite populations [25].

Our meta-analysis has several advantages. First, this meta-analysis comprehensively summarizes the evidence data on an anti-leishmanial activity resveratrol and its derivatives. Second, the included studies were conducted in different countries that are including the high prevalence of leishmaniasis. Third, our search strategy was very detailed and spanned multiple databases.

This systematic review and meta-analysis have several limitations: First, due to the small number of included studies for stilbenes and its derivatives, we were not able to better evaluate the leishmanicidal effects of stilbenes and its derivatives. Second, we had a substantial heterogeneity between included studies which does not allow a reliable assessment, although we used random-effect models to combine the pooled mean of IC₅₀ among included studies. Third, the sample sources for

eligible studies were countries which may differ in the prevalence of *Leishmania*. Finally, given the limitations the findings should be interpreted with caution, because the reliability of this meta-analysis is limited by scarcity of large studies.

Conclusions

Available evidence from combined primary studies showed a significant reduction in *Leishmania* viability in both promastigote and amastigote stages. However, significant reducing effect in *Leishmania* viability support RSV and its derivatives as an adjunct to pharmacologic management of leishmaniasis.

Abbreviations

L:*Leishmania*; CL:Cutaneous leishmaniasis; VL:Visceral leishmaniasis; RSV:Resveratrol; PRISMA:Preferred reporting items for systematic reviews and meta-analysis statement; NOS:Newcastle-ottawa quality assessment; PS:Phosphatidylserine; DC:Dendritic cell.

Declarations

Ethics Approval and consent to participate

The ethical approval was not necessary for the reason that our study was a meta-analysis belonging Availability of data and materials

Consent for publication

Not applicable.

Availability of data and materials

Data of this study are included in the article and the primary data can be provided from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

All the authors drafted the manuscript. Nasrin Amiri-Dashatan, Mehdi Koushki and Marzieh Ashrafmansouri contributed to the improvement of the selection criteria, the risk of bias assessment strategy and data extraction criteria. Nasrin Amiri-Dashatan, Mehdi Koushki and Marzieh Ashrafmansouri started selecting publications and assessing the eligibility and quality. Nasrin Amiri-Dashatan, Marzieh Ashrafmansouri and Nayebali Ahmadi promoted the search strategy. Nasrin Amiri-Dashatan, Mehdi Koushki and Nayebali Ahmadi provided statistical expertise. All authors read, provided feedback and approved the final manuscript.

Acknowledgement

This project was supported by Proteomics Research Center, Shahid Beheshti University of Medical Science.

