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Cytotoxic Activities of Different Solvent Extracts of *Tirmania nivea* and *Terfezia claveryi* against HepG2 and L929 Cells

E A Elsayed[1,2]*, F D Alsahli[3,4], H A El Enshasy[5,6] and M A Wadaan[1]

[1] Bioproducts Research Chair, Zoology Department, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

[2] Natural and Microbial Products Department, National Research Centre, Dokki, Cairo 12311, Egypt

[3]Zoology Department, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

[4] Life Science and Environment Research Institute, National Centre for Genome Technology, KACST, Riyadh, Kingdom of Saudi Arabia

[5] Institute of Bioproduct Development, Universiti Teknologi Malaysia (UTM), Johor 81310, Malaysia

[6] City of Scientific Research and Technology Applications, New Burg Al Arab, Alexandria 21934, Egypt

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Desert truffles constitute an unexplored source for naturally occurring bioactive compounds. In the current work, different solvents have been used to extract bioactive components from *T. nivea* and *T. claveryi*. All tested extracts showed cytotoxic activities against HepG2 and L929 cells in a dose-dependent manner. Hexane extracts of *T. nivea* and *T. claveryi* gave highest toxicities of 91.23±0.03 and 92.7±0.01%, respectively, at 1 mg/mL, while the ethyl acetate extract resulted in toxicity of 94.5±0.02at 1 mg/mL in case of *T. claveryi*, which is higher by about 6% than that obtained for *T. nivea*. Furthermore, morphological examination showed that cells are gradually affected by increasing extract concentration.

Keywords: Truffles, Biological activity, Cytotoxic, HepG-2, L929

Introduction

The history of traditional and complementary medical applications of natural resources goes back to the time of Sumerian civilization, and many documents have been reported about the use of different desert truffles in ancient times[1]. Originally, truffles were used as precious food components and served as delicate food in European coutries[2]. They are rich in different dietary constituents, including carbohydrates, proteins, lipids, minerals and vitamins[3-5]. Desert truffles have been widely used in treating many microbial diseases[2]. Moreover, they have drawn a great scientific attention for their various bioactive characteristics; i.e. antimicrobial, antioxidant, anti-inflammatory and anticancer[6-8]. According to World Health Organization, cancer is considered as one of the most threatening diseases affecting a great percentage of human population[9]. Cancer treatment involves many therapeutic drugs which have severe side effects on human health[10]. Nowadays, there is constituent search for alternative therapies that provide a promising potential to replace medically applied cancer therapeutics[11]. This is motivated by the huge psychological and physiological side effects of such cancer treatment practices. Therefore, natural resources (microorganisms and plants) provide a more suitable alternative, where the major treatment side effects can be avoided[12]. Until now, there is little information found in the literature about different biological activities of truffles, especially desert truffles. Accordingly, the current work aimed at investigating the anticancer activities of different solvent extracts of two desert truffle species found in Saudi Arabia habitats; namely *Tirmania nivea* and *Terfezia claveryi*.

Materials and methods

Samples and extraction

Samples of *Tirmania nivea* and *Terfezia claveryi* were collected from Riyadh province, KSA with the help of mycologists in the Department of Microbiology, KSU. Sample preparation and extraction using methanol, 70% methanol, water, ethyl acetate and hexane were performed as previously described[13]. Stock solutions were prepared in DMSO.

Cell cultivation and cytotoxic assay

MCf-7 cancer cells were grown in DMEM supplemented with 10% serum, 100x, 1%

*Author for Correspondence
E-mail: eaelsayed@ksu.edu.sa

antibioticsolution, in a humid-CO_2 incubator (ShelLab, USA) at5% CO_2, 37°C. On the day of assay, cells were detached from growing surface, centrifuged andwashed twice with sterile PBS buffer solution. Viable cellconcentration was determined using Trypan Blueexclusion[3]. Grown cells were treated with different workingsolutions of truffle extracts ranging from 0.0 to1000 µg/mL.

Afterwards, the cytotoxic activities were determined screened using standard MTT assay. Control wells were only treated with DMSO ≤ 0.5%). The assay was performed as we previously described[14,15]. Prepared cells were seeded in 96 wellculture plates with a final concentration of 10^4 cells/100 µL/well, and then were grown for 24 h. Afterwards, the medium was aspirated and substituted with fresh medium supplemented with differentconcentrations of different prepared extracts, and then grown further for 24 h. Plateswere microscopically examined for morphological changes. Then, MTT (10 µL, 5 mg/mL in PBS) was added for4 h, and the developed formazan crystals were dissolved using 200 µL of DMSO. Absorbance was measured at 550 nm. Cell viabilitywas calculated in relation to the control. Concentrationsproducing 50% decrease in cell growth were taken as IC_{50} values.

