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Original Research Article

In Vitro Effect of *Capparis spinosa* Extract on HSP70 and LACK Gene Expression in *Leishmania tropica* Promastigotes

Ali Mohammed Abed[1]*, Rawan Hassan Al-Hamdani[1], Noor Tala Ali[2]

[1]Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq
[2]College of Nursing, University of Tikrit, Tikrit, Iraq

***Corresponding Author:** Ali Mohammed Abed
Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq

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Abstract: ***Background*:** Leishmaniasis is a globally widespread parasitic infection, and the resistance of the parasite causing this infection to commonly used drugs has become a serious problem. Therefore, it has become necessary to conduct studies on the role of alternative medicines as a treatment for this disease. ***Objective*:** This study aimed to determine the effect of using Capparis spinosa extract on the gene expression of HSP70 and LACK genes in promastigote. ***Method*:** The parasite was cultured in RPMI-1640 culture medium in the laboratory under suitable working condition. Subsequently, the parasite was exposed to different concentration of Capparis spinosa extract, and then the gene expression of the parasites HSP70 and LACK was measured by used RT-PCR assay. ***Results*:** Exposure of promastigote to different concentrations of Capparis spinosa extract resulted in a significant increase in the Gene expression of HSP70 and LACK genes compared to the control group suggesting the potential use of this extract as a treatment for leishmaniasis. ***Conclusion*:** The current study showed that Capparis spinosa extract has an effect on HSP70 and LACK genes expression through the effect of some chemical compounds of the plant extract on protein synthesis and iron chelation.

Keywords: Leishmania, Capparis Spinosa Extract, HSP70, LACK, Promastigote, *in Vitro*.

INTRODUCTION

Cutanoues Leishmaniasis is the world's second deadliest neglected tropical disease, with only malaria causing a higher number of global deaths among this class of diseases. Clinically, it is classified into three categories: cutaneous leishmaniasis, which only affects the site of skin inoculation; visceral leishmaniasis, which impacts internal organs; and mucocutaneous leishmaniasis [9]. The tropical Leishmania protozoan (*Leishmania tropica*) causes cutaneous leishmaniasis in endemic regions of the Old World. It rarely leads to the visceral-tropic form of the disease, and can also trigger recurrent leishmaniasis, a condition characterized by new skin lesions that emerge at the edge of scars when initial treatment is not fully curative [6].

Caper (Capparis spinosa) is the main cultivated species of the genus Capparis. In this study, we sorted out the basic background of this species and found that it has localized common names across Iraq: it is called Kabar in Basra, kifri in the Kurdish region, and the universal common name used across the whole country is Shfallah. Previous studies have confirmed that this species contains active components including alkaloids, lipids, and flavonoids, and has medicinal activities such as antifungal effects, hepatotoxicity regulation, and anti-leishmanial activity [1].

This study selected the Leishmania homologue of receptors for activated protein kinase C (LACK) as its core research subject, and plans to observe the RNA expression level of this antigen in clinical isolates of Leishmania tropica (L. tropica) that are sensitive and resistant to the first-line drug Glucantime. Existing studies have confirmed that LACK is highly conserved among Leishmania species with high genetic diversity, exists throughout the parasite's entire life cycle, can stimulate immune responses and induce the generation of type 1 T helper cells, and is a core target for vaccine

development [4]. HSP70 function as a molecular chaperone facilitating protein homeostasis under stress, while LACK participates in signal transduction and immune evasion. Their expression dynamic are interconnected with parasite survival, infectivity, and drug resistance, forming a critical axis for therapeutic targeting [4].

MATERIALS AND METHODS

Parasite Isolates

The promastigote strain of the isolated parasite used for primary culture in this study was isolated from two patients in Iraq, and was provided by the Medical Research Center at the College of Medicine, Al-Nahrain University.

