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Research Article

Diagnostic of Fatty Acid Extracted from Peganum Harmala Seed and Effect Study on The Growth the Leishmania Tropica Promastigotes In Vitro

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Abstract:

Fatty acids were isolated from the seeds of Peganum harmala, a legume, with petroleum ether. The fatty acids were analyzed using HPLC and were determined to comprise 48.9% Hexadecanoic acid, 2.3% Heptadecanoic acid, 13.7% Octadecanoic acid, and 2.8% Tetradecanoic acid. The current investigation demonstrated that the fatty acids extracted from the seeds of Peganum harmala are more advantageous. The current study's data revealed significant differences $p<0.05$ across various doses of the petroleum ether oil extract of Peganum harmala and differing exposure durations. The study's findings indicated that the 4 mg/dl dose of the oil extract exhibited the highest inhibitory activity against Leishmania tropica at 24 h 22.50 ± 1.04, 48 h 41.50 ± 1.19, 72 h 64.75 ± 2.66, and 96 h 101.25 ± 0.25. Conversely, the lowest inhibitory dose of 1 mg/dl was recorded at 24 h 66.00 ± 2.20, 48 h 108.75 ± 1.65, 72 h 154.50 ± 1.71, and 96 h 207.00 ± 1.91, in comparison to the control groups 75.25 ± 0.85, 156.00 ± 1.83, 320.00 ± 2.16, 522.50 ± 9.31respectively.

Fatty acids exhibited a beneficial effect in suppressing the proliferation of the Leishmaniasis cutanea parasite, namely Leishmania tropica promastigotes, ex vivo.

This research presents a possible alternative to pharmaceutical substances, such as streptolyticum, utilized in treating the parasite responsible for cutaneous Leishmaniasis.

This study concludes that fatty acids extracted from harmful plant seeds enhance the parasiticidal efficacy with increasing concentration, and prolonged treatment duration correlates with a more significant percentage of parasite elimination.

Key words: Leishmania tropica, promastigotes, fatty acid Peganum harmala, HPLC, Leishmaniasis

Introduction

Harmala is a perennial herbaceous plant from the Nitrariaceae family, predominantly in the arid Mediterranean and Asian areas. This plant possesses therapeutic and ecological significance (Zha et al., 2020). P. harmala, commonly called Syrian Rue, is a medicinal herb in the semi-arid regions of North-West India, North Africa, and Central Asia. This plant is referred to as "Espand" in Iran, "Harmel" in North Africa, and "African Rue," "Mexican Rue," or "Turkish Rue" in the United States , bushy wildflowers attain a height of 60-90 cm and possess a short creeping root (Mahmoudian *et al*., 2002). The Harmal species exhibit antibacterial activities against diverse microorganisms (Saeidi *et al*., 2015; Tehrani *et al*., 2014), effectively eliminating or suppressing pathogenic pathogens. Various antimicrobial agents are employed for this purpose, including antibacterial, antiviral, and antifungal substances. Each exhibits distinct mechanisms of action to inhibit infection (Saeidi *et al*., 2015; Tong *et al*., 2015).

Leishmaniasis is one of the six principal infectious illnesses globally, predominantly found in tropical and subtropical regions, transmitted by sand flies as vectors (Karimkhani *et al*., 2017). Leishmaniasis is a worldwide endemic illness and a public health concern in seven developing nations: Afghanistan, Iran, Algeria, Peru, Brazil, Saudi Arabia, and Syria (Karimkhani *et a*l., 2017). The World Health Organization (WHO) classified leishmaniosis as a neglected tropical illness, reporting approximately 2 million new cases annually and over 350 million individuals at risk (Alvar *et al*., 2012).