References

1. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*. 2012;7(5):e35671.
2. Ahmadi NA, Modiri M, Mamdohi S. First survey of cutaneous leishmaniasis in Borujerd county, western Islamic Republic of Iran. *East Mediterr health J*. 2013;19(10):847–53.
3. Amiri-Dashatan N, Koushki M, Ahmadi N. Comparison of gene expression of pyruvate kinase and trypanothione synthase in metacyclic promastigote forms of *Leishmania (L.) tropica* and *L. major* by real-time PCR. *Ann Parasitol*. 2020;66(1):13–8.
4. Amiri-Dashatan N, Rezaei-Tavirani M, Ahmadi N. A quantitative proteomic and bioinformatics analysis of proteins in metacyclogenesis of *Leishmania tropica*. *Acta Trop*. 2020;202:105227.
5. Ashrafmansouri M, Sadjjadi FS, Seyyedtabaei S, Haghighi A, Rezaei-Tavirani M, Ahmadi N. Comparative Two-dimensional Gel Electrophoresis Maps for Amastigote-like Proteomes of Iranian *Leishmania tropica* and *Leishmania major* Isolates. *Galen Med J*. 2019;8:1520.
6. Tabrizi F, Seyyed Tabaei S, Ahmadi N, Arefi Oskouie A. A nuclear magnetic resonance- based metabolomic study to identify metabolites differences between Iranian isolates of *Leishmania major* and *Leishmania tropica*. *Iran J Med Sci*. 2020; 46: (In Press).
7. Dashatan NA, Tavirani MR, Zali H, Koushki M, Ahmadi N. Prediction of *Leishmania Major* Key Proteins via Topological Analysis of Protein-Protein Interaction Network. *Galen Med J*. 2018;7:1129.
8. Walker J, Gongora R, Vasquez JJ, Drummelsmith J, Burchmore R, Roy G, et al. Discovery of factors linked to antimony resistance in *Leishmania panamensis* through differential proteome analysis. *Mol Biochem Parasitol*. 2012;183(2):166–76.
9. Lindoso JAL, Costa JML, Queiroz IT, Goto H. Review of the current treatments for leishmaniasis. *Res Rep Trop Med*. 2012;3:69–77.
10. Wiwanitkit V. Interest in paromomycin for the treatment of visceral leishmaniasis (kala-azar). *Ther Clin Risk Manag*. 2012;8:323–8.
11. Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. *Clin Infect Dis*. 2012;55(4):543–50.
12. Hamidi M, Ziaee M, Delashoub M, Marjani M, Karimitabar F, Khoramie A. The effects of essential oil of *Lavandula angustifolia* on sperm parameters quality and reproductive hormones in rats exposed to cadmium. *J Rep Pharm Sci*. 2015;4(2):121–8.
13. Zain W, Ahmat N, Norizan NH, Nazri N. The evaluation of antioxidant, antibacterial and structural identification activity of trimer resveratrol from Malaysia's Dipterocarpaceae. *Aust J Basic Appl Sci*. 2011;5(5):926–9.
14. Chen X, Qiao H, Liu T, Yang Z, Xu L, Xu Y, et al. Inhibition of herpes simplex virus infection by oligomeric stilbenoids through ROS generation. *Antivir Res*. 2012;95(1):30–6.
15. Fuchino H, Kiuchi F, Yamanaka A, Obu A, Wada H, Mori-Yasumoto K, et al. New leishmanicidal stilbenes from a Peruvian folk medicine, *Lonchocarpus nicou*. *Chem Pharm Bull*. 2013;61(9):979–82.
16. Koushki M, Amiri-Dashatan N, Ahmadi N, Abbaszadeh HA, Rezaei-Tavirani M. Resveratrol. A miraculous natural compound for diseases treatment. *Food Sci Nutr*. 2018;6(8):2473–90.
17. Wu Y, Liu F. Targeting mTOR: evaluating the therapeutic potential of resveratrol for cancer treatment. *Anti-cancer Agent Me*. 2013;13(7):1032–8.

18. Castelli G, Bruno F, Vitale F, Roberti M, Colomba C, Giacomini E, et al. In vitro antileishmanial activity of trans-stilbene and terphenyl compounds. *Exp Parasitol.* 206;166: 1–9.
19. Lucas IK, Kolodziej H. In vitro antileishmanial activity of resveratrol originates from its cytotoxic potential against host cells. *Planta Med.* 2013;79(1):79, 20 – 6.
