



In Vitro and In Vivo Anti-*Toxoplasma* Activities of *Dracocephalum kotschy* Extract in Experimental Models of Acute Toxoplasmosis

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Abstract

Background The problem of resistance to antiparasitic drugs, associated with their side effects, suggest exploring other alternatives, including medicinal plants. *Dracocephalum kotschy* (*D. kotschy*), for example, from Lamiaceae family, is a plant widely used in Iran and in many countries, and to which interesting pharmacological properties have been attributed. This study aimed to investigate in vitro and in vivo anti-*Toxoplasma* activities of *D. kotschy* extract in experimental models of acute toxoplasmosis.

Methods Anti-*Toxoplasma* activity of the extracts in vitro was performed on Vero Cell, using the MTT test. Vero cell were infected with (3×10^5 tachyzoites/well) following treatment with Dichloromethane (F1), dichloromethane: methanol (F2), methanol (F3), methanol: water (F4), and deionized water (F5) extracts of *D. kotschy*, and pyrimethamine and sulfadiazine (positive control). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to measure cell viability. Effects of extracts on tachyzoites viability were evaluated by trypan blue exclusion method, followed by light microscopy. For in vivo test, 18 groups of 8–10-week-old Inbred Balb/c mice weighing 18–20 g, were intraperitoneally infected with 2×10^3 tachyzoites and then treated with sterile PBS (negative control), pyrimethamine (25 mg/kg) and sulfadiazine (500 mg/kg) as positive controls, and F1 to F5 extracts (at doses 50, 100 and 200 mg/kg).

Results The 50% Inhibitory Concentration of F1 extract, F2 extract, Sulfadiazine (Positive control) and Pyrimethamine (Positive control) were 8.77 μ g, 7.1 μ g, 391.18 μ g, and 84.20 μ g, respectively, while the selectivity indices were 15.667, 30.197, 1.552 and 4.064, respectively. In vivo anti-*Toxoplasma* activity test showed that Methanol: water (F-4) 50 extract was more active than the positive control.

Conclusions Indeed, the extract allowed a survival rate of 10% of the mice, compared to 0% for all the other groups. *D. kotschy* has the potential to be valorized in the management of toxoplasmosis.

Keywords Antiparasitic agents · *Toxoplasma* · Plants · Medicinal · Survival rate · Iran

Abbreviations

D. kotschy *Dracocephalum kotschy*

MTT Thiazolyl blue tetrazolium bromide

T. gondii *Toxoplasma gondii*

IC50 Half-maximal inhibitory concentration

PBS Phosphate-buffered saline

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Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular parasite of a large number of homeothermic animals including humans [1]. The most common routes of transmission are the consumption of food or drink (usually water) contaminated with cat feces (containing the parasite's oocytes) or the ingestion of undercooked meat containing the parasite's cysts [2]. In a pregnant woman, *T. gondii* can cross placental barriers, infecting the fetus, causing death in most cases. If the fetus survives, it can suffer from severe birth defects, growth retardation, mental retardation, eye disease, and many other clinical manifestations [3]. Estimates report that up to one-third of the world's human population has been exposed to the parasite [4]. The overall annual incidence of congenital toxoplasmosis has been estimated at 190,100 cases [5].

The therapeutic management of toxoplasmosis involves antiparasitic drugs, such as Sulfadiazine and Pyrimethamine, but problems of ineffectiveness, parasite resistance, toxicity, and side effects have been reported [6, 7]. Among the possibilities to be explored or alternatives to antiparasitic drugs, natural substances from medicinal plants occupy a prominent place. Indeed, several medicinal plants have been reported in the traditional treatment of toxoplasmosis. Also, several pharmacological studies demonstrated the ability of medicinal plant extracts to inhibit in vitro and in vivo the growth of *T. gondii* [1, 7–11].

Dracocephalum kotschyi (*D. kotschyi*), a member of the Lamiaceae family, is vulgarly called Zarrin-giah and Badrandjboie-Dennaie in Iran [12]. The species is widely used in Iran for its many medicinal properties, due to the presence of bioactive compounds [13], especially high content and diversity of polyphenols and flavonoids. Also, its antispasmodic [12], immunomodulatory [14] properties have been demonstrated. Unfortunately, very little data are available on its antiparasitic properties, in vivo and in vitro, in particular against *T. gondii*. Indeed, no scientific study has focused on the scientific valorization of *D. kotschyi* in the search for an alternative to the insufficiencies of classical antiparasitic drugs against toxoplasmosis. This study aims to investigate in vitro and in vivo anti-*Toxoplasma* activities of *D. kotschyi* extract in experimental models of acute toxoplasmosis. This study is based on the hypothesis that given the very high use of *D. kotschyi*, its numerous biological and pharmacological properties and its high content of bioactive compounds, this medicinal plant is likely to be valued in the fight against toxoplasmosis. The ability of several extracts of *D. kotschyi* to inhibit in vitro and in vivo the tachyzoites of *T. gondii* are explored using standard and appropriate methodologies to provide scientific data on the anti-*Toxoplasma* properties of the medicinal plant.

