Research Article

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Biological evaluation of CH_3OH and C_2H_5OH of *Berberis vulgaris* for *in vivo* antileishmanial potential against *Leishmania tropica* in murine models

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Abstract: Leishmaniasis is one of the global health issues and is still being handled with costly and sometimes unsuccessful compounds having serious side effects, highlighting the importance of seeking new potent antileishmanial compounds. Herbal medicines have been considered as the main source of prevention and treatment for a wide variety of diseases as well as other photogenetic diseases over the last few centuries. The current study was designed to investigate the *in vitro* and *in vivo* antileishmanial efficacy of ethanolic and methanolic extracts of *Berberis vulgaris* root collected from Kurez region of District Orakzai, Khyber Pakhtunkhwa, Pakistan. Stationary growth-phase metacyclic promastigotes form of *Leishmania tropica* was incubated *in vitro* in methanolic and ethanolic extracts using amphotericin B as a positive control. The antileishmanial activity of *B. vulgaris* extracts was measured after the incubation period using methoxynitrosulfophenyl-tetrazolium carboxanilide assay. For the *in vivo* study, BALB/c mice were infected with metacyclic *L. tropica* promastigotes, and lesions appeared after 28 days of inoculum. The results of study research showed that *B. vulgaris* extract had a powerful antileishmanial activity on promastigotes of *L. tropica* at different concentrations (5, 10, 25, 50, and 75 µg/mL). The inhibition concentration 50 values for ethanolic and methanolic extracts of *B. vulgaris* were determined to be 15.37 and 17.55 µg/mL, respectively. In the *in vivo* activity, it was observed that *B. vulgaris* ethanolic extract at the concentration of 1.5 mg/kg decreased the lesion diameter in BALB/c infected mice. The ethanolic extract topically and orally reduced the lesion size 0.51 ± 0.023 and 0.56 ± 0.008 mm, while methanolic extract both topically and orally decrease the lesion diameter of 0.63 ± 0.008 and 0.68 ± 0.009 mm relatively, in comparison with negative control (1.45 ± 0.016 mm). Hematological parameters of mice including blood red blood cells, hematocrit, and hemoglobin in mice groups (infected non-treated) were found to be decreased, while the mice group treated with extract demonstrated nearly similar results to non-infected group. It is concluded from the current study that antileishmanial activity of *B. vulgaris* from Pakistan against *L. tropica in* both *in vitro* and *in vivo* showed a promising antileishmanial activity. This study might be helpful in the control strategies of cutaneous leishmaniasis caused by *L. tropica*.

Keywords: *Berberis vulgaris*, *L. tropica*, Kurez region, BALB/c, AmpB

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1 Introduction

Leishmaniasis is a cluster of diseases induced by more than 20 different species of *leishmania* protozoan parasites that

cause various forms of human leishmaniasis, ranging from dermal ulcers to fatal visceral forms. Leishmaniasis is transmitted through the bites of infected female *Phlebotomus* or *Lutzomyia* sand flies. In 98 different countries and territories, it is endemic, impacting 12 million individuals and threatening more than 350 million people around the globe. Clinically, leishmaniasis exists in three major forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, and visceral leishmaniasis [1,2].

In Pakistan, around 21,000–35,000 cases, including both anthroponotic (ACL) and zoonotic forms of CL, are reported annually [3]. ACL, primarily associated with *Leishmania tropica*, occurs unpredictably and is prevalent in urban areas of Baluchistan, Azad Jammu Kashmir, Punjab, Khyber Pakhtunkhwa (KP), and the former Federally Administered Tribal Areas (FATA) [3,4]. Leishmaniasis is characterized by sporadic outbreaks attributed to *L. tropica* in KP, the northwestern province of Pakistan [5]. Transmission of CL occurs through blood-sucking sand flies [6], with Pakistan recording around 37 species and sub-species of these insects [7]. Sand flies of the genera *Phlebotomus* and *Lutzomyia* transmit Leishmania parasites in the old and new worlds, respectively, and are found in diverse habitats ranging from tropical rainforests to deserts [8].

Currently, drug therapy is the primary prevention method for leishmaniasis due to the absence of an effective vaccine [6,9]. Pentavalent antimony compounds, including meglumine antimony and sodium stibogluconate, despite their toxicity, high costs, and resistance issues, remain the first-line therapy for CL [10]. Ancient civilizations relied on medicinal plants to combat diseases such as cancer and infections. Today, in drug development, animal models play a crucial role in understanding disease mechanisms, assessing treatment safety, and confirming drug effectiveness. However, it is challenging to find an animal model that perfectly mimics human disease. Rodent models, like the mouse model for various *Leishmania* species, help researchers study CL and understand immune responses, although they do not fully replicate human pathology [11].

Given the challenges associated with existing antileishmanial drugs, researchers are exploring new compounds, both natural and synthetic, for their potential efficacy. One promising natural option is European barberry (*Berberis vulgaris*), known as "Zereshk" in Persian. European barberry contains bioactive compounds such as berbamine, palmatine, and berberine, traditionally used for various ailments including cardiovascular, gastrointestinal, respiratory, skin, renal, and infectious diseases. Previous literature showed that *Berberis* species demonstrated multiple biological activities [12–15]. Studies have shown that European barberry and its derivatives possess antifungal, antibacterial, and antiparasitic properties against certain pathogens. Some research indicates promising *in vitro* antileishmanial activity of European barberry, prompting further investigation into its *in vivo* potential. Therefore, this study aims to evaluate the *in vivo* antileishmanial and lesion control effects of European barberry against both promastigote and amastigote forms of *L. tropica*.