20. Ahmadi Jouybari TA, Ghobadi KN, Lotfi B, Alavi Majd H, Ahmadi NA, Rostami-Nejad M, et al. Evaluating effect of albendazole on *Trichuris trichiura* infection: A systematic review article. *Iran J Parasitol.* 2016;11:441–7.
21. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med.* 2009;151(4):264–9.
22. Wells GA, Tugwell P, O'Connell D, Welch V, Peterson J, Shea B, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. 2015.
23. Kedzierski L, Curtis JM, Kaminska M, Jodynys-Liebert J, Murias M. In vitro antileishmanial activity of resveratrol and its hydroxylated analogues against *Leishmania major* promastigotes and amastigotes. *Parasitol Res.* 2007;102(1):102, 91–97.
24. Ferreira C, Soares DC, do Nascimento MTC, Pinto-da-Silva LH, Sarzedas CG, Tinoco LW, et al. Resveratrol is active against *Leishmania amazonensis*: in vitro effect of its association with amphotericin B. *Antimicrob Agents Chemother.* 2014;58(10):6197–208.
25. Passos CLA, Ferreira C, Soares DC, Saraiva EM. Leishmanicidal effect of synthetic trans-resveratrol analogs. *PLoS One.* 2015;10(10):1–16.
26. Coimbra ES, Santos JA, Lima LL, Machado PA, Campos DL, Pavan FR, et al. Synthesis, antitubercular and leishmanicidal evaluation of resveratrol analogues. *J Brazil Chem Soc.* 2016;27(12):2161–9.
27. Bruno F, Castelli G, Vitale F, Giacomini E, Roberti M, Colomba C, et al. Effects of trans-stilbene and terphenyl compounds on different strains of *Leishmania* and on cytokines production from infected macrophages. *Exp Parasitol.* 2018;184:31–8.
28. Antinarelli LMR, Meinel RS, Coelho EAF, da Silva AD, Coimbra ES. Resveratrol analogues present effective antileishmanial activity against promastigotes and amastigotes from distinct *Leishmania* species by multitarget action in the parasites. *J Pharm Pharmacol.* 2019;71(12):1854–63.
29. Tolomeo M, Roberti M, Scapozza L, Tarantelli C, Giacomini E, Titone L, et al. TTAS a new stilbene derivative that induces apoptosis in *Leishmania infantum*. *Exp Parasitol.* 2013;133(1):37–43.
30. Borenstein M, Hedges L, Higgins J, Rothstein H. *Comprehensive meta-analysis (Version 2.2. 064)*. Englewood: Biostat; 2011.
31. Ashrafmansouri M, Amiri-Dashatan N, Ahmadi N, et al. Quantitative Proteomic Analysis to Determine Differentially Expressed Proteins in Axenic Amastigotes of *Leishmania tropica* and *Leishmania major*. *IUBMB Life.* 2020 (Accepted).
32. Devaux PF. Static and dynamic lipid asymmetry in cell membranes. *Biochemistry.* 1991;30(5):1163–73.
33. Weingartner A, Kemmer G, Muller FD, Zampieri RA, Gonzaga dos Santos M, Schiller J, et al. *Leishmania* promastigotes lack phosphatidylserine but bind annexin V upon permeabilization or miltefosine treatment. *PLoS One.* 2012;7(8):e42070.
34. Wanderley JL, Pinto da Silva LH, Deolindo P, Soong L, Borges VM, Prates DB, et al. Cooperation between apoptotic and viable metacyclics enhances the pathogenesis of Leishmaniasis. *PLoS One.* 2009;4(5):e5733.
35. Freitas Balanco JM, Moreira MEC, Bonomo A, Bozza PT, Amarante-Mendes G, Pirmez C, et al. Apoptotic mimicry by an obligate intracellular parasite downregulates macrophage microbicidal activity. *Curr Biol.* 2001;11(23):1870–3.
36. Wanderley JLM, Thorpe PE, Barcinski MA, Soong L. Phosphatidylserine exposure on the surface of *Leishmania amazonensis* amastigotes modulates in vivo infection and dendritic cell function. *Parasite Immunol.* 2013;35(3–4):109–19.

