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Research Article

Evaluation of *in vitro* biocompatibility of tropical tasar (*Antheraea mylitta*) pupal oil using normal mammalian cell lines

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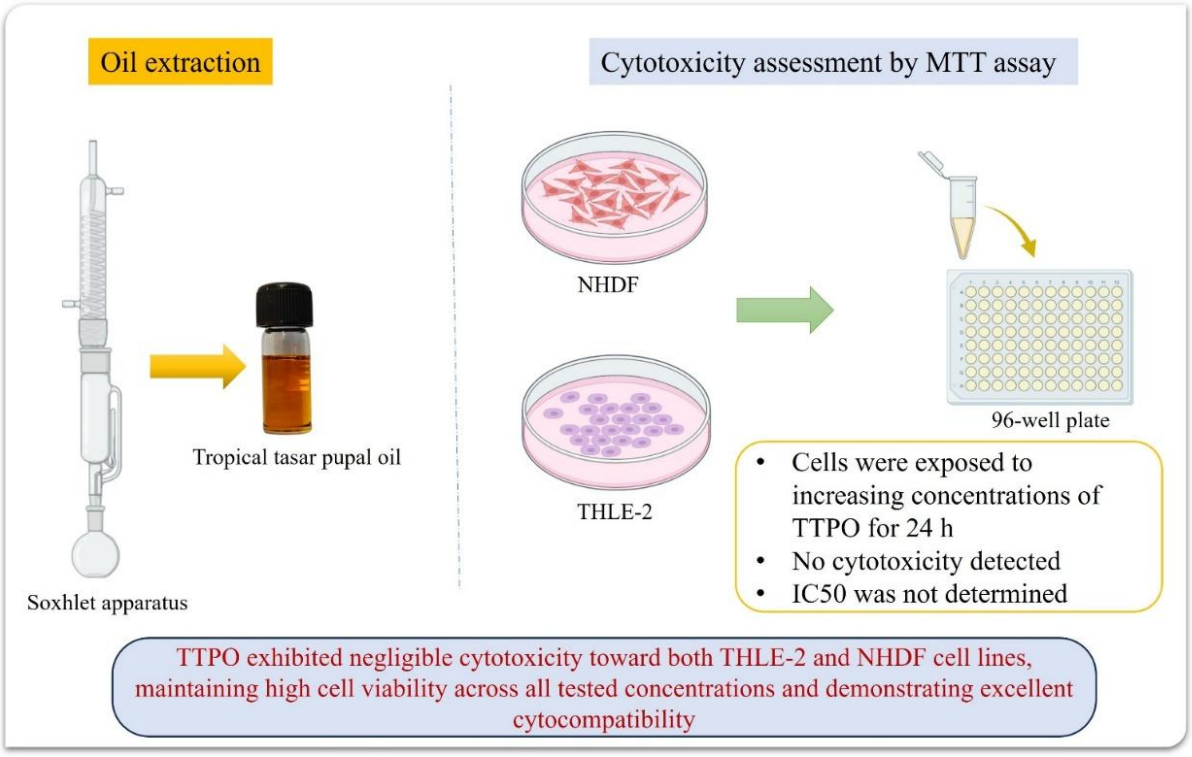
Abstract

Tropical tasar pupal oil (TTPO), extracted from *Antheraea mylitta* pupae, has gained increasing attention as a sustainable source of nutritionally valuable lipids due to its high content of bioactive unsaturated fatty acids and potential health-promoting properties. Before its application in food, nutraceutical, or pharmaceutical products, evaluation of its biological safety is essential. This study aimed to assess the *in vitro* cytotoxicity of TTPO in normal human dermal fibroblasts (NHDF) and human liver epithelial cells (THLE-2) through the MTT assay. The results demonstrated that TTPO exhibited negligible cytotoxicity toward both cell lines, with no significant reduction in cell viability, indicating excellent cytocompatibility and no adverse effects under the experimental conditions. These findings suggest that TTPO is biologically safe for normal mammalian cells and support its further development as a functional food, nutraceutical, and pharmaceutical formulation. Nevertheless, comprehensive *in vivo* toxicity studies and long-term safety assessments are required to further validate its safety and facilitate its future commercial applications.