2 Materials and methods

2.1 Study area

Fresh roots of *B. vulgaris* were collected from the Kurez region (coordinates: 33°–33′ to 33°–54′ N and 70°–36′ to 71°–22′ E) in District Orakzai, KP, Pakistan (previously a part of the region called FATA). Orakzai District is characterized by its mountainous terrain and valleys, with elevations ranging from 6,000 to 7,000 feet [16] (Figure 1).

2.2 Collection of plant materials

The fresh root of *B. vulgaris* samples was identified through botanical keys [17]. The root was washed under the tap water to remove debris, and those specimens were numbered and kept in the laboratory of Molecular Parasitology and Virology Department of Zoology, Kohat University of Science and Technology (KUST), and dried at 30–40°C undershade for 10–15 days. The dried roots were size-reduced to coarse powder with the aid of electric grinder [18].

2.3 Preparation of extracts

The plant material was first air-dried and then ground into a fine powder. Approximately 50 grams of the powdered root of *B. vulgaris* was subjected to extraction using a Soxhlet apparatus with 500 mL of 95% ethanol and methanol as solvents, respectively. The extraction process continued for 6–8 h until the solvent in the Soxhlet apparatus became colorless, indicating complete extraction.

After the extraction, the crude extracts were filtered using a solvent filtration apparatus. The solvent was then removed from the filtrate by evaporating it under reduced pressure using a rotary vacuum evaporator at a temperature not exceeding 40°C [17,18]. This ensured the preservation of heat-sensitive phytochemicals. The percentage yield of the dried extracts is recorded in Table 1, with the ethanolic extract yielding approximately 14.38% and the methanolic extract yielding 10.22%, according to the given formula:

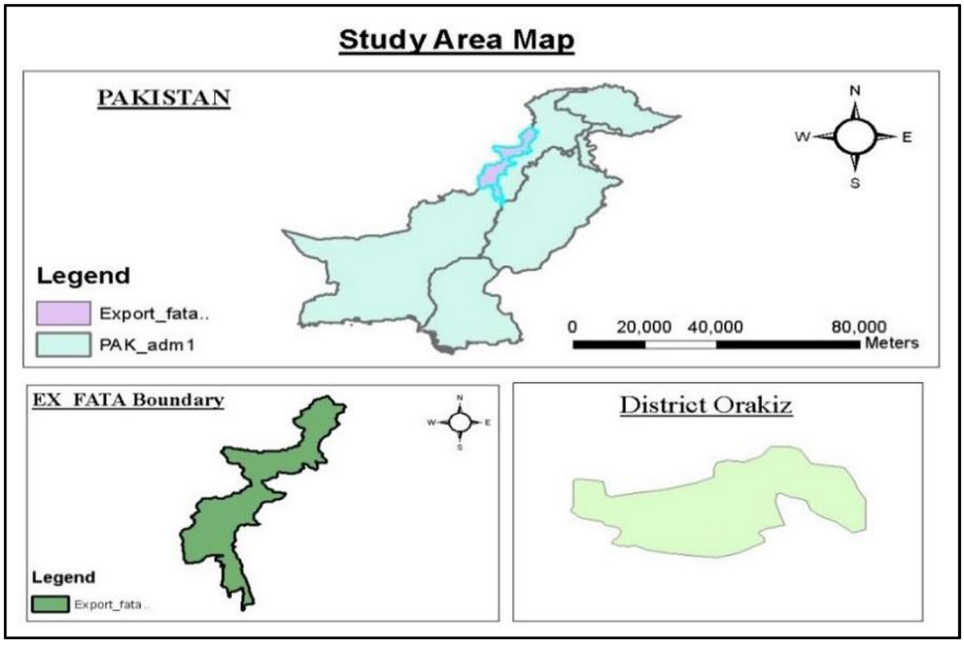

Figure 1: Map shows the area of Orakzai from where *B. vulgaris* root was collected.

$$\text{Average yield\%} = \frac{\text{Weight of the extract adter solvent removal}}{\text{Dry weight of plant sample}} \times 100$$

2.4 Parasitic culture

Preserved isolates of *L. tropica* KWH23, acquired from the Cell Culture Lab at the Institute of Zoological Sciences (formerly Department of Zoology), Peshawar University, were cultured in M199 growth medium supplemented with 10% heat-inactivated fetal calf serum (HI-FCS), 100 µg/mL penicillin, 100 µg/mL streptomycin, 50 µg/mL kanamycin, and 5 µg/mL hemin. Culturing was conducted in a bio-level two-crop laboratory under appropriate laboratory conditions. The culture was incubated in an anaerobic environment at 24 to 26°C, and growth was recorded daily for 10 days by counting using a Neubauer Hemocytometer (Sigma-Aldrich BR717810-1EA BLAUBRAND). During the logarithmic growth phase, there was a significant increase in promastigote count. It was at this stage that the promastigotes were harvested by centrifugation and used for *in vitro* assays at 1×10^5 cells/mL [19].