Materials and Methods

Parasite

Tachyzoites from the RH strain of *T. gondii* were provided by the Toxoplasmosis Research Center (TRC) in Iran.

Vero Cells Culture

In this experimental study, samples were run in triplicate to avoid any biased error. The kidney cell lines "Vero" was initiated from a green monkey kidney and were obtained from the National Cell Bank of Iran (NCBI, Pasteur Institute of Iran, Tehran, Iran). Vero cells were cultured at 37 °C with 5% CO₂, in an RPMI-1640 (Gibco) medium supplemented with 100 µg/ml streptomycin, 2 mM L-glutamine, 10% fetal bovine serum (FBS), and 100 units/ml penicillin.

Plant Material

Dracocephalum kotschyi aerial parts were collected from Semirom, Isfahan province, Iran, in October 2019. It was authenticated by Professor of Pharmacognosy: Mustafa Ghanadian, Department of Pharmacognosy, Isfahan University of Medical Sciences, Isfahan, Iran, where a voucher specimen was deposited (No. 1519).

Animal Model and Ethics

In vivo experiments were conducted on 8–10-week-old Inbred Balb/c mice weighing 18–20 g. The animal protocols used in the study were approved by the Ethics Committee before starting the study. All mice were kept in cages under standard laboratory conditions including an average temperature (20–25 °C), humidity (60 ± 10%), light (12 h per day), given drinking water, and regular diet. The study protocol was approved by the ethical committee of the Shiraz University (IR.Shirazu.REC.1399.1214). Guidelines of Animal Ethics Committees (AECs) from Iran, have been used.

Chemicals and Reagents

Chemicals and reagents used in this study include methanol (Chromasolv, Sigma-Aldrich), dichloromethane (Chromasolv, Sigma-Aldrich) and distilled water (Water for chromatography LiChrosolv).

Plant Extraction

The extraction of the plant material was done by direct extraction from raw materials using different solvents in the order of increasing polarity. The fine pulverized plant

material (200 g) was extracted with dichloromethane (1.2 L, 11 g, F-1); dichloromethane: methanol 80:20 (1.2 L, 13 g, F-2); methanol (1.2 L, 12 g, F-3); methanol: water 60:40 (1.2 L, 8 g, F-4); and deionized water (1.2 L, 7 g, F-5). Each extraction was done using the maceration method with continuous shaking for 48 h. Each extract was then filtered through Whatman® Grade 1 filter paper, and concentrated under reduced pressure using a rotary evaporator. Concentrated extracts were lyophilized using a freeze dryer, placed into a sterile glass container, and kept at $-20\text{ }^{\circ}\text{C}$ until use.

Phytochemical Analysis, Identification of Bioactive Compounds and NMR Spectroscopy

Chromatographed on a SC6 polyamide column (20 × 300 mm) using gradient mixtures. Major fractions were selected and injected to recycle HPLC pump using Shimpak-RP C₁₈ column (250 × 20 mm) and acetonitrile: DMSO: water (69:9:22) as mobile phase to yield compounds, as the main bioactive compounds. ¹H-NMR spectra of bioactive isolated compounds were taken by a regular 5 mm diameter probe using Bruker 400 MHz (Bruker Biospin, Rheinstetten, Germany), operating at 400 MHz for ¹H-NMR and 100 MHz for ¹³C-NMR spectrometry.

Anti-Toxoplasma Activity of the Extracts In Vitro

Cytotoxicity Assessment

All extracts and control drugs (pyrimethamine and sulfadiazine) were dissolved in complete culture medium RPMI-1640 containing dimethyl sulfoxide (DMSO) less than 1% to improve the dilution. Vero cells were cultured (2×10^4 cell/well/180 μl/ml) in 96 well plates for 24 h. After that, the cells were exposed to the agents at a final concentration of 1000, 500, 100, 50, 10, and 1 μg. After 24 h, cell viability was measured by adding 20 μl of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Then, the 50% cytotoxic concentration (CC₅₀) was measured using Graph Pad Prism 6.0 software [15]. Then, the selectivity index (SI) was determined (CC₅₀/IC₅₀ = SI). All data points represent the average of three independent experiments [16].