Parasite Culture

The Roswell Park Memorial Institute 1640 (RPMI-1640) culture medium was used as previously described by Moore and Woods [14]. Fetal bovine serum collecting serum in South America only. Promastigotes of *Leishmania tropica* were used in this study. The parasites were cultured under standard laboratory conditions in appropriate culture medium and maintained at optimal temperature for promastigote growth, as previously described in leishmanial culture protocols [10, 12]. Parasites were subcultured regularly to ensure logarithmic-phase growth before experimental use.

Collection and Preparation of Plant Extract (C. Spinosa):

The leaves of C. spinosa were meticulously collected from various locations in Tikrit city and transported to the laboratory at the College of Science, University of Tikrit, for botanical identification.

Fifty grams of air-dried and finely crushed C. spinosa leaves were placed into a cellulose whipping and extracted using a Soxhlet extractor (Germany) with 250 mL of 96% ethanol. The solvent was subsequently evaporated using a rotary evaporator at 45°C until the process was completed. The extract was then subjected to lyophilization using a Lyophilizer (KARL KOLB, Alpha 1-2 LD plus, Germany) for freeze-drying, and the residues were stored at 4°C [13].

The analysis of the active compounds in the ethanolic extract of C. spinosa leaves was conducted utilizing GC-MS technology at Samarra University, College of Applied Sciences, Central Laboratory.

Experimental Design

Log phase promastigotes were seeded at comparable densities and treated with plant extract for indicated exposure time in vitro. Control cultures were kept under the same conditions as *Capparis spinosa* free and all experiments were performed in biological replicates for reproducibility. After treatment, RNA from both treated and untreated promastigotes were extracted using a commercial kit of RNA extraction as recommended by the manufacturer. The concentration and purity of the RNA were determined by visualization in a spectrometer congener using optical density measurements at 260 nm (absorbance) for quantification, while samples with adequate purity only were used for subsequent analyses. Equal amounts of purified RNA were reverse transcribed to complementary DNA (cDNA) with a reverse transcription kit according to the manufacturer's instructions and stored at −20 °C for further use.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from treated -promastigote with drug (50 and 100 µg/ml) as well as from untreated control cultures after 48 h of incubation. The RNA isolation was conducted with commercial kit and according to company protocol. Concentration and purity of the isolated RNA was determined by spectrophotometry using absorption at 260 and 280 nm, any samples with acceptable purity were used for subsequent analyses [3].

The same amounts of high quality RNA were reversely transcribed into cDNA by using the first-strand cDNA synthesis Kit according to the standard protocol supplied by manufacturer. The synthesized cDNA was then served as template for reverse transcription-qPCR (RT- qPCR) assays to analyze the relative gene expression levels of HSP70 and LACK genes, with a housekeeping reference gene used in normalization.

Primer Construction and Quantitative Real-Time PCR (qRT-PCR)

Gene expression of HSP70 and LACK was assessed after 48 h of treatment with plant extract at concentrations of 50 and 100 µg/ml using quantitative real-time PCR.

Two sets of the primers for qRT-PCR were designed as follows.

HSP70, LACK and house-keeping gene sequence from Leishmania tropica were used for designing target genes primers as well as normalizer gene using GenBank database. Primers were designed with Primer-BLAST to allow specificity, optimal Tm and the absence of secondary primer–dimer structures. The specificity of each primer pair was also confirmed in silico before the assay.

qRT-PCRs were performed in a Light Cycler instrument, using the standard light cycler protocol and SYBR Green chemistry. The reaction mixture in each well consisted of synthesized cDNA templates, gene-specific forward and reverse primers, SYBR green master mix, nuclease-free water to a final volume as indicated by the manufacturer. Premedication amplification in an optimized condition with initial heat denaturation, cycle's steps composed of heat denaturation primer binding and extension [2-5]. Reactions were performed in triplicate, with no-template controls to rule out contamination. A melting curve analysis was carried out at the end of each run to verify that only specific products were amplified. Primer information is provided in Table 1.