2.5 Stock solutions and dilutions of extracts

Each ethanolic and methanolic extract was diluted into five different concentrations (5, 10, 25, 50, and 75 µg/mL), prepared through serial dilutions in 0.5% dimethyl sulfoxide (DMSO) using the formula $C_1V_1 = C_2V_2$ [20].

2.6 *In vitro* activity of *B. vulgaris* against promastigote

For the assessment of the antileishmanial activity of *B. vulgaris* extracts, an *in vitro* assay was conducted using 96-well flat-bottom plates. Promastigotes were quantified in culture suspension using an enhanced Neubauer Hemocytometer prior to the application of the extracts. From a viable bulk culture

Table 1: Methanolic extract of *B. vulgaris* against *L. tropica* promastigote

Concentrations µg/mL	Inhibition 1 (%)	Inhibition 2 (%)	Inhibition 3 (%)	Mean value (%) SD	IC_{50} µg/mL
75	95.0	99.0	99.5	97.83 ± 2.01	17.55
50	89.2	93.6	96.0	92.93 ± 2.81	
25	39.2	43.0	44.6	42.26 ± 2.26	
10	29.0	28.5	30.8	29.43 ± 0.98	
5	16.4	18.0	19.5	17.96 ± 1.26	

of promastigotes (3×10^6 parasites/mL), 1×10^6 promastigotes per well in 200 µL of fresh Roswell park memorial institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (FBS) were seeded in each well of a 96-well dish. Approximately 20 mL of promastigote culture was needed for each 96-well plate. The five different concentrations of both ethanolic and methanolic extracts of *B. vulgaris* were applied. The negative control group was treated with 0.5% DMSO. Each concentration of the extracts was tested in triplicate. The 96-well plate was then incubated for 48 h at 26°C. Following the 48 h incubation period, the number of promastigotes in each well (both treated and control groups) was counted using a microscope and an Improved Neubauer Hemocytometer [17].

2.7 Methoxynitrosulfophenyl-tetrazolium carboxanilide (XTT) assay

Following the incubation period, a solution containing XTT (4 mg/mL) in phosphate buffered saline (PBS) (pH 7.0 at 37°C) with peroxymonosulfate (0.06 mg/mL) was applied (20 µL per well), and the plate was reincubated for an additional 4 h at 26°C. After the 4 h incubation, the percentage inhibition within each well (both treated and control) was assessed using an HT microplate reader, with a test wavelength of 450 nm and a reference filter of 650 nm [19]. The % age inhibition was calculated from these absorbance values using the following formula:

$$\%\text{Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100,$$

where the OD represents the optical density.

The 50% inhibitory concentrations were calculated using the nonlinear regression in GraphPad prism software.

2.8 Animal model

Male BALB/c mice aged 4 to 6 weeks, with an average weight of approximately 30 g, were procured from the Peshawar Veterinary Research Institute and transported to the Molecular Parasitology and Virology Laboratory at the Department of Zoology, KUST, Kohat. They were treated in accordance with applicable protocols for the ethical use of laboratory animals. Every effort was made to minimize the discomfort of the mice throughout the duration of the research. The mice were housed in ventilated plastic cages under controlled conditions of 23 ± 2°C temperature, 55 to 60% humidity, and a 12 h light–dark cycle (as shown in Figure 2). They were provided with ample food and purified water in the animal care facility managed by the laboratory [21].

2.9 Mice inoculation

The obtained promastigotes form of *L. tropica* were collected in sterile tubes until they were needed for infecting the mice. The promastigotes were counted using a hemocytometer under an inverted microscope. Once counted, the promastigotes were centrifuged at 2,000 rpm for 10 min at 4°C. After centrifugation, the supernatants were removed, leaving behind the pellet, which was then diluted in 10 mL of fresh RPMI-1640 medium containing 10% FBS. Subsequently, 10 µL of the promastigotes of *L. tropica* (2×10^6 cells/mL) was injected subcutaneously into the base of the tail and the footpad of the mice to induce infection [21].

2.10 *In vivo* activity of *B. vulgaris* against infected mice

After 28 days of inoculation, the development of lesions was observed. The mice were then divided into groups and subgroups. A total of 21 mice were randomly assigned into 4 groups as follows: Group 1 consisted of 3 untreated infected mice, Group 2 consisted of 3 infected mice treated

Figure 2: Use of Balb/C mice model for *in vivo* activity under favorable condition.

with PBS, Group 3 consisted of 12 infected mice further subdivided into 2 subgroups: subgroup 1 contained 6 infected mice treated with methanolic extract (3 mice treated orally and 3 topically), subgroup 2 contained 6 infected mice treated with ethanolic extract (3 mice treated orally and 3 topically), and Group 4 consisted of 3 healthy mice (as shown in flow sheet diagram in Figure 3). Treatment with pre-determined doses of *B. vulgaris* extract began 4 weeks post-infection and continued daily for 8 weeks [19].

2.11 Lesion size measurement

The diameter of the lesion was measured using a Vernier caliper at two diameters (*D* and *d*) at right angles to each other both before and after treatment. The lesion size (in millimeters) was calculated using the formula: $S = (D + d)/2/2$ [19].