The primary motivations for addressing leishmaniosis are to mitigate the infected wound's severity and alleviate the patient's psychological and emotional distress (Arana, 2018). CL creates largely deformed lesions on exposed body regions like the face, arms, and legs, but it is not fatal. Thus, people affected are humiliated and ostracized, preventing them from marrying, studying, or working correctly, causing economic and psychological harm. (Bailey *et al*. 2019). CL has leishmaniasis, host, and transition immunity-based clinical manifestations. CL typically causes a painless papule or ulcer where the female sand fly is fed. A variable percentage of cases self-heal within 3-18 months, often forming an ulcer with a crust of dried secretions. Most Old World CL comes from *L. major, L. aethiopica*, and *L. tropica*. All species can produce several slow-healing lesions with massive, misshapen scars. (Burza *et al*. 2018). Clinical trials have failed to justify CL therapies, which have limited efficacy (Gonzalez *et al*., 2009)Two recent

studies comprehensively analyzed these treatments (Burza *et al*., 2018). Current harmful drugs were used for an extended period of treatment. CL-specific systemic treatments include pentavalent antimonials, amphotericin B, and miltefosine. Even though VL patients have distinct pharmacokinetic features from CL patients, drugs that work for VL are investigated and adapted for CL patients (Guery et al., 2017;Solomon *et al*., 2013)

Despite the availability of synthetic chemical compounds designed to combat the parasite, research has demonstrated that these treatments have toxic effects that manifest throughout therapy, in addition to the substantial financial burden associated with these manufactured drugs. Consequently, there is a necessity to explore effective natural substances that are both safe and productive. Scientific investigations have also validated the efficacy of plant-derived compounds with antimicrobial properties, highlighting their medicinal significance in treating various ailments, often with minimal side effects (Al Doori ,2020 ; Oyi *et al*., 2002). This study aimed to extract fatty acids from harmala plant seeds, characterize them using HPLC, and subsequently compare their effects on the growth of Leishmania tropica promastigotes in a laboratory setting. This research aims to identify and develop safe, accessible, cost-effective alternative treatments for inhibiting parasite growth.

Materials and Methods

Name	Con . %
Hexadecanoic acid	48.9
Heptadecanoic acid	2.3
Octedecanoic acid	13.7
Tetradecanoic acid	2.8

Harmal seeds collection

The Harmal seeds were procured from the local markets of Tikrit city and subsequently classified at the Medicinal Plants Center for Mosul Dam Development, which is associated with the Iraqi Ministry of Agriculture. The seeds were purged of soil and adhering debris, subsequently packed in paper bags, and stored in moisture-free conditions until required.

Preparation of plant extract using Soxhlet

The seeds of the harmala plant were pulverized using an electric grinder. Fifty grams of harmala powder and harmala seeds were placed in a filter paper batch within a Soxhlet apparatus, followed by adding 500 milliliters of petroleum ether to extract the fatty acids. The extraction persisted for 7 hours each day until the solvent became colorless, after which the alcoholic extract was concentrated using a rotary evaporator under a vacuum at 40 °C (Hasan *et al*., 2019).

Parasite Culture

The reference strain L. tropica was acquired from the Biotechnology Research Center at Al-Nahrain University. The promastigotes of the indigenous Iraqi Leishmania strain (MHOM/IQ/1992) were effectively cultivated in RPMI-1640 media, supplemented with 10% fetal calf serum, at a temperature of 25°C.

Viability assays on promastigotes

Parasites in the promastigote stage were moved from the stock culture medium to RPMI-1640 enriched with 10% fetal calf serum (FCS), pH 7.2.

To determine the LC50 of the fatty acids extracted from C. longa and its seeds, equivalent concentrations of the fatty acids (4, 3, 2, and 1 mg/dl) were employed in this study to assess their efficacy against L. tropica promastigotes in vitro. These concentrations were introduced into the promastigote culture medium (10 ml) within test tubes. Consequently, 23.2×10^4 promastigotes/ml were introduced into each tube containing 10 ml of media and cultured at 25 °C for 96 hours. Negative controls (cultures devoid of fatty acids, serving as a baseline for comparison) were employed, and each tube was thoroughly mixed at the conclusion of every 24-hour period.