37. Fadeel B, Xue D. The ins and outs of phospholipid asymmetry in the plasma membrane: roles in health and disease. *Crit Rev Biochem Mol.* 2009;44(5):264–77.
38. Ambit A, Fasel N, Coombs GH, Mottram JC. An essential role for the *Leishmania major* metacaspase in cell cycle progression. *Cell Death Differ.* 2008;15(1):113–22.

Figures

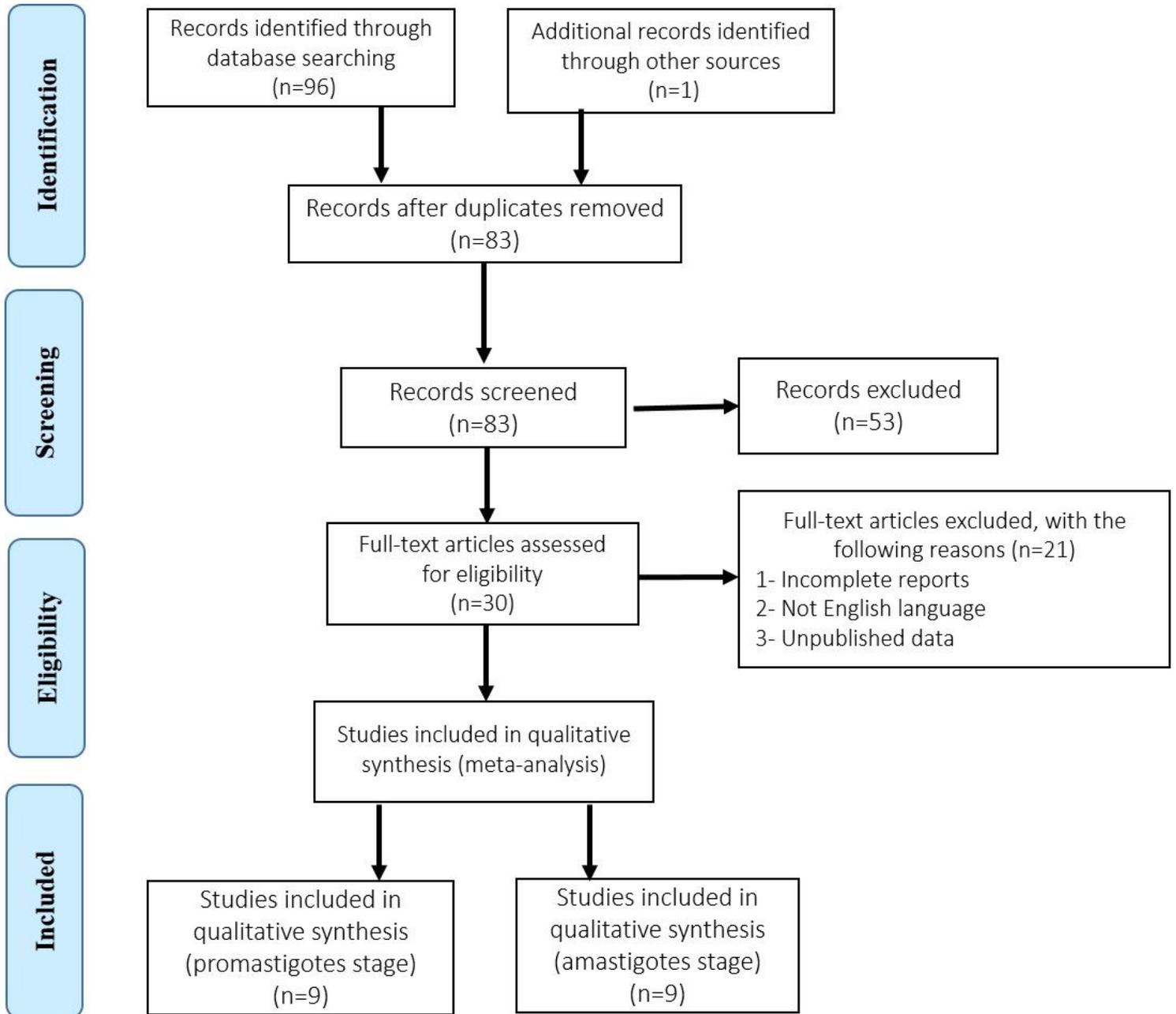
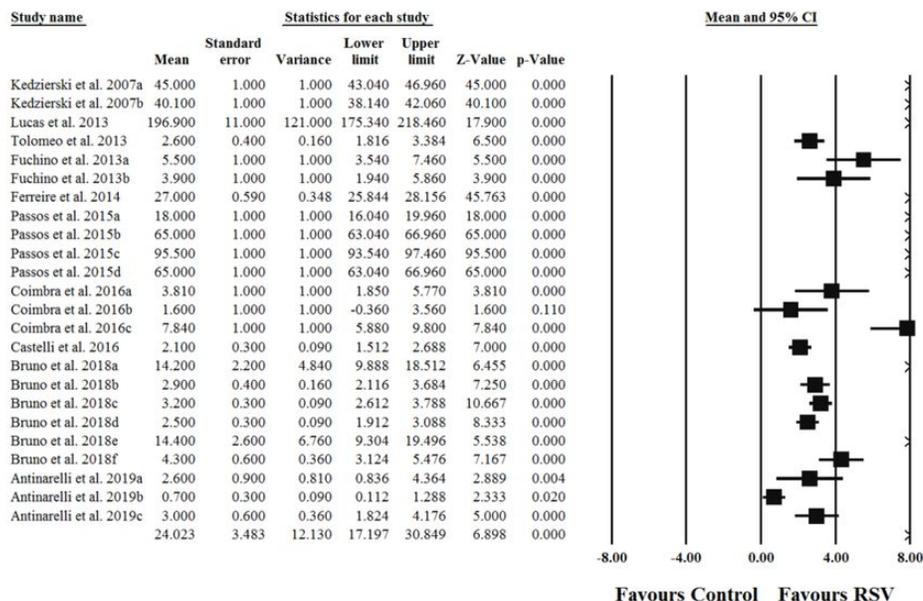