Keywords: *Antheraea mylitta*, normal cell lines, cytotoxicity, *in vitro*, tasar pupal oil

Graphical Abstract



1. Introduction

In recent years, edible insects have gained increasing attention as a sustainable nutritional resource, supported by growing consumer acceptance and favorable regulatory developments in several countries[1,2]. Edible insects are rich in valuable nutrients, including high-quality proteins, beneficial fatty acids, vitamins, and minerals, making their nutritional composition comparable to that of conventional plant- and animal-derived foods[3,4]. Fatty acids are biologically important macromolecules that play indispensable roles in maintaining cellular integrity and physiological functions [5]. They serve as essential structural components of cell membranes and participate in numerous cellular signalling pathways that regulate metabolism, inflammation, and immune responses [6,7]. Among fatty acids, polyunsaturated fatty acids (PUFAs) have attracted considerable attention owing to their well-documented health-promoting properties and potential applications in functional foods and nutraceuticals [8]. Traditionally, omega-3 fatty acids are obtained from dietary sources such as flaxseed, walnuts, leafy green vegetables, and fish oil. However, growing demand for sustainable and alternative sources of omega-3 fatty acids has prompted researchers to explore insect-derived lipids [9]. Owing to their unique lipid composition, insect oils exhibit favourable bioavailability and have emerged as promising candidates for functional food development.

Among these, silkworm pupae oil has recently gained considerable interest as a novel source of alpha-linolenic acid (ALA) [10,11]. Recent investigations have extensively characterised the lipid composition and biological properties of oils extracted from different silkworm species [12–14]. In addition to their high omega-3 content, insect-derived lipids are rich in several bioactive compounds, namely tocols, phytosterols, and carotenoids, contributing to their nutritional & therapeutic value [15]. These bioactive constituents have been associated with several beneficial biological activities, including antioxidant, anticancer, antimicrobial, vasculoprotective, hypocholesterolemic, antidiabetic, hepatoprotective, and anti-ulcerative effects [16–19].

Silkworm pupae oil is rich in 60-70% unsaturated fatty acids, with ALA as the principal component. Its nutritional profile and bioactive compounds support its potential in functional foods and therapeutic applications [20,21]. Various advanced analytical techniques, including gas chromatography-mass spectrometry (GC-MS), gas chromatography with flame ionization detection (GC-FID), high-performance liquid chromatography (HPLC), and Fourier transform infrared spectroscopy (FTIR), have been widely employed for the qualitative and quantitative characterization of edible oils [9,22,23]. In our previous studies, the effect of drying temperature (50-80 °C) on the quality of tasar silkworm pupae oil (TPO) was systematically investigated [9].

Although several studies have been conducted on the characterization, antioxidant, and antimicrobial potential of TTPO, limited information is available on its cytocompatibility with normal mammalian cell lines. Such data are essential to establish the biosafety of TTPO and facilitate its translation into commercial applications. Therefore, the present study evaluated the *in vitro* cytotoxicity of tasar pupal oil against normal cell lines using the MTT assay to assess its safety profile.

2. Experimental section

2.1 Sample procurement

Tasar cocoons were obtained from local Seri-farmers, State Silk Board Unit, Fatehpur, Uttar Pradesh, India.

2.2 Chemicals required

DMEM media, fetal bovine serum, MTT reagent, Human Fibroblast Expansion Basal Medium, Trypsin EDTA 0.05%, and DMSO from Gibco were of analytical grade.

2.3 Drying of pupae

Initially, pupae were removed from the tasar cocoons and dried at 50 °C in a hot air oven (Scientech: SE-127) until to achieve constant weight. Further, the dried pupae powder proceeded to the extraction procedure [9].

2.4 Oil extraction

TTPO was extracted by employing a Soxhlet technique using n-hexane [24] as a solvent. Following extraction, n-hexane was discarded using a vacuum rotary evaporator (Hanshin SN. -HS-300SN). Thereafter, the TTPO was transferred to an airtight Amber bottle and stored in the refrigerator for further investigations.

2.5 MTT cytotoxicity assay

Preparation of the TTPO sample

A 32 mg/mL stock solution was made in DMSO. Plain culture media were used to create serial two-fold dilution ranges (1000-5 µg/mL). The vehicle control consisted of plain media supplemented with 1% DMSO.

Cell culture

THLE-2 and NHDF cell lines were procured from ATCC and maintained under appropriate culture conditions. 10% inactivated FBS, penicillin (100 IU/mL), and streptomycin (100 µg/mL) were added to the culture medium used to culture the stock cells. Cultures were maintained in standard conditions containing 5% CO_2 at 37°C until they reached confluence. The cell lines were then separated using trypsin (0.05%) and centrifuged at 1000 rpm for five minutes. The supernatant was discarded, and the cell pellet was carefully resuspended in 1 ml of culture medium. Cell viability was assessed by preparing a homogeneous single-cell suspension.

MTT assay protocol

The cytocompatibility of the THLE-2 and NHDF was assessed using the MTT assay. Briefly, 100 µL of the prepared cell suspension was dispensed into each well of a pre-labelled 96-well plate and incubated for 24 h at 37 °C in an incubator maintained with 5% CO_2 to facilitate cell attachment and growth. After the incubation period, the spent culture medium was gently discarded, and the cell monolayer was gently rinsed

with plain culture medium to eliminate any residual components.