Statistical Analysis

The data from the current study were statistically examined employing Tukey's test to compare the means. The significance level of 0.05 was utilized in the test. The analysis of the current data was conducted using SPSS version 22.

Results

Analysis of fatty acid components in harmala seeds via HPLC methodology The HPLC analysis of the petroleum ether extract post-saponification revealed the following constituents: Hexadecanoic acid 48.9%, heptadecanoic acid 2.3%, octadecanoic acid 13.7%, and tetradecanoic acid 2.8%, as presented in Table No. (1).

Table (1): Fatty acids discovered via the HPLC method in the petroleum ether extract of Peganum harmal

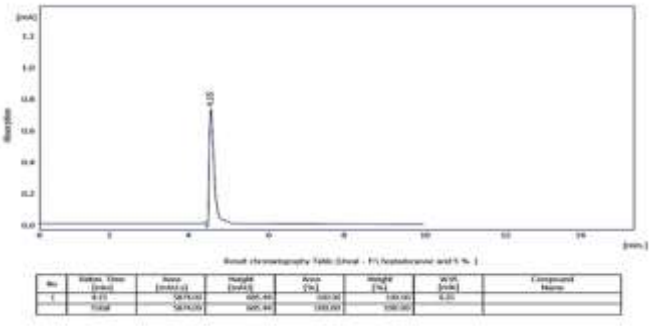

Figure (1): result chromatography table (uncal-f:\heptadecanoic acid 5%)

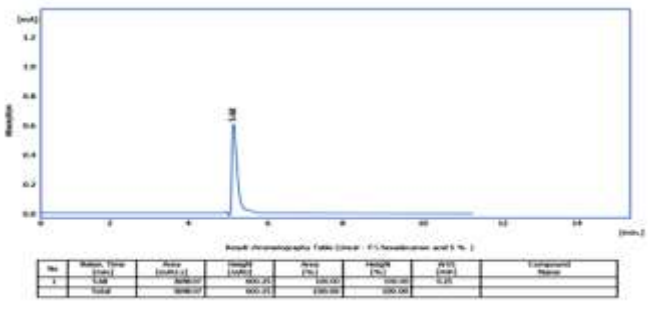

Figure (2) result chromatography table (uncal-f:\Hexadecanoic acid 5%)

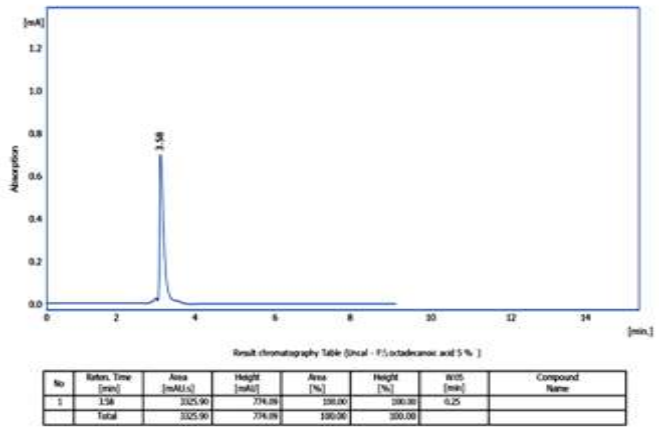

Figure (3) result chromatography table (uncal-f:\Octedecanoic acid 5%)

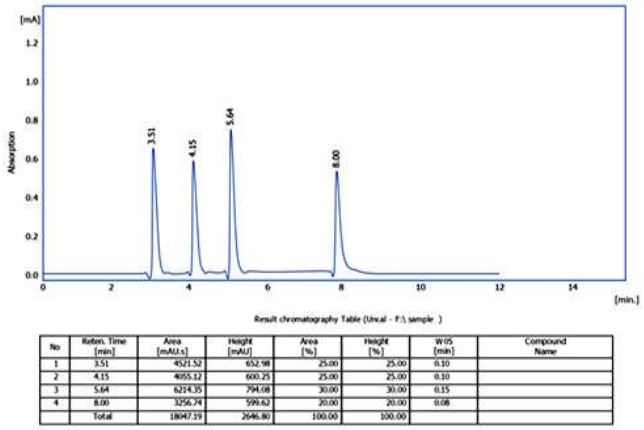

Figure (4) result chromatography table (uncal-f:\sample)