Effects of Extracts on Tachyzoites Viability by Trypan Blue Exclusion

The tachyzoite viability test was performed, in vitro using the method proposed by Cover and Gutteridge [17]. Six concentrations (1, 10, 50, 100, 500, and 1000 μg) of *D. kotschy* extracts were incubated for 30, 90, and 180 min. The trypan blue stain was used to assess the viability of the tachyzoites. 45 μl of tachyzoite suspension containing 10^6 cells/ml and *D. kotschy* extracts at six different concentrations (1, 10, 50,

100, 500, and 1000 μg) in 96-well microplates are brought together. The whole is incubated at 37 °C. After 30-, 90-, and 180-min incubation in 5% CO₂ at 37 °C, a trypan blue dye exclusion test for tachyzoites was carried out under the microscope. Results were expressed as % viability. Positive controls containing pyrimethamine and sulfadiazine, while PBS were the negative controls. The plates were then spread on a glass slide followed by an examination under an optical microscope. The experiments were repeated three times.

Light Microscopy of Tachyzoites in Cell Lines

Vero cell culture (2×10^5 cells/ml) was carried out on a glass slide in a 35 mm cell culture dish up to the confluence and then infected with 1×10^6 tachyzoites/box. After 4 h incubation, the monolayers were washed with Hanks' Balanced Saline Solution (HBSS; Gibco Inc., USA), followed by the addition of the extracts contained in RPMI-1640. The glass lids were removed from the dishes at 48 h after adding the extracts, pyrimethamine, sulphadiazine, and 1% DMSO (negative control). All glass slides were washed with HBSS and fixed with methanol before staining with Giemsa (Sigma Inc., USA). The samples were observed under an oil-immersion objective lens by an optical microscope.

Anti-Toxoplasma Activity of the Extracts in vivo

BALB/c mice ($n = 180$), were housed into 18 groups (10 mice each). One group was kept as an uninfected control. 18 groups were intraperitoneally infected with 2×10^4 tachyzoites. The infected control group was administered sterile PBS (negative control), the positive controls received pyrimethamine (25 mg/kg), sulfadiazine (500 mg/kg), and other groups were treated with F1 to F5 extracts (at doses 50, 100, and 200 mg/kg). The mice were monitored daily for mortality and morbidity. The survival periods were recorded daily until all mice died. Initially, for controlling drug side effects, a preliminary experiment was done on Balb/c mice receiving the same dose of drugs, and no mortality or clinically significant toxicity was observed.

Statistical Analysis

Results were analyzed using Graph Pad Prism 6.0 software. Differences between test and control groups were evaluated by analysis of variance (ANOVA) one tailed and Newman–Keuls multiple-comparison test. The P value < 0.05 was considered significant.

Results

In Vitro Anti-Toxoplasma Activity of the Extracts

Cytotoxicity and Antiparasitic Effect

For all extracts, cytotoxicity on Vero cells varied according to the concentrations of the extracts (Fig. 1).

CC₅₀, IC₅₀, and SI of the extracts and positive controls are shown in Table 1. Antiparasitic assessment of extracts and positive controls showed a high inhibitory activity of Dichloromethane extract and Dichloromethane: Methanol extract of *D. kotschy* on tachyzoite with IC₅₀ = 8.77 µg and 7.1 µg, respectively, in comparison to positive control

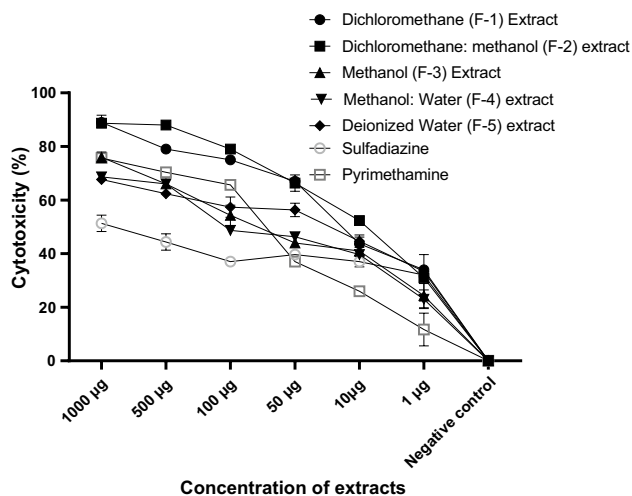


Fig. 1 Effects of concentration on cytotoxicity of each plants extracts. Vero cell infected with 3×10^5 tachyzoites/well of RH *Toxoplasma gondii* and treated with Sulfadiazine and Pyrimethamine (Positive control), and +Dichloromethane (F1), dichloromethane: methanol (F2), methanol (F3), methanol: water (F4), and deionized water (F5) extracts of *Dracocephalum kotschy*, at different concentration (1, 10, 50, 100, 500, 1000 µg); Negative control = PBS

Table 1 In Vitro, the IC₅₀/CC₅₀ value for Vero cells (CC₅₀ vero + extract), anti-*T. gondii* RH strain activity, and selectivity of *D. kotschy* extracts, sulfadiazine and pyrimethamine