2.12 Hematological analysis

A one-use 1 mL syringe and a 26 × 6 mm needle were used to collect 0.8 mL of whole blood from each mouse post-treatment via an intracardiac route. The blood was then transferred into ethylenediamine tetraacetic acid tubes for hematological variable analysis. Absolute measurements for red blood cells (RBCs), hematocrit, hemoglobin, white blood cells (WBCs), and lymphocytes were recorded using an auto Hematology Analyzer following the standard protocols [22].

2.13 Statistical analysis

Statistical analysis of the data obtained from both the *in vitro* and *in vivo* assays was conducted using Minitab to analyze the results of the antileishmanial activity of *B. vulgaris* extracts. This included calculating the mean and standard deviation and determining variations in different parameters. Additionally, for the *in vitro* assay, the inhibition concentration 50 (IC_{50}) values were calculated through linear regression analysis using GraphPad Prism 5 Software.

3 Results

3.1 Activity of methanolic extract of *B. vulgaris* against promastigote form of *L. tropica*

The activity of the methanolic extract of *B. vulgaris* against the promastigote form of *L. tropica* was investigated and found to be lower compared to the ethanolic extract. Similar to the ethanolic extract, five concentrations were

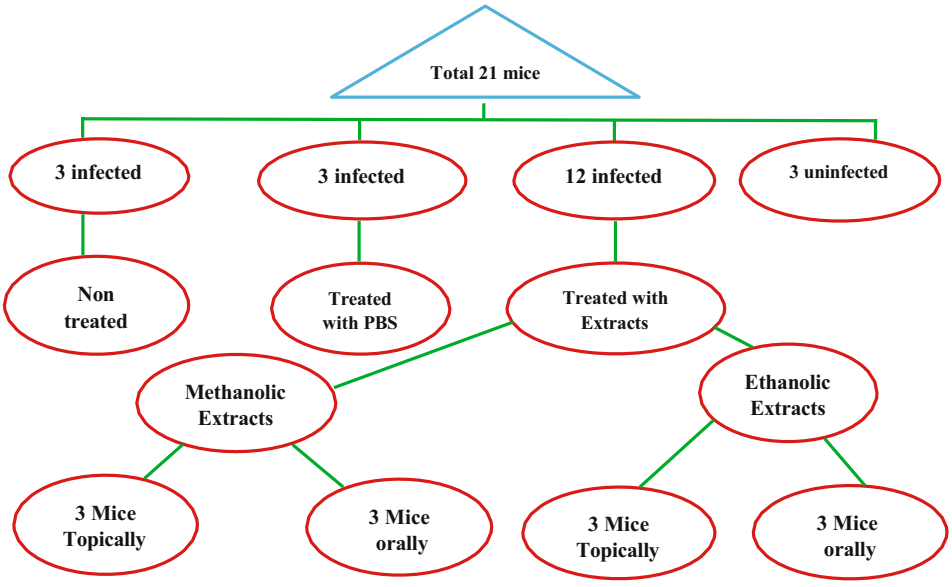

Figure 3: Flow sheet diagram of the control and experimental groups and subgroups for the *in vitro* activity.

tested for the methanolic extract to measure its anti-promastigote activity. The inhibition rates were highest at the concentration of 75 µg/mL (97.83 ± 2.01%), followed by 50 µg/mL (92.93 ± 2.81%), with the lowest inhibition observed at 5 µg/mL (17.96 ± 1.26%). Statistical analysis revealed that the inhibition at 50 µg/mL and 75 µg/mL concentrations were significantly similar (Figure 4). The IC_{50} value, representing the concentration at which 50% inhibition is achieved, was calculated to be 17.55 µg/mL, as shown in Table 1.

3.2 Activity of amphotericin B against promastigote

In the *in vitro* model against *L. tropica* promastigote, amphotericin B was used as a positive control. Five different concentrations of 1, 2.5, 5, 7.5, and 10 µg/mL were employed to assess its activity against *Leishmania sp*. The maximum inhibitory potential of the standard amphotericin B was observed at 10 µg/mL, followed by 7.5 µg/mL, with the lowest inhibition seen at 1 µg/mL (as shown in Figure 5). The IC_{50} value for the control compound was determined to be 2.52 µg/mL.

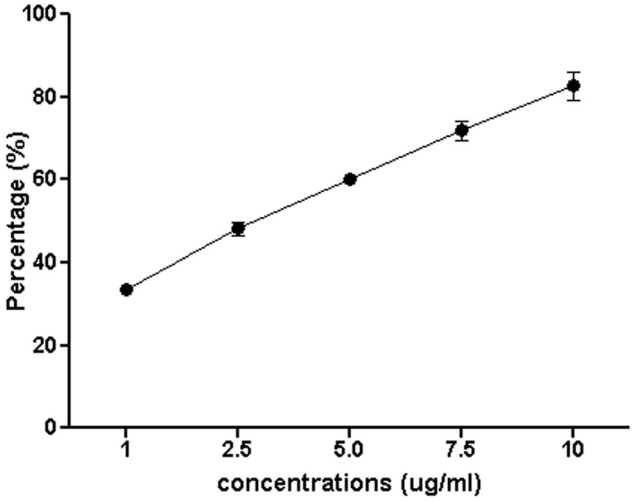

Figure 5: Amphotericin B-positive control activity against promastigotes.