Table 1: Primers for *Leishmania tropica /major* gen expression (RT-PCR)

5il	Primer name	Sequence (5'-3')	Size bp	References
LACK	F	GAACTACGAGGGTCACCTGAA	165	8
	R	AGACCGTAGTCGCTGTCCAC		
HSP70	F	GCAACCAGATCACCATCACC	150	8
	R	AGTACGCGTAGTTCTCCAGG		
18s (housekeeping)	F	GCGAAACGCCAAGCTAATAC	116	designed
	R	CCAGTGAAGGCATTGGTTTT		

Relative Gene Expression Analysis

Relative expression levels of HSP70 and LACK genes were calculated using the comparative threshold cycle ($2^{-}\Delta\Delta Ct$) method.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using appropriate statistical software. Differences between treated and control groups were evaluated using suitable statistical tests, and a p-value of ≤ 0.05 was considered statistically significant (GraphPad Prism, San Diego, CA, USA).

RESULT

Using the plant extract as a treatment for L. tropica promastigotes led to a significant change in the expression of HSP70 and LACK genes compared to the gene expression of the control group.

Expression Gene of HSP70

Using the plant extract at a concentration of 50ug/ml resulted in a significant increase in the gene expression of the HSP70 gene (3.741 ± 0.9261) compared to the gene expression of the control group (1.426 ± 0.3839) when using the same concentration of the Capparis Spinosa extract as shown in Table 2 and Figure 1.

Table 2: Descriptive statistics of HSP70 gene expression in *Leishmania tropica* promastigotes treated with Capparis Spinosa extract 50 ug/ml compared with the control group

Group	N	Mean ± S.E	P-value
Control	10	1.426 ± 0.3839	0.04
Ivermectin (50 µg/ml)	10	3.741 ± 0.9261	

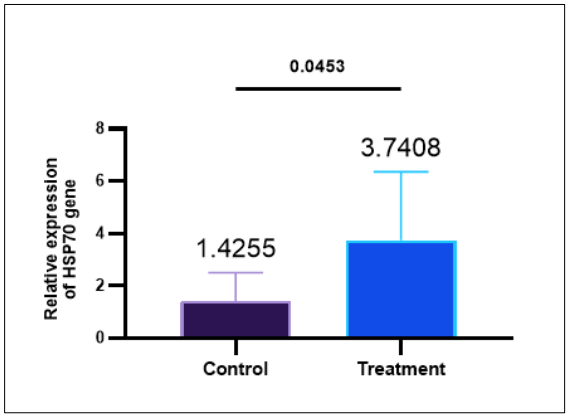

Figure 1: Capparis Spinosa extract 50 ug/ml

Figure 1. Effect of Capparis Spinosa extract 50ug/ml on HSP70 gene expression in *Leishmania tropica* promastigotes under in vitro conditions.

Using the plant extract at a concentration of 100 ug/ml resulted in a significant increase in the gene expression of the HSP70 gene (4.78 ± 0.7119) compared to the gene expression of the control group (1.02 ± 0.05926) when using the same concentration of the Capparis Spinosa extract as shown in Table 3 and Figure 2.

Table 3: Descriptive statistics of HSP70 gene expression in *Leishmania tropica* promastigotes treated with Capparis Spinosa extract 100 ug/ml compared with the control group

Group	N	Mean ± S.E	P-value
Control	10	1.02 ± 0.05926	0.04
Ivermectin (100 µg/ml)	10	4.78 ± 0.7119	