Name	Tested drugs name	Cytotoxicity (CC ₅₀ , µg)	In Vitro anti-Toxoplasma assay (IC ₅₀ , µg)	Selectivity index (SI) ^a
F1	Dichloromethane extract	137.40	8.77	15.667
F2	Dichloromethane: methanol extract	214.40	7.1	30.197
F3	Methanol extract	123.45	48.11	2.565
F4	Methanol: water extract	604.146	71.72	8.423
F5	Deionized water extract	82.19	28.48	2.885
C1	Sulfadiazine (positive control)	607.18	391.18	1.552
C2	Pyrimethamine (positive control)	342.23	84.20	4.064

IC₅₀, median inhibitory concentration

^aRatio of the IC₅₀ value for Vero cells to the CC₅₀ value for *T. gondii* tachyzoites

(Sulfadiazine and Pyrimethamine) (IC₅₀ = 391.18 µg and 84.20 µg, respectively).

Dichloromethane: Methanol extract and Dichloromethane extract of *D. kotschy* had the highest Selectivity Indices, illustrating better selectivity, with low effect for host cell: 30.197 and 15.667, respectively. On the other hand, the positive control (Sulfadiazine, Positive control) had the lowest selectivity index (1.552). Sulfadiazine was the most toxic treatment for the host cells.

Effects of Extracts on Tachyzoites Viability by Trypan Blue Exclusion

The trypan blue stain was used to assess the viability of the tachyzoites. The results showed that *D. kotschy* has acceptable efficacy in vitro, and the parasiticidal effect of F1 (Dichloromethane) and F2 (Dichloromethane: Methanol) extracts were significantly better than positive control in all exposure times (Table 2). Viability was evaluated based on concentration at 30 min, 90 min, and 180 min. It should be noted that cell viability for all extracts and positive controls decreases over time and as the concentration of the extracts or reference molecule increases.

Effects of the Extracts on *T. gondii* in vivo

Clinically, the number of mice in the untreated infected group (negative control) began to decrease on day 7 of the study and all mice died until day 9 post-infection.

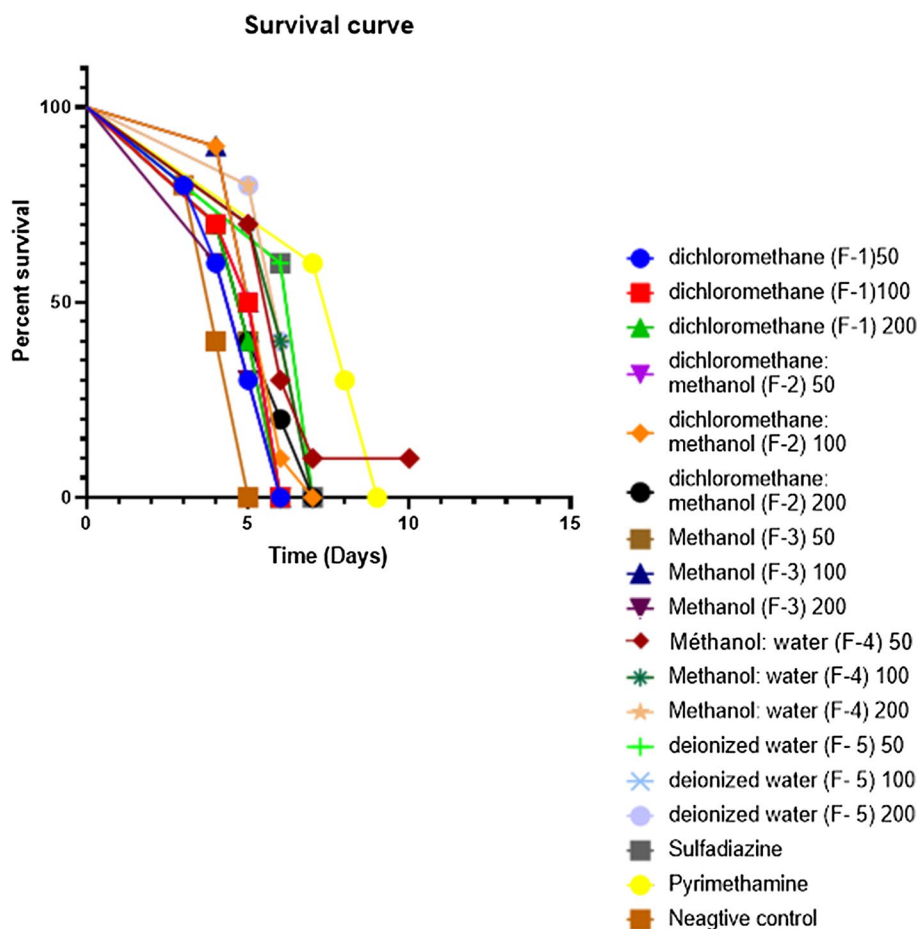
Mice in the Pyrimethamine group (positive control) began to die on day nine and all died by day eleven. Between the sixth and seventh days, all mice in the Sulfadiazine (positive control) group died (Fig. 2).