3.3 Comparison of ethanolic and methanolic extracts in *in vitro* assay

The comparison between the ethanolic and methanolic extracts revealed that the percentage inhibition rate was higher in the ethanolic extracts compared to that in the methanolic extracts of *B. vulgaris* in the *in vitro* activity against the promastigote form of *L. tropica* (as shown in Figure 6). Another notable finding was that increasing the concentration of the ethanol extract marginally slowed down the inhibition rate, although this difference was not statistically significant when compared to the methanol extract.

3.4 *In vivo* antileishmanial effect of *B. vulgaris*

In vivo study evaluated the efficacy of ethanolic and methanolic extract of *B. vulgaris* root against *L. tropica*

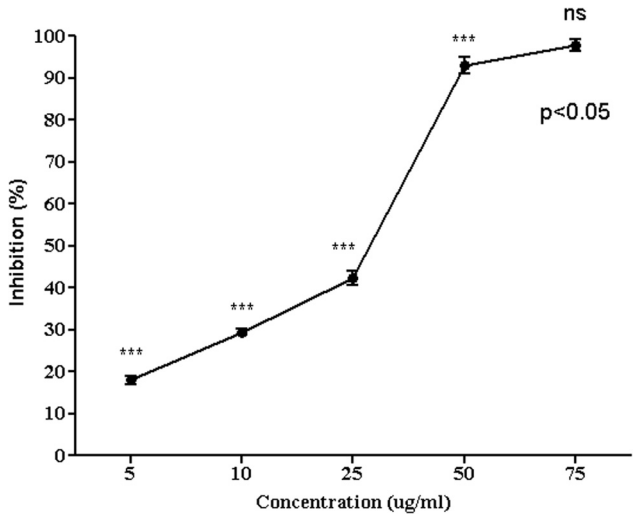

Figure 4: Activity of *B. vulgaris* methanolic extract against promastigote form of *L. tropica*.

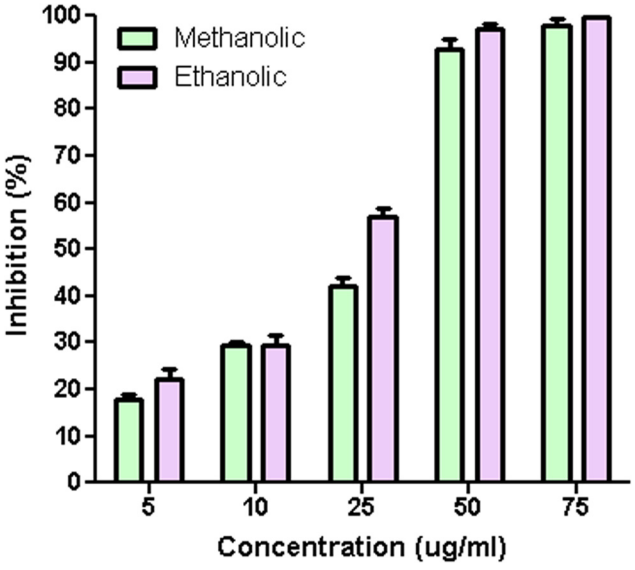

Figure 6: Comparison of ethanolic and methanolic extracts against *L. tropica* promastigote.

Table 2: Antileishmanial activity of *B. vulgaris* in ethanolic and methanolic solvents against *L. tropica* promastigote both topically and orally

Weeks	Concentrations (mg/kg/day)	Extract	Mean lesion size (mm) ± SD		Extract	Mean lesion size (mm) ± SD		Negative control
			Topically	Orally		Topically	Orally	
1	1.5	Ethanolic	0.90 ± 0.040	0.89 ± 0.009	Methanolic	0.93 ± 0.023	0.94 ± 0.032	0.85 ± 0.001
2	1.5	Ethanolic	0.87 ± 0.020	0.88 ± 0.023	Methanolic	0.91 ± 0.023	0.92 ± 0.004	0.88 ± 0.023
3	1.5	Ethanolic	0.84 ± 0.009	0.86 ± 0.014	Methanolic	0.89 ± 0.009	0.91 ± 0.012	0.94 ± 0.023
4	1.5	Ethanolic	0.80 ± 0.009	0.82 ± 0.020	Methanolic	0.86 ± 0.014	0.89 ± 0.009	1.01 ± 0.014
5	1.5	Ethanolic	0.74 ± 0.008	0.76 ± 0.012	Methanolic	0.81 ± 0.023	0.85 ± 0.000	1.11 ± 0.029
6	1.5	Ethanolic	0.68 ± 0.009	0.70 ± 0.009	Methanolic	0.76 ± 0.012	0.80 ± 0.009	1.23 ± 0.032
7	1.5	Ethanolic	0.60 ± 0.016	0.63 ± 0.008	Methanolic	0.70 ± 0.009	0.74 ± 0.008	1.33 ± 0.045
8	1.5	Ethanolic	0.51 ± 0.023	0.56 ± 0.008	Methanolic	0.63 ± 0.008	0.68 ± 0.009	1.45 ± 0.016