Subsequently, 100 µL of the test samples prepared at different concentrations was added to the designated wells, followed by a further 24 h incubation under identical culture conditions. At the end of the treatment period, each well was supplied with 100 µL of freshly prepared MTT reagent (5 mg MTT dissolved in 10 mL of 1× PBS). The plates were returned to the incubator for an additional 4 h at 37 °C in a 5% CO_2 atmosphere, permitting viable cells to generate insoluble purple formazan crystals through mitochondrial metabolic activity. After removing the MTT solution, the crystals were dissolved by adding 100 µL of DMSO and the plates were gently shaken to ensure complete solubilization before measuring the absorbance at 590 nm using a multimode microplate reader (SpectraMax i3X, Molecular Devices) [25,26].

2.6 Statistical approach

The mean values ± S.D. were used to report the experimental data. Graphs were generated through OriginPro 24 (OriginLab, Northampton, MA, USA).

3. Results and Discussion

3.1 TTPO yield

The present report involved the extraction of TTPO from tasar pupae dried at 50 °C using the Soxhlet apparatus. The oil yield obtained was around 17.35 %, as our previous study reported [9].

3.2 Cytotoxicity assessment

The safety assessment of TTPO against THLE-2 and NHDF cell lines was evaluated using the MTT assay after 24 h of exposure at concentrations ranging from 1.526 to 100 µg/mL. TTPO exhibited minimal cytotoxic effects across the tested concentration range, with only a slight dose-dependent reduction in cell viability (Tables 1 and 2). Cell viability remained greater than 50% even at the maximum tested concentration (100 µg/mL), preventing the calculation of the IC_{50} value (Figure 1). At 100 µg/mL, the mean percentage inhibition was 37.17 ± 0.23% for THLE-2 cells and 37.79 ± 1.449% for NHDF cells, indicating that TTPO possesses good cytocompatibility and does not exert significant toxic effects on normal mammalian cells under the experimental conditions.

Table 1. *In vitro* cell viability assessment of TTPO in NHDF cell lines. The experimental data are represented as mean ± SD based on duplicate independent experiments (n=2)

NHDF								
Sample name	Conc. µg/ml	n=1		n=2		Mean % Inhibition	S.D.	IC_{50} (µg/ml)
		Abs at 590 nm	% Inhibition	Abs at 590 nm	% Inhibition			
Vehicle control	0	0.727	0.00	0.752	0.00	0.00	0.000	
TTPO (µg/ml)	1.562	0.715	1.58	0.734	2.39	1.99	0.574	IC_{50} not calculated due to lesser inhibition
	3.125	0.703	3.31	0.726	3.45	3.38	0.095	
	6.25	0.650	10.66	0.683	9.12	9.89	1.087	
	12.5	0.609	16.23	0.631	16.14	16.19	0.062	
	25	0.563	22.54	0.595	20.92	21.73	1.151	
	50	0.528	27.39	0.517	31.29	29.34	2.761	
	100	0.460	36.77	0.460	38.82	37.79	1.449	

Table 2. *In vitro* cytocompatibility assessment of TTPO in THLE-2 cell lines. The experimental results are observed as mean ± SD from two independent datasets (n=2)

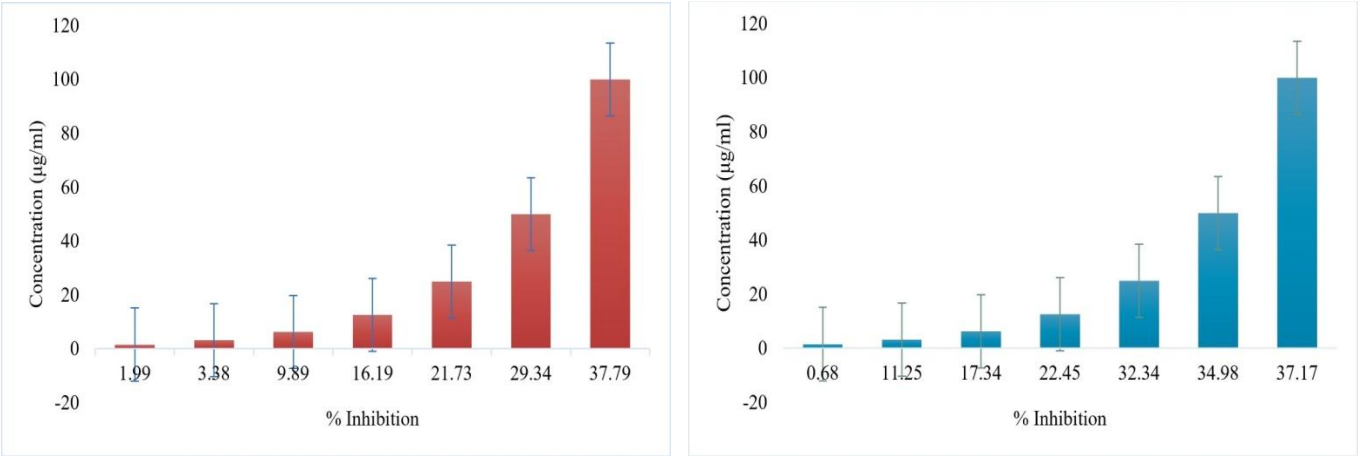
THLE-2								
Sample name	Conc. µg/ml	n=1		n=2		Mean % Inhibition	S.D.	IC_{50} (µg/ml)
		Abs at 590 nm	% Inhibition	Abs at 590 nm	% Inhibition			
Vehicle control	0	1.161	0.00	1.157	0.00	0.00	0.000	
	1.562	1.158	0.21	1.143	1.16	0.68	0.674	IC_{50} not calculated
	3.125	1.030	11.26	1.027	11.23	11.25	0.018	