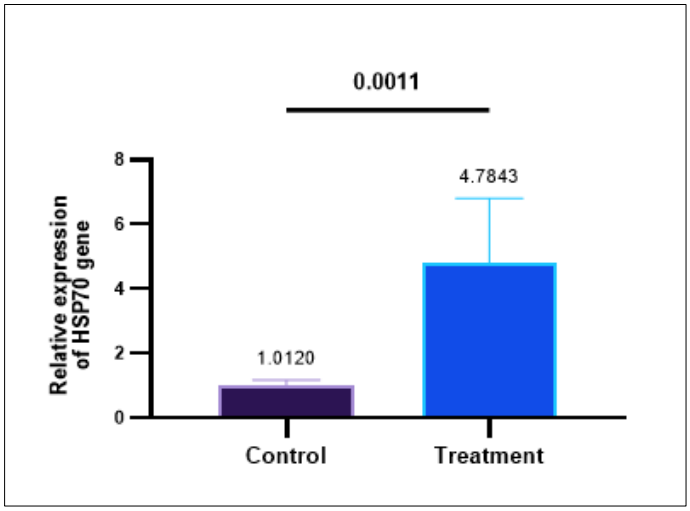

Figure 2: Capparis Spinosa extract 100 ug/ml

Figure 2. Effect of Capparis Spinosa extract 100ug/ml on HSP70 gene expression in *Leishmania tropica* promastigotes under in vitro conditions.

LACK Gene Expression

This study conducted molecular biology experiments, using 50 µg/ml Capparis spinosa extract for intervention. The measured LACK gene expression level in the experimental group was 3.180 ± 0.7831, which was significantly elevated compared with the 0.9905 ± 0.1334 recorded in the control group. These results correspond to Table 4 and Figure 3.

Table 4: Descriptive statistics of LACK gene expression in *Leishmania tropica* promastigotes treated with Capparis Spinosa extract 50 ug/ml compared with the control group

Group	N	Mean ± S.E	P-value
Control	10	0.9905 ± 0.1334	0.04
Ivermectin (50µg/ml)	10	3.180 ± 0.7831	

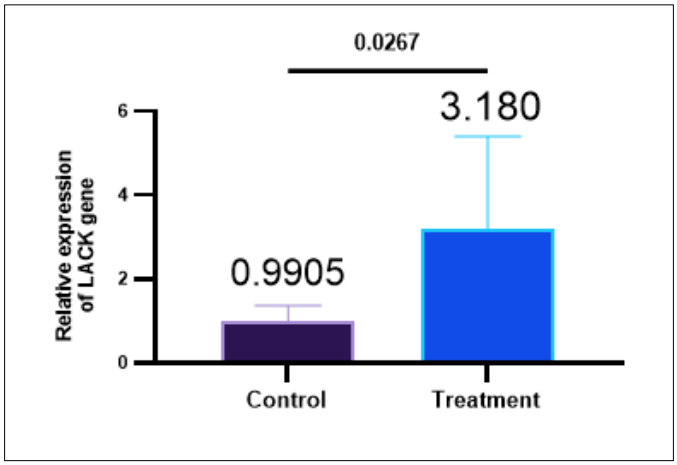

Figure 3: Capparis Spinosa extract 50 ug/ml

Figure 3. Effect of Capparis Spinosa extract 50 ug/ml on LACK gene expression in *Leishmania tropica* promastigotes under in vitro conditions.

In this study, we treated samples with 100 µg/ml Capparis spinosa extract, and measured the expression level of the LACK gene as 4.519 ± 0.6183. This value was significantly higher than the 0.3999 ± 0.002930 recorded for the control group. For full details of the related results, see Table 5 and Figure 4.

Table 5: Descriptive statistics of LACK gene expression in *Leishmania tropica* promastigotes treated with Capparis Spinosa extract 100 ug/ml compared with the control group

Group	N	Mean ± S.E	P-value
Control	10	0.3999 ± 0.002930	0.04
Ivermectin (100 µg/ml)	10	4.519 ± 0.6183	