amastigote form in mice model. To examine the lesion size control effects of *B. vulgaris in vivo*, male BALB/c mice were affected by *L. tropica* amastigote, and the two extracts were administered 1.5 mg/kg daily topically and orally for 2 months post-infection. As shown the Table 2, the mean lesion diameter of mice group treated with ethanolic extract topically administered was measured to be their highest value (0.51 ± 0.023) and lowest value (0.90 ± 0.040) in comparison with negative control (0.85 ± 0.001 to 1.45 ± 0.016). In another mice group that received the same ethanolic extract orally, the mean lesion diameter was measured and their highest value was 0.56 ± 0.008 and the lowest was 0.89 ± 0.009. The mean lesion size in the mice group treated topically reduced more compared with that in the mice group receiving the same extract orally. The lesion size decreased in the mice group treated topically with methanolic extract, which were found to have the highest value 0.63 ± 0.008 and the lowest value of 0.93 ± 0.023, whereas the orally treated group have the highest value of 0.68 ± 0.009 and the lowest value of 0.94 ± 0.032. In the first week of treatment, the lesion diameter was not decreased in all groups of mice, while the lesion size grew more dramatically as the weeks increased. The highest activity of *B. vulgaris* extract against *L. tropica* was observed in the ethanolic extract as compared to that in the methanolic extract in both mice group treated with topically and orally in *in vivo* mice model (Figures 7 and 8).

3.5 Hematological analysis

The hematological analysis of mice blood was conducted after 8 weeks of infection, comparing both treated and untreated infected mice with a control group of uninfected mice. The blood parameters included RBCs, hematocrit, hemoglobin, WBCs, and lymphocytes, as shown in Table 3, Figure 9.

In the untreated infected mice group, the mean value of RBCs decreased (6.33 ± 0.28), whereas in the treated

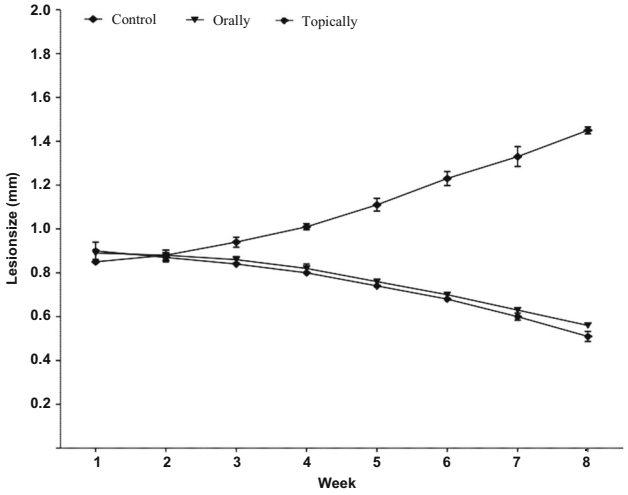

Figure 7: *In vivo* activity of ethanolic extract of *B. vulgaris* against *L. tropica*.

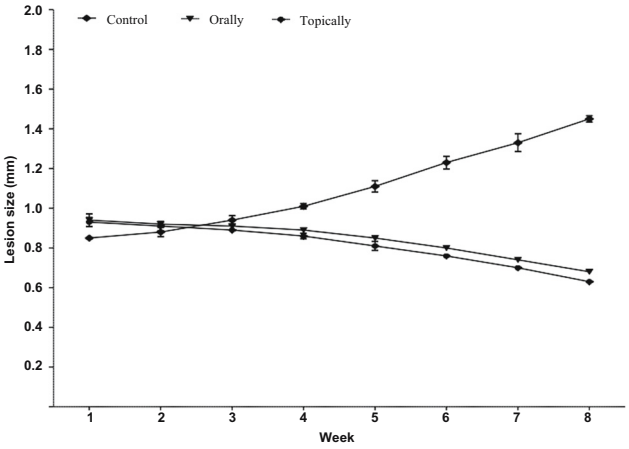

Figure 8: *In vivo* activity of methanolic extract of *B. vulgaris* against *L. tropica*.

Table 3: Hematological analysis of blood parameters of mice groups infected and uninfected

Blood parameters	Noninfected	Hematological analysis after 8 weeks of infection	
		Infected without treatment	Infected with treatment
RBCs ($\times 10^{12}$/mL)	7.66 ± 1.52	6.33 ± 0.28	7.00 ± 1.00
Hematocrit (%)	37.66 ± 1.52	34.66 ± 1.15	36.00 ± 0.57
Hemoglobin (g/dl)	12.66 ± 1.52	10.66 ± 1.52	11.33 ± 0.57
WBC (mm^3)	8,321 ± 145	7,866 ± 188	8,210 ± 81.64
Lymph ($\times 10^3/mm^3$)	29.12 ± 11.21	32.6 ± 13.32	28.20 ± 11.02

infected mice group, it increased (7.00 ± 1.00) compared to the control group of uninfected mice. Similarly, the mean values of hematocrit and hemoglobin decreased in the untreated infected mice group (34.66 ± 1.15 and 10.66 ± 1.52, respectively), but slightly increased in the treated infected mice group (36.00 ± 0.57 and 11.33 ± 0.57, respectively).