Morphological examination

Cells exposed to different concentrations of prepared truffle extracts were microscopically examined for morphological changes using an inverted contrast microscope (Nikon Eclipse T500, Japan) at 20x magnification.

Statistical analysis

Statistical analysis was performed using completely randomized design by MSTAT-C software by One Way Analysis of Variance (ANOVA) followed by Duncan test. Results arepresented as means ± standard deviation (S.D), and significant differences were denoted at $p \leq 0.05$.

Result and Discussion

Anticancer activities of different extracts of *T. nivea* and *T. claveryi*

Different solvent extracts of *T. nivea* and *T. claveryi* have been evaluated for their cytotoxic effects against both HepG2 (Fig. 1) and L929 (Fig. 2) cells. Both cell lines were treated with increasing concentrations of extracts 0.0-1000 µg/mL. Generally, it can be seen that the biological activity of the extracts for both truffle types increased gradually with increasing extract concentration. It can be seen that all tested extracts exhibited variable cytotoxic activities against both cell lines. For HepG2 cells, the MeOH, 70% MeOH and H_2O extracts of both *T. nivea* and *T. claveryi* showed only moderate activities, for which we were not able to obtain IC_{50} values. Furthermore, the hexane and ethyl acetate extracts of *T. nivea* showed only their cytotoxic activities at concentrations ≥ 500 µg/mL. On the other hand, the same extracts for *T. claveryi* showed potential activities starting from 62.5 µg/mL. Additionally, both hexane extracts of *T. nivea* and *T. claveryi* showed comparable toxicities at 1 mg/mL (91.23±0.03 and 92.7±0.01%, respectively), while the highest ethyl acetate extract obtained at 1 mg/mL in case of *T. claveryi* (94.5±0.02) was higher by about 6% than that obtained for *T. nivea* (89.4±0.01%).

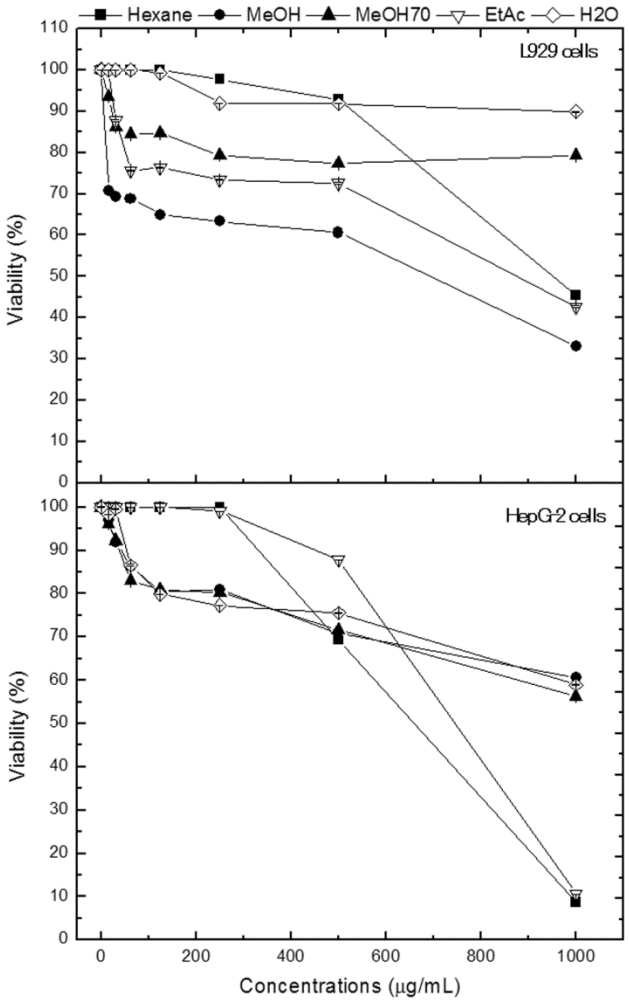

Fig.1 — Effect of different solvent extracts of *T. nivea* on the viability of HepG-2 and L929 cells. Data are expressed as means±SD