Figure 1

Flow chart of the number of studies detected and selected into the meta-analysis

a



b

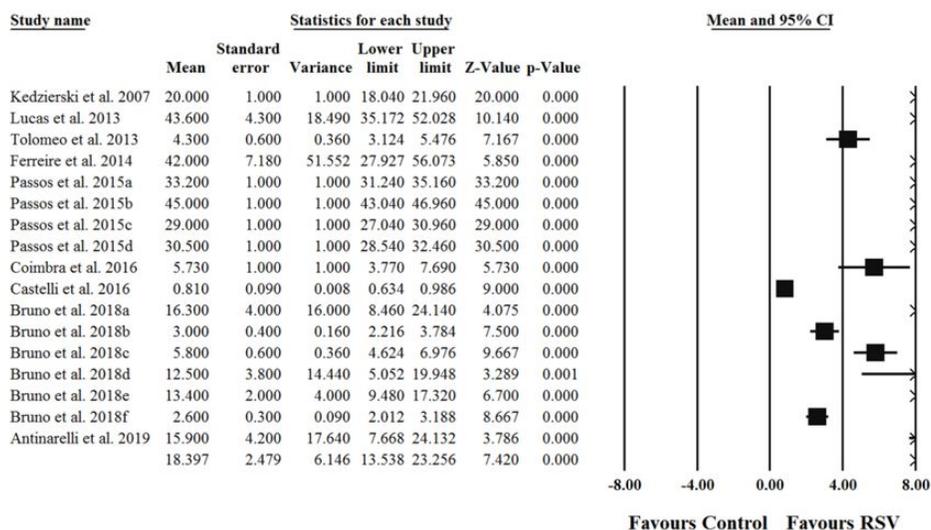


Figure 2

Forest plot evaluating mean of IC50 and 95% confidence intervals for the leishmanicidal effects of RSV and other stilbenes derivatives in A) promastigote and B) amastigote stages of leishmania. Meta-analysis was performed using a random-effects model with inverse variance weighting.

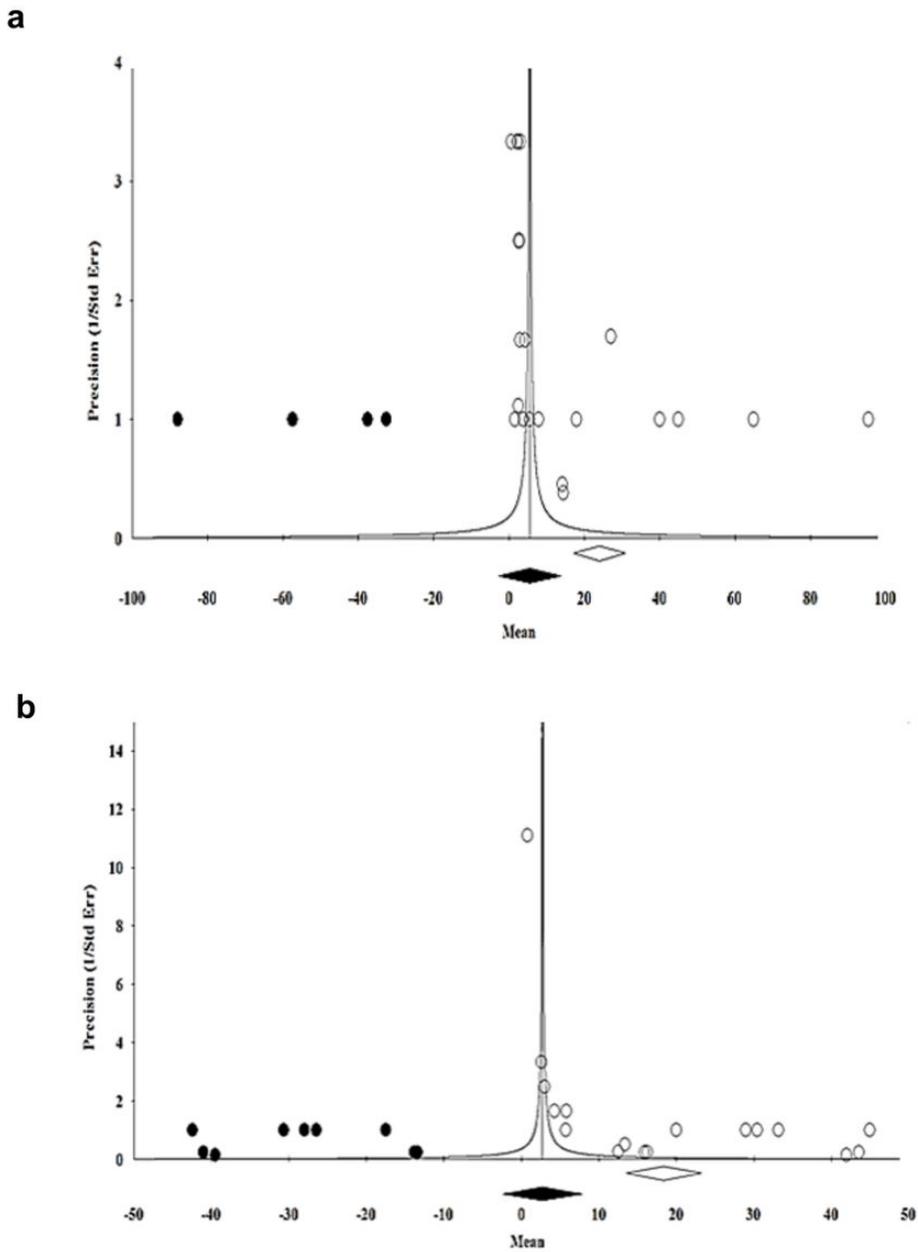
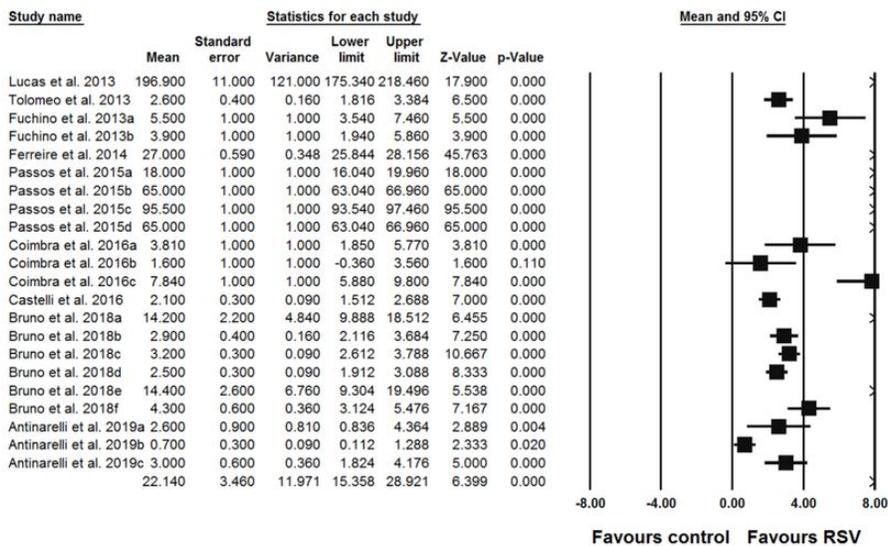