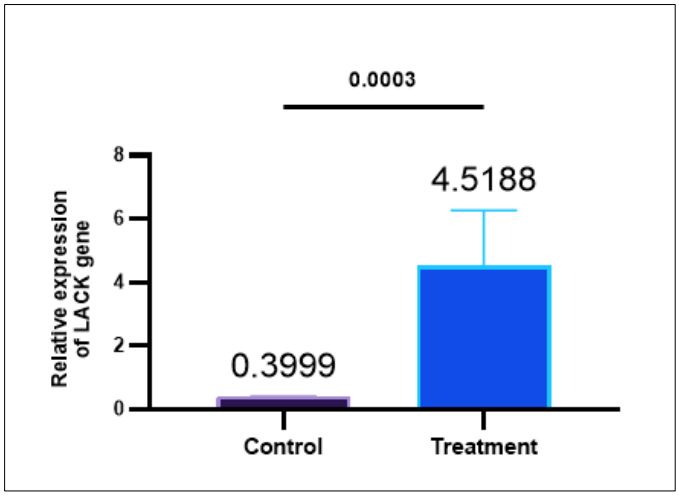

Figure 4: Capparis Spinosa extract 100 ug/ml

Figure 4. Effect of *Capparis Spinosa* extract 100 ug/ml on LACK gene expression in *Leishmania tropica* promastigotes under in vitro conditions.

DISCUSSION

The present study demonstrates a significant upregulation of both HSP70 and LACK gen expression in Leishmania promastigotes following treatment with *C.spinosa* ethanolic extract at concentration of 50 ug/ml and 100 ug/ml.The observed dose -dependent increase in gen expression provides valuable insights into the molecular mechanisms underlying the antileishmanial activity of this medical plant.

According to existing research [11], heat shock protein Hsp70 is a core component of cellular networks, and participates in protein folding and molecular chaperone regulation processes. the preliminary screening of caper bush (Capparis spinosa) extracts conducted in this study detected polyphenolic substances(including flavonoids and alkaloids) likely contribute to the observed effects through multiple mechanisms such as , Disruption of Protein Synthesis: phenolic compounds can interfere with protein synthesis by binding to ribosomal subunits or by modifying the cellular redox environment, leading to accumulation of misfolded proteins that trigger the heat shock response, Oxidative Stress Induction: The production of reactive oxygen species (ROS) by phenolic compounds can cause oxidative degeneration to proteins, lipids, and DNA, further activating the stress response pathways and upregulating HSP70 expression and Membrane Perturbation : The interaction of flavonoids compounds with the parasite membrane may alter membrane fluidity and permeability, compromising cellular integrity and triggering stress signaling cascades [1].

The academic community has confirmed that these compounds exert antibacterial effects by disrupting the outer membrane of microorganisms, interfering with protein synthesis to inhibit the growth of microbial populations. At present, the precise mechanism underlying the anti-leishmanial activity of *C. spinosa* remains unclear. According to reference [7], its antiparasitic activity derives from the polyphenolic compounds it contains; lacanin, a component found in its extracts, can block the protozoan's sugar uptake, leading to the parasite's aggregation [1].

Existing research [4], has confirmed that the LACK gene is involved in signal transduction, RNA processing, and cell cycle regulation. Literature [7], records that caper phenolics arrest the cell cycle of Leishmania promastigotes via iron chelation, and the present study speculates that this mechanism is the trigger for the concentration changes that occur during the temporal expression of the LACK gene. The increased expression might represent an adaptive response to the stressful conditions ,as the parasite attempts to modulates its signaling pathways to survive the toxic effects of the extract.

Both HSP70 and LACK genes showed similar patterns of upregulation, suggesting coordinated stress responses. However, the higher fold-change observed for *LACK* at 100 µg/mL (approximately 11.3-fold increase) compared to *HSP70* (approximately 4.7-fold increase) indicates that different regulatory mechanisms may be involved or that LACK is more sensitive to the specific effects of the extract components.

CONCLUSIONS

This study demonstrates the effect of different concentration of Capparis spinosa extract on the Hsp70 and Lack genes of *leishmania tropica* under in vitro.

This effect of the chemical compounds in Capparis spinosa extract on the gene expression concentration of the *leishmania tropica* highlights the importance of conducting broader studies on the effect of the chemical compounds in this extract on the gene expression of the *leishmania tropica.*

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