TTPO (µg/ml)	6.25	0.984	15.24	0.932	19.44	17.34	2.968	due to lesser inhibition
	12.5	0.930	19.87	0.867	25.02	22.45	3.640	
	25	0.780	32.81	0.788	31.87	32.34	0.668	
	50	0.763	34.26	0.744	35.69	34.98	1.011	
	100	0.731	37.01	0.725	37.33	37.17	0.230	

According to ISO 10993-5 guidelines for the evaluation of biomedical samples, a reduction in cell viability of below 30% is generally regarded as non-cytotoxic, while greater reductions indicate increasing levels of cytotoxicity. Although a mild concentration-dependent decline in viability was observed at higher TTPO concentrations, cell survival remained above 50% throughout the tested range, demonstrating that the oil exhibits only low cytotoxic potential and maintains acceptable cytocompatibility under the present experimental conditions [27].

The low cytotoxic response of TTPO towards NHDF and THLE-2 cell lines suggests that it exerts minimal toxic effects on both cell lines, indicating good compatibility with skin and liver cells, even at the highest concentration tested. These results highlight the potential safety of TTPO for cosmetic applications and demonstrate the importance of cytotoxicity assays in determining the suitability of topical formulation ingredients [28,29]. The favourable cytocompatibility observed in both hepatic and dermal normal cell lines is particularly important because these cell types are commonly employed for preliminary safety evaluation of bioactive compounds intended for nutraceutical, pharmaceutical, and cosmetic applications. Maintaining high metabolic activity in these normal cells suggests that TTPO is unlikely to induce acute cellular damage at biologically relevant concentrations. The observed cytocompatibility may also be associated with the characteristic lipid composition of TTPO. Previous compositional analyses have demonstrated that tasar pupal oil is rich in α-linolenic acid, along with other nutritionally important fatty acids [9,10,30]. PUFAs are known to contribute to membrane integrity and cellular homeostasis and generally exhibit good biocompatibility when administered at appropriate concentrations [31], which may partly explain the minimal cytotoxic response observed in the present study. However, the precise mechanisms underlying the cellular compatibility of TTPO require further investigation through oxidative stress, apoptosis, and inflammatory biomarker analyses.

Srivastava et al. reported that tasar silkworm pupal oil did not adversely influence cell proliferation, and the determined IC_{50}:180 µg/mL further confirms its low cytotoxicity [10]. Similarly, in a more recent investigation on eri pupal oil, Mishra et al. observed comparable cytotoxicity patterns in both THLE-2 and NHDF cell lines, where no IC_{50} value was detected, indicating negligible cytotoxic effects [16]. Additionally, similar observations have been documented for other insect-derived oils; namely, black soldier fly oil displayed no significant cytotoxic effects on HaCaT keratinocytes, peripheral blood mononuclear cells and primary human dermal fibroblasts with IC_{50} values above 200 µg/ml [32–34]. Moreover, the low cytotoxicity observed for several essential oils provides additional evidence supporting the application of insect-derived oils in topical and cosmetic formulations [35].



(a) (b)

Fig 1. Dose-dependent cytotoxic effect of TTPO on normal mammalian cell lines determined by the MTT assay. (a) NHDF (b) THLE-2 cell lines showing percentage inhibition following 24 h exposure to increasing concentrations of TTPO. Values are presented as mean ± SD (n=2).

Conclusion

The present study demonstrated that tropical tasar pupal oil (TTPO) exhibits favorable *in vitro* biocompatibility toward normal mammalian cell lines (THLE-2 and NHDF) as determined by the MTT assay, indicating minimal cytotoxicity within the tested concentration range. These findings provide important preliminary evidence supporting the biological safety of TTPO and suggesting its potential as a natural lipid source for future food products, nutraceuticals, and biomedical applications. However, as the current investigation was limited to *in vitro* cytocompatibility assessment, further studies involving *in vivo* toxicity evaluations are required to comprehensively establish its safety profile and facilitate its translation into practical applications.

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