Mice in the extract groups also had, for the most part, 100% mortality, except for the methanol: water (F-4) 50 extract group, where mice had a 10% survival rate. Indeed, the mice started to die on day 5 and there was no more death after day 7. At the end of the in vivo test, methanol: water

Table 2 Effects of extracts on tachyzoite viability

Extracts	Concentrations (μg)	Tachyzoite viability (in percentage) at different time			P value
		30 min	90 min	180 min	
F1 (dichloromethane)	1	96.13 \pm 1.32	90.46 \pm 0.95	86.46 \pm 1.12	< 0.05
	10	94.70 \pm 0.80	86.03 \pm 1.12	82.56 \pm 1.88	
	50	90.46 \pm 1.12	86.56 \pm 1.12	79.80 \pm 1.80	
	100	90.03 \pm 0.95	80.70 \pm 0.80	78.03 \pm 2.31	
	500	87.23 \pm 0.95	76.80 \pm 1.80	70.56 \pm 2.31	
	1000	79.46 \pm 0.95	72.56 \pm 1.88	63.46 \pm 2.31	
F2 (dichloromethane: methanol)	1	95.43 \pm 1.12	89.66 \pm 0.95	85.66 \pm 1.12	< 0.05
	10	94.10 \pm 0.80	87.23 \pm 1.12	81.56 \pm 1.88	
	50	89.46 \pm 1.12	85.76 \pm 1.12	79.10 \pm 1.80	
	100	89.43 \pm 0.95	80.10 \pm 0.80	77.23 \pm 2.31	
	500	86.13 \pm 0.95	76.10 \pm 1.80	69.76 \pm 2.31	
	1000	78.76 \pm 0.95	71.56 \pm 1.88	62.56 \pm 2.31	
F3 (methanol)	1	98.23 \pm 1.12	92.76 \pm 0.95	88.76 \pm 1.12	< 0.05
	10	97.10 \pm 0.80	88.23 \pm 1.12	84.56 \pm 1.88	
	50	92.56 \pm 1.12	88.76 \pm 1.12	82.10 \pm 1.80	
	100	92.43 \pm 0.95	82.90 \pm 0.80	80.23 \pm 2.31	
	500	89.23 \pm 0.95	79.10 \pm 1.80	72.76 \pm 2.31	
	1000	81.76 \pm 0.95	74.76 \pm 1.88	65.56 \pm 2.31	
F4 (methanol: water)	1	98.83 \pm 1.12	93.16 \pm 0.95	89.16 \pm 1.12	< 0.05
	10	97.50 \pm 0.80	88.83 \pm 1.12	85.16 \pm 1.88	
	50	93.16 \pm 1.12	89.16 \pm 1.12	82.50 \pm 1.80	
	100	92.83 \pm 0.95	83.50 \pm 0.80	80.83 \pm 2.31	
	500	89.83 \pm 0.95	79.50 \pm 1.80	73.16 \pm 2.31	
	1000	82.16 \pm 0.95	75.16 \pm 1.88	66.16 \pm 2.31	
F5 (deionized water)	1	97.23 \pm 1.12	91.56 \pm 0.95	87.66 \pm 1.12	< 0.05
	10	96.90 \pm 0.80	87.23 \pm 1.12	83.66 \pm 1.88	
	50	91.56 \pm 1.12	87.56 \pm 1.12	80.90 \pm 1.80	
	100	91.23 \pm 0.95	81.90 \pm 0.80	79.23 \pm 2.31	
	500	88.23 \pm 0.95	77.90 \pm 1.80	71.56 \pm 2.31	
	1000	80.56 \pm 0.95	72.56 \pm 1.88	64.56 \pm 2.31	
Pyrimethamine (positive control)	1	95.66 \pm 1.52	92.00 \pm 2.00	84.66 \pm 2.51	< 0.05
	10	93.33 \pm 2.08	87.33 \pm 2.08	81.66 \pm 2.08	
	50	93.33 \pm 3.05	84.33 \pm 3.51	77.33 \pm 2.51	
	100	92.00 \pm 2.00	84.33 \pm 1.52	76.66 \pm 1.52	
	500	90.00 \pm 2.00	80.33 \pm 2.51	75.33 \pm 2.51	
	1000	86.33 \pm 1.52	75.33 \pm 2.08	73.00 \pm 3.00	
Sulfadiazine (positive control)	1	97.66 \pm 0.57	92.33 \pm 1.52	90.66 \pm 1.52	< 0.05
	10	97.33 \pm 0.57	96.00 \pm 1.00	90.00 \pm 1.00	
	50	95.66 \pm 1.52	94.66 \pm 0.57	88.66 \pm 1.15	
	100	95.00 \pm 2.00	89.66 \pm 1.52	87.66 \pm 1.52	
	500	93.66 \pm 1.52	87.33 \pm 2.08	85.66 \pm 2.08	
	1000	90.33 \pm 2.08	84.66 \pm 1.52	79.33 \pm 1.52	
Negative	–	97.00 \pm 1.00	95.33 \pm 2.51	95.33 \pm 1.15	> 0.05

Fig. 2 Survival curves of mice following acute toxoplasmosis. Balb/c mice infected with *T. gondii* RH strain were treated with *Dracocephalum kotschyi*, sulfadiazine, and pyrimethamine



(F-4) 50 had the best in vivo activity compared to the other extracts and positive control.