Furthermore, the mean value of WBCs decreased in the untreated infected mice group (7,866 ± 188), while it increased in the treated infected mice group (8,210 ± 81.64). Additionally, the mean value of lymphocytes increased in the untreated infected mice group (32.6 ± 13.32), but decreased after treatment, with all these mean values compared to the control group of uninfected mice.

4 Discussion

Leishmaniasis poses a significant public health challenge in tropical and subtropical regions, where it is transmitted through the bite of infected female sand-flies. Endemic in 98 countries and territories, leishmaniasis affects 12 million people and threatens around 350 million individuals worldwide. CL accounts for a majority of cases, affecting 1.5 million people annually, with approximately 90% of cases occurring in countries such as Pakistan, Iraq, Syria, Saudi Arabia, Peru, Iran, and Afghanistan [23]. Despite being a global health concern, leishmaniasis is still treated with costly and sometimes ineffective compounds that carry serious side effects, underscoring the need for new therapeutic options. Pentavalent antimonials, pentamidine, and amphotericin B are among the standard agents used for leishmaniasis treatment [24]. However, these drugs have several limitations, including the need for regular intramuscular

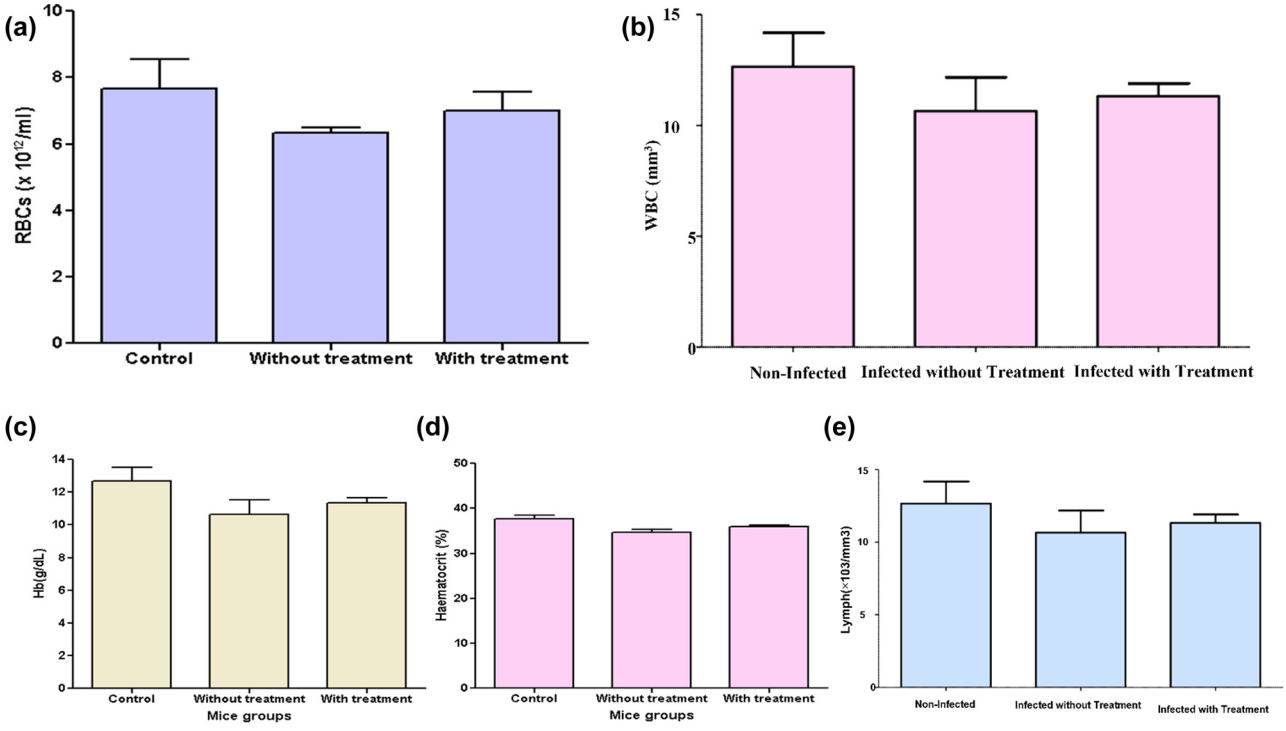

Figure 9: Comparative hematological analysis of mice's blood post 8 week infection shows (a) RBCs, (b) WBCs, (c) hemoglobin, (d) hematocrit, and (e) lymphocyte.

or intravenous injections over a period of 20–28 days, potential toxicity, parasite resistance, and high treatment costs [24–26]. Due to the adverse effects associated with pentavalent antimonials, there is growing interest in natural remedies. Plant-based components and extracts offer promising alternatives due to their lower side effects, cost-effectiveness, and widespread availability. In this study, we evaluated the *in vitro* and *in vivo* antileishmanial effects of ethanolic and methanolic extracts of *B. vulgaris* against promastigote and amastigote forms of *L. tropica*.