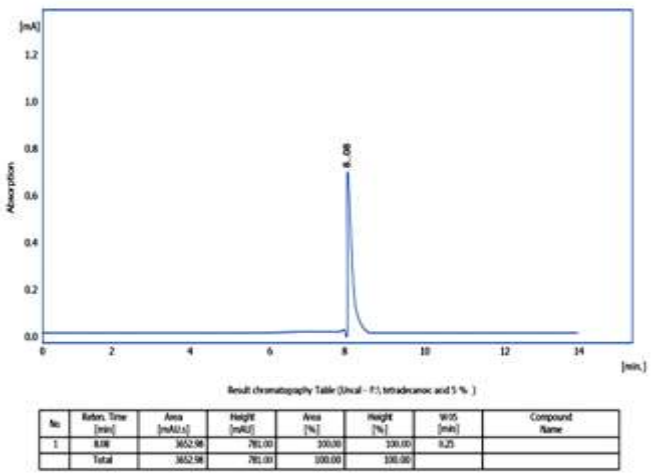

Figure (5) result chromatography table (uncal-f: Tetradecanoic acid 5%)

Data of current study showed there is significant differences ($p<0.05$) between different doses of Oil Petrelium ether Peganum harmala L extraction and different times of exposure. Our results showed (4 mg/dl) dose of oil extraction scored highest activity inhibition against Leishmania tropicalis at 24 hour (22.50 ±1.04), 48 hour (41.50 ±1.19), 72 hour (64.75 ±2.66), and 96 hour (101.25 ±0.25), while least dose inhibition was (1 mg/dl) at 24 hour (66.00 ±2.20), 48 hour (108.75 ±1.65), 72 hour (154.50 ±1.71), and 96 hour (207.00 ±1.91), compared to controls (75.25 ±0.85, 156.00 ±1.83, 320.00 ±2.16, and 522.50 ±9.31) respectively (table (2) and figure (6).

Table 2; inhibition effect of Oil Petrelium ether *Peganum harmala* L extraction on *Leishmania tropicalis* with different rats

Dose	Oil ***Petrelium ether Peganum harmala L*** extraction							
	$10^4 \times 25$ Zero dose							
	Time exposure (hour)							
	After 24 hour		After 48 hour		After 72 hour		After 96 hour	
	Mean	Error	Mean	Error	Mean	Error	Mean	Error
1 mg	66.00 [c] A	2.20	108.75 [c] B	1.65	154.50 [c] C	1.71	207.00 [c] D	1.91
2 mg	53.25 [b] A	1.65	83.75 [b] B	1.44	106.75 [b] B	1.31	145.00 [b] C	1.08
3 mg	41.00 [b] A	1.08	68.75 [b] B	1.03	104.00 [b] C	1.29	142.25 [b] D	1.89
4 mg	22.50 [a] A	1.04	41.50 [a] B	1.19	64.75 [a] C	2.66	101.25 [a] D	2.36
controls	75.25 [c] A	0.85	156.00 [d] B	1.83	320.00 [d] C	2.16	522.50 [d] D	9.31

Small letters compare with vertical view
Capital letters compare with horizontal view
Different letters refer to significant different ($p<0.05$)
Duncan test used to detect differences among means

Lethal Concentration-50 (LC50) of fatty acids Was Extracted from harmala seeds following a duration of 96 hours of Incubation at a concentration of 20 mg/dL, yielding a recorded value of 56%.