Phytochemical Analysis and Identification of Bioactive Compounds from Most Active Extract

Antiprotozoal effects of the resulted extracts F1–F5 were compared in Table 1. F2, extracted by dichloromethane: methanol, with more anti-*T. gondii* activity (IC₅₀ of 7.1 µg/ml) was selected and chromatographed on a SC6 polyamide column (20 × 300 mm) using gradient mixtures of chloroform: methanol as follows: F2.1 (100:0); F2.2 (96:4); F2.3 (94:6); F2.4 (90:10); F2.5 (88:12); F2.6 (86:14); F2.7 (80:20). Major fractions F2.2 and F2.4 were selected and injected to recycle HPLC pump using Shimpak-RP C₁₈ column (250 × 20 mm) and acetonitrile: DMSO: water (69:9:22) as mobile phase to yield compounds 1–4, respectively, as the main bioactive compounds.

NMR Spectroscopy

¹H-NMR spectra of bioactive isolated compounds were taken by a regular 5 mm diameter probe using Bruker

400 MHz (Bruker Biospin, Rheinstetten, Germany), operating at 400 MHz for ¹H-NMR and 100 MHz for ¹³C-NMR spectrometry. Spectra were collected at 25 °C in DMSO-d₆. The sugar linkage was detected through Heteronuclear multiple bond correlation (HMBC) between anomeric proton H-1" of sugar and its location on aglycone structure. The configurations of anomeric proton was deduced to be β-form based on its anomeric large coupling constants ($J = 7.0\text{--}8.0$ Hz). Chemical shifts are reported in delta (δ_{H} or δ_{C}), after shimming by topshim command in TOPSPIN software. The data were processed using 2018 Mestrelab Research program (www.mestrelab.com). Isolated compounds (Fig. 3) were identified according to data reported in literature [18–21].

Apigenin: Pale yellow powder, ¹H-NMR in pyridine-d₆ (400 MHz) ppm: 6.10 (1H, d, $J = 2.0$ Hz, H-8), 6.36 (1H, d, $J = 2.0$ Hz, H-6), 6.49 (1H, s, H-3), 6.83 (2H, d, $J = 8.8$ Hz, H-6',2') and 7.75 (2H, d, $J = 8.8$ Hz, H-5',3'); ¹³C-NMR (100 MHz, pyridine-d₆) δ_{C} : 183.2 (C4), 166.4 (C2), 163.8 (C7), 163.3 (C5), 163.2 (C4'), 159.0 (C9), 129.4 (C5', C3'), 117.3 (C6', C2'), 122.8 (C1'), 106.5 (C10), 104.4 (C3), 100.5 (C6), 95.3 (C8). ESI negative mass m/z 269 [M–1].

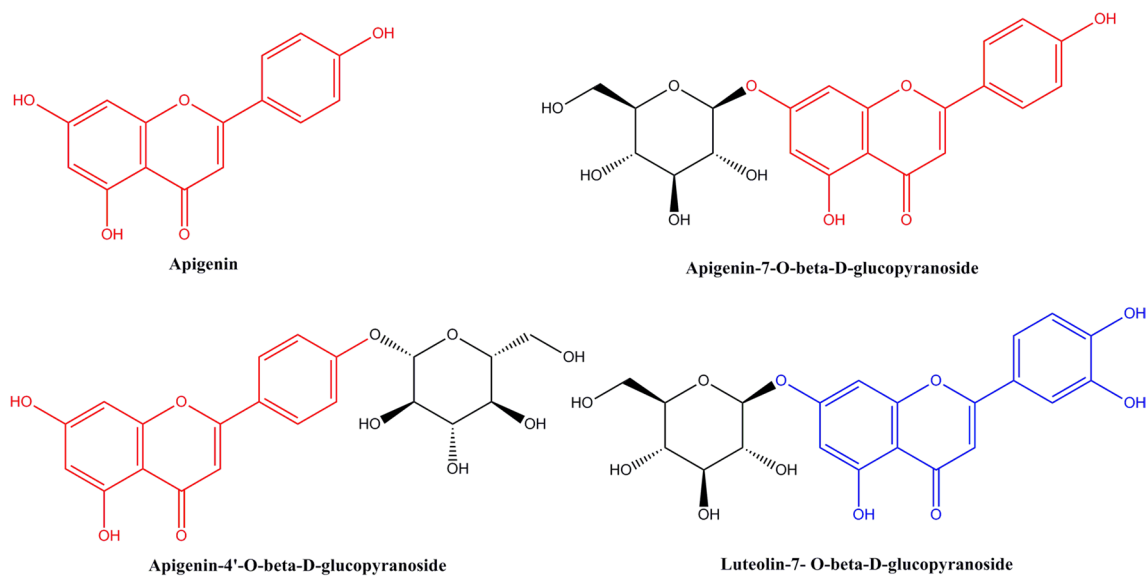


Fig. 3 Identification of phytochemicals from the most active extract: dichloromethane: methanol of *D. kotschy*