Our results demonstrated potent antileishmanial activity of *B. vulgaris* extract against promastigotes of *L. tropica* at concentrations ranging from 5 to 75 µg/mL, with parasite inhibition ranging from 22.23 to 99.38% and an IC_{50} value of 15.37 µg/mL. Interestingly, the ethanolic extract showed higher inhibition rates compared to the methanolic extract at the same concentrations, with inhibition rates of 97.83 and 17.96%, respectively, and an IC_{50} value of 17.55 µg/mL for the methanolic extract. Overall, our findings suggest that ethanolic extract of *B. vulgaris* exhibits superior antileishmanial activity compared to its methanolic counterpart in *in vitro* assays against *L. tropica* promastigotes. Several studies have investigated the antimicrobial effects of *B. vulgaris* and its bioactive compounds, consistently showing effective activity against various microorganisms. For instance, research conducted by Mahmoudvand et al. [17] demonstrated that the extract of *B. vulgaris* and berberine effectively inhibited the growth of *L. tropica* and *L. major* promastigotes in a dosage-dependent manner, with IC_{50} values ranging from 2.1 to 26.6 µg/mL. Another study by Ozbilgin et al. [27] observed a percentage inhibition of parasites between 88.0 and 100.0% in the presence of *B. vulgaris* ethanol extract, with an IC_{50} value of 444.81 ± 2.12 µg/mL.

Our study aligns with previous research, showing that the chloroform extract of *N. sativa* and ethanolic extracts of *B. vulgaris* significantly inhibit the efficiency of *L. tropica* promastigotes compared to the standard medication, with IC_{50} values of 7.83 and 4.83 µg/mL, respectively [17]. Furthermore, both extracts dramatically reduce the growth rate of amastigotes compared to the positive control ($p < 0.05$), suggesting the efficient antileishmanial activity against *L. tropica in vitro*. Previous studies have also highlighted the anticandidal effects of *B. vulgaris* extract, particularly from ethanol extracts, attributed to bioactive compounds such as berberine. While the mechanism influencing the antifungal function of berberine is not fully understood [28], our findings support the notion of significant anticandidal impact.

Interestingly, berberine has shown inhibition effects on the proliferation of hepatoma and leukemia cell lines *in vitro* [29], yet it is not considered toxic at medical dosages. However, some side effects have been reported at high dosages [30]. Nevertheless, *B. vulgaris* extracts with selectivity indexes ≥ 10 revealed protection to macrophages and clarity to parasites, suggesting safety for cell line consideration despite considerable cytotoxicity at high concentrations [31].

It is understood that there is a future for natural products in trying to seek appropriate and selective agents for the Leishmaniasis therapy. A significant advantage of the systematic diversity of this product screening is the substances that could generate natural products a source of novel antileishmaniasis lead compounds. The findings of our research show that the extract of *B. vulgaris* has a strong suppression effect on leishmaniasis and after treatment decrease in lesion size with an increased in concentration of ethanolic *B. vulgaris* extracts in infected BALB/c mice with the *L. tropica* amastigotes.

There is no one single study that has been reported of *B. vulgaris* against *L. tropica* in *in vivo* model. But some studies of *B. vulgaris* are present against different species of *Leishmania* that they supported our study against *L. tropica*. The results of the researcher study show that 20% root bark extracts of *B. vulgaris* have a powerful reducing effect on leishmaniasis [32]. Fatah et al. [33] reported a reduced lesion size following treatment with increasing concentrations of ethanolic extracts from the root, stem, and leaf of *B. vulgaris* in BALB/c mice infected with the promastigote form of *L. major*. The aqueous extracts of *B. vulgaris* fruits have been reported that had an effective activity against Echinococcus granulosus at lower concentration (4 mg/mL) and short time of viability [34].

Some other studies of different plant species extracts show their activities against *L. tropica.* Ozbel and Ozbilgin [35] reported that while the acidified extract of *Haplophyllum myrtifolium* showed promising results in an in vitro study, it had limited effectiveness in reducing lesion size in mice infected with *L. tropica* during in vivo testing at a concentration of 50 mg/kg. Our parasite burden and cytokine manufacturing findings support the safe and feasible therapy of leishmaniasis with the new formulation of PM-SLNs (paromomycin sulfate–solid lipid nanoparticles), and the use of SLN as drug delivery framework could even regulate the propagation form of *L. tropica* parasites in mice that have been infected [36].

In contrast, it is stated that *B. vulgaris* had shown toxicity against different parasites but it is proven from the study that the reduction in the density of the lesion, the amount of parasites, and the weight gain of BALB/c mice first infected with *L. major*, at 20, 40, and 80% of ethanolic stem bark extracts, was reported, followed by treatment. The 20% of extract was found to be effective, but 80% of the extract was harmful [37].

5 Conclusion

Therefore, the aforementioned studies suggested that our conclusion of the current study revealed that *B. vulgaris* ethanolic and methanolic extracts had promising antileishmanial activity in both *in vitro* and *in vivo* models and can regulate CL in mice infected with *L. tropica*. This outcome has also created a scientific proof that herbal plants can be used for the prevention and therapy of CL in traditional medicine. In summary, our findings underscore the significant effectiveness of *B. vulgaris* ethanol extracts against *L. tropica*, likely attributed to bioactive compounds such as polyphenols and berberine. However, further research is needed to elucidate the mechanisms of intervention of *B. vulgaris* and its components against *Leishmania sp*.

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