Figure 3

Random-effects Funnel plot detailing publication bias in the studies investigating the leishmanicidal effects of RSV and other stilbenes derivatives in A) promastigote and B) amastigote stages of leishmania after trimming and filling. Circles represent observed published studies; closed circles represent imputed unpublished studies.

a



b

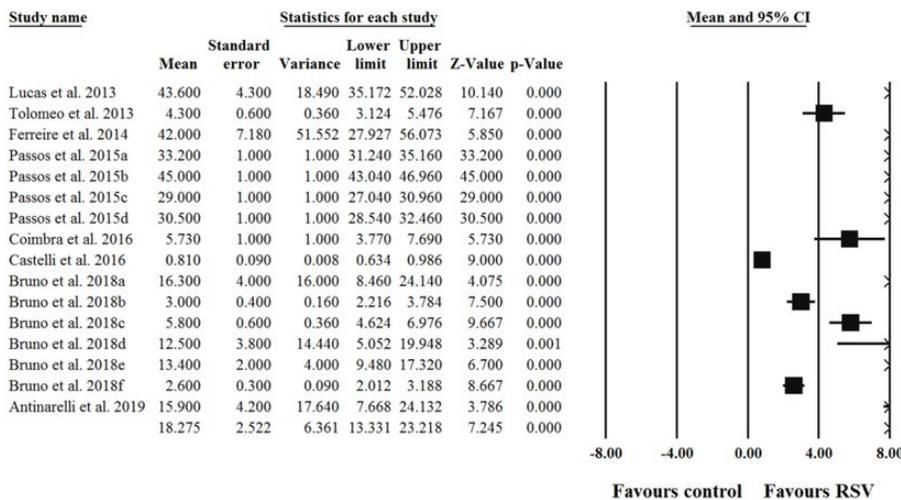


Figure 4

Leave-one-out sensitivity analysis of the leishmanicidal effects of RSV and other stilbenes derivatives in A) promastigote and B) amastigote stages of leishmania. Meta-analysis was performed using a random-effects model with inverse variance weighting.

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