Apigenin 4'-O- β -D-glucopyranoside: Pale yellow powder, $^1\text{H-NMR}$ in DMSO- d_6 (400 MHz) ppm: δ 3.2 to 3.8 (6H, overlapped, H-1'' to H-6''), 4.72 (1H, d, $J=7.2$ Hz, H-1''), 6.67 (1H, s, H-3), 6.75 (1H, d, $J=2.0$ Hz, H-8), 6.81 (1H, d, $J=2.0$ Hz, H-6), 6.92 (2H, d, $J=8.8$ Hz, H-6',2') and 7.90 (2H, d, $J=8.8$ Hz, H-5',3'); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) δC : 177.0 (C4), 162.7 (C2), 161.2 (C5), 160.1 (C7), 158.5 (C4'), 158.3 (C9), 128.1 (C6', C2'), 121.1 (C1'), 115.1 (C5', C3'), 108.1 (C10), 105.6 (C3), 104.5 (C1''), 104.3 (C6), 98.3 (C8), 77.5 (C5''), 75.5 (C3''), 73.6 (C2''), 69.6 (C4''), 60.8 (C6''). ESI negative mass m/z 431 [M-1].

Apigenin 7-O- β -D-glucopyranoside: Pale yellow powder, $^1\text{H-NMR}$ in DMSO- d_6 (400 MHz) ppm: δ 3.2 to 3.8 (6H, overlapped, H-1'' to H-6''), 5.08 (1H, d, $J=7.6$ Hz, H-1''), 6.46 (1H, d, $J=2.0$ Hz, H-8), 6.84 (1H, d, $J=2.0$ Hz, H-6), 6.88 (1H, s, H-3), 6.94 (2H, d, $J=8.8$ Hz, H-6',2') and 7.96 (2H, d, $J=8.8$ Hz, H-5',3'); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) δC : 181.1 (C4), 164.2 (C2), 162.9 (C5), 162.3 (C7), 161.1 (C9), 156.1 (C4'), 128.5 (C6', C2'), 120.1 (C1'), 116.0 (C5', C3'), 105.3 (C10), 103.1 (C3), 99.1 (C1''), 99.5 (C6), 94.1 (C8), 77.1 (C5''), 76.3 (C3''), 73.0 (C2''), 69.5 (C4''), 60.5 (C6''). ESI negative mass m/z 431 [M-1].

Luteolin 7-O- β -D-glucopyranoside: Pale yellow powder, $^1\text{H-NMR}$ in DMSO- d_6 (400 MHz) ppm: δ 3.2 to 3.8 (6H, overlapped, H-1'' to H-6''), 5.08 (1H, d, $J=7.2$ Hz, H-1''), 6.44 (1H, d, $J=2.0$ Hz, H-8), 6.74 (1H, s, H-3), 6.79 (1H,

d, $J=2.0$ Hz, H-6), 6.94 (1H, d, $J=8.8$ Hz, H-5'), 7.42 (1H, d, $J=2.0$ Hz, H-2'), 7.46 (1H, dd, $J=8.8, 2.0$ Hz, H-6'); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) δC : 181.8 (C4), 164.6 (C2), 162.9 (C7), 161.1 (C5), 156.9 (C9), 149.7 (C4'), 145.3 (C3'), 120.9 (C1'), 119.3 (C6), 116.0 (C5'), 113.0 (C2'), 105.3 (C10), 103.1 (C3), 99.9 (C1''), 99.7 (C6), 94.7 (C8), 77.1 (C5''), 76.3 (C3''), 73.1 (C2''), 69.5 (C4''), 60.5 (C6''). ESI negative mass m/z 447 [M-1].

Discussion

This study was aimed to investigate in vitro and in vivo anti-*Toxoplasma* activities of *D. kotschy* extract in experimental models of acute toxoplasmosis.

For the investigation, we opted for RH strain of *T. gondii*, because this strain has been successfully used in several studies devoted to the anti-*Toxoplasma* activity of natural substances, especially because it produces acute toxoplasmosis [22]. Furthermore, the choice of Vero cells as host cells is due to the fact that this model allows optimal growth of *T. gondii* with good replication efficiency [23, 24].