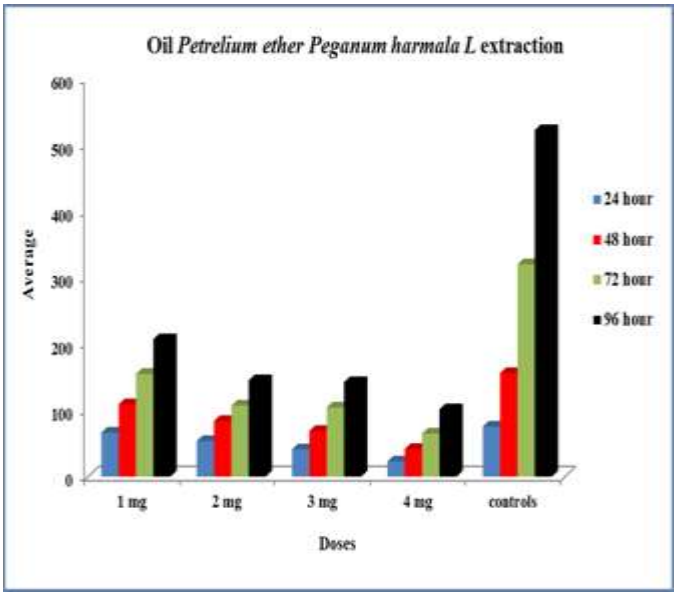

Figure 6; inhibition effect of Oil Petrelium ether Peganum harmala L extraction on Leishmania tropicalis with different rats

Discussion:

This study examined the anti-leishmaniasis efficacy of Promistigot, derived from petroleum ether fatty acids of Harmal plant seeds, on the proliferation of *Leishmania tropica* in a laboratory setting.

This indicates the effective activity of the Harmal plant on the cells of *Leishmania tropica*.

The results of the current research showed the effect of the Harmal plant directly on the mortality of Promistigot for the Leishmania tropica parasite. The time period of 96 and 72 hours affected the parasite more for a concentration of 1 ml/dl, the concentration ratio was 207.00 ± 1.91 and 154.50 ± 1.71 respectively compared to the control group 522.50 ± 9.31 and 320.00 ± 2.16, respectively.

The second concentration of 2 ml/dl of the plant extract showed an increase in the mortality rate, as the parasite concentration in the 24-hour period was 53.25 ±1.65 and the highest mortality rate was in the 96 and 72-hour period, where the parasite concentration was 106.75 ±1.31 and 145.00 ±1.08 respectively compared to the control group. As for the concentration of 3 ml/dl, the highest mortality rate was in 96 and 72 hours 104.00 ±1.29 and 142.25 ±1.89 respectively for the control group. The highest mortality rate was given in the 96 and 72-hour period at the concentration of 4 ml/dl, where it was 101.25± 2.36 and 64.75±2.66. The data of the current study showed significant differences ($p<0.05$) between different doses of the ethereal birch oil extract *Peganum harmala L.* and different exposure times respectively (Table (2) and Figure (6).

Since our study is experimental and no similar study has been conducted before, the current results were unique and do not have similar results to other studies. The reason for the increased mortality is attributed to the fact that the obtained compounds Hexadecanoic acid, Heptadecanoic acid, Octedecanoic acid and Tetradecanoic acid may have been linked in the metabolic pathway of the promastigote stage in the parasite, which led to the poisoning and death of the organism. Chemical connections may have occurred on the cell membrane, preventing the exchange of nutrients or preventing the entry and exit of nutrients into the cell, thus killing the parasite.

Peganum harmala seed extracts showed in vitro activity against the L. tropica parasite. The active compounds of fatty acids have a greater inhibitory effect than other extracts. As a result, the compounds may be responsible for this Effect. The present results support the results of (Roknai, *et. al*., 2021) that harmala is effective in treating leishmaniasis and its toxic effect is much less than the effects of Amphotericin B and Glucantime.

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