One of the problems encountered in the management of toxoplasmosis is the toxicity of chemical drug molecules, hence the need to resort to natural substances. Our study

confirmed this observation. Indeed, at the end of their *in vitro* investigations, it was observed that Sulfadiazine (reference drugs and positive control) was the most toxic treatment for the host cells (Selectivity Index = 1.552). Sulfadiazine is one of the molecules used in the treatment of toxoplasmosis [6, 7]. It is, therefore, highly toxic to host cells. Pyrimethamine also presented a low SI (4.064). This constitutes a strategic disadvantage for these reference molecules and justifies the interest in using natural substances. Indeed, we can observe that Dichloromethane: Methanol extract and Dichloromethane extract of *D. kotschy* had the highest Selectivity Indices, illustrating better selectivity, with low effect for host cell: 30.197 and 15.667, respectively. The data are consistent with the results of Montazerri *et al.* [7] who also showed that Pyrimethamine was toxic to the host cell in comparison to Hydroalcoholic Extract of some Brassicaceae Species. This toxicity of the reference drugs is probably linked to their reduced efficacy reported in several studies, which requires the use of high concentrations that are, therefore, toxic for the cells.

About anti-*Toxoplasma* activity, the 50% Inhibitory Concentration of Dichloromethane extract, Dichloromethane: Methanol, extract, Sulfadiazine (Positive control), and Pyrimethamine (Positive control) were 8.77 µg, 7.1 µg, 391.18 µg, and 84.20 µg, respectively. All the plant extracts were more active than the positive control. This confirms that medicinal plants such as *D. kotschy* can be used in the management of toxoplasmosis, an alternative to conventional drugs. The extracts of *D. kotschy* were distinguished by a strong antiparasitic activity, associated with low toxicity. This is the first report of the anti-*Toxoplasma* activity of *D. kotschy*, but several data confirm the high activity and low toxicity of natural substances from medicinal plants against *Toxoplasma*. In a study dedicated to anti-*Toxoplasma* activity of some Brassicaceae, extracts of *L. sativum*, *L. perfoliatum*, *N. officinale*, *A. homolocarpum*, *C. bursa-pastoris* presented 50% inhibitory concentrations of 5.1, 14.67, 32.49, 37.31, 71.35 and selectivity indices of 8.06, 2.59, 0.74, 0.78, respectively, in comparison to pyrimethamine (IC₅₀ = 63 µg/ml) [7].

At the organism level (*in vivo*), the survival test showed high mortality in all groups, illustrating a low activity of extracts and reference molecules. However, it is striking to note that the methanol: water (F-4) 50 extract was more active than the positive control, having allowed a survival rate of 10% of the mice against 0% for all other groups. This result confirms the results of the *in vitro* tests and attests that *D. kotschy* has a better activity *in vitro* and *in vivo* against *Toxoplasma* strains, in comparison with classical antiparasitic agents, such as Sulfadiazine (Positive control) and Pyrimethamine (Positive control). Also, high variability

was observed in the activity of the extracts. This may be due to the variability of the solvents used.

The activity of the extract is due to the quality of the active molecules present in the extract, which is related to the affinity that these molecules have for the extraction solvent.

This study was not focus on chemical composition of *D. kotschy*; however, the most bioactive extract, F-2, was submitted for phytochemical analysis for identification of its phytochemicals. Four compounds were separated and identified as apigenin (compound 1), Apigenin-4'-*O*-β-D-glucopyranoside (compound 2), apigenin-7-*O*-β-D-glucopyranoside (compound 3) and luteolin-7-*O*-β-D-glucopyranoside (compound 4). Previous study reported the presence of actives compounds in the plant extract or oil. Shakib *et al.* [25] found that *D. kotschy* essential oils contains Oxygenated Sesquiterpenes, Monoterpene Hydrocarbons, Sesquiterpene Hydrocarbons and Oxygenated Monoterpenes. Flavonoids such as calycopterin, acacetin 7-*O*-β-D-glucopyranoside, xanthomicrol, apigenin 4'-*O*-β-D-glucopyranoside, isokaempferide, luteolin 3'-*O*-β-D-glucuronide, luteolin, luteolin 7-*O*-β-D-glucopyranoside, apigenin have also been identified elsewhere [18–21].

Conclusions

We report for the first-time anti-*Toxoplasma* activity of *D. kotschy*, *in vitro*, and *in vivo*. This potential is associated with a low toxicity for the host cell. Supplementary works are necessary to identify actives compounds associated with anti-*Toxoplasma* activity.

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Data Availability The data sets from the present study are available from the corresponding author upon request.

Declarations

Conflict of Interest All authors declare that there are no conflicts of interest.

Ethics Approval and Consent to Participate The study protocol was approved by the ethical committee of the Shiraz University (IR.Shirazu.REC.1399.1214).

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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