Comparing these results with those obtained for L929 cells, it can be seen that L929 cells exhibited the same trend as HepG2 cells for MeOH, 70% MeOH and H_2O extracts. Moreover, both hexane and ethyl acetate produced comparable toxicities at the highest dose tested (54.62±0.06 and 57.3±0.14%, respectively) in case of *T. nivea*. On the other hand, in case of *T. claveryi*, the highest toxicity obtained by ethyl acetate extract (94.13±0.02) was higher by 9.32% than the highest toxicity obtained using hexane solvent (84.81±0.02). Concerning IC_{50} values, results showed that the ethyl acetate and hexane solvents of *T. claveryi* gave the most effective solvent extracts IC_{50} values, ranging from 376.2±0.03 to 595.85±0.91 µg/mL. The morphological examination of treated cells with different solvent extracts of both truffles showed that cells were adversely affected upon using increasing concentrations of the applied extracts. Images obtained for both cells affected by different extracts of *T. nivea* (Fig. 3) showed that cells were detached from the surface of culturing plate with concurrent blubbing and deformation and finally suffered from death. Although results showed similar trends in cell response to different extracts, however, the response of the cells varied to a great extent. Our results are consistent with our earlier reports on the cytotoxic activities of different bioactive compounds isolated from various natural resources[13,16,17]. Different cell types vary in their biological behavior according to their inherent differences in membrane structures and organization. Furthermore, preliminary works carried out on desert truffles showed similar results against cancer cells. Dahham *et al*.[1] found that the hexane and ethyl acetate extracts of *T. claveryi* showed potential cytotoxic effects against U-87 MG cells, while methanolic and ethanolic extracts showed moderate activities. The cytotoxic activities of desert truffles have been attributed to the presence of different polysaccharides, which exhibited potential anti-proliferative activities against PC3 and MCF-7 cell lines[18-20].

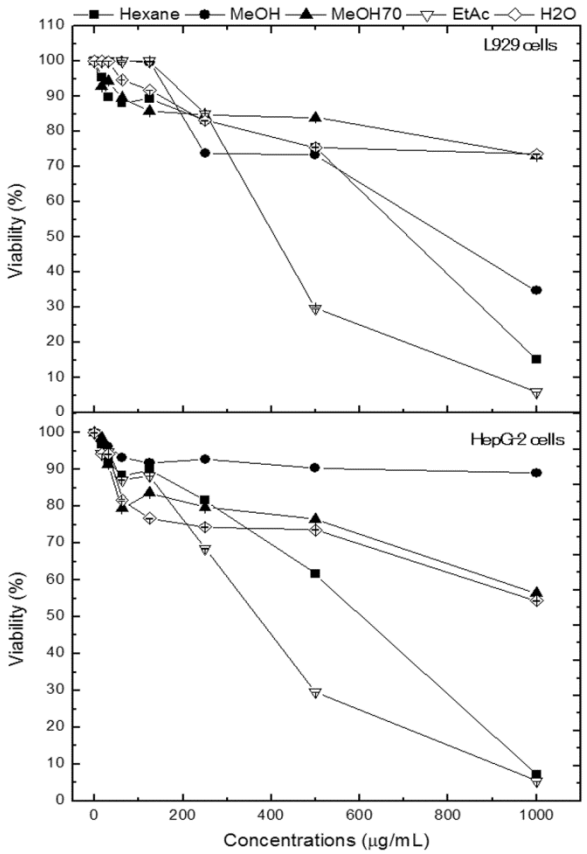

Fig. 2 — Effect of different solvent extracts of *T. claveryi* on the viability of HepG-2 and L929 cells. Data are expressed as means±SD

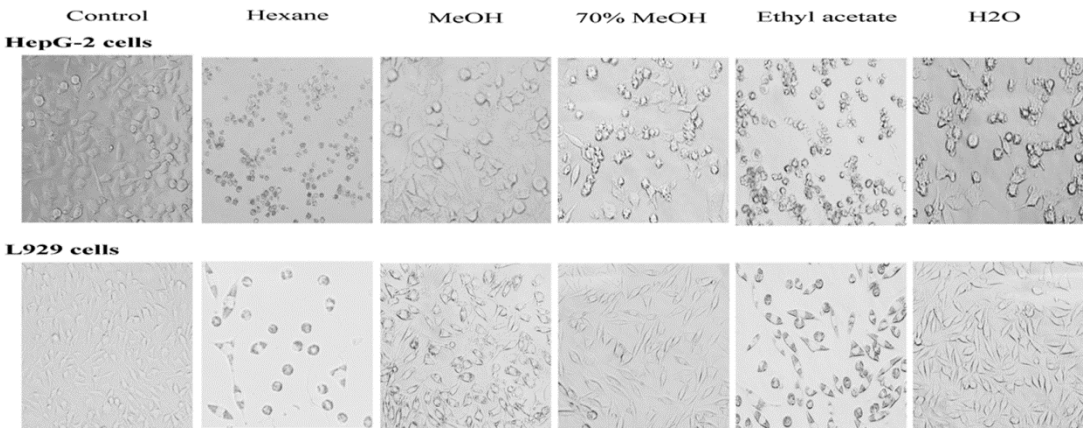

Fig. 3 — Effect of different solvent extracts (1 mg/mL) of *T. nivea* on the morphological characteristics of both HepG-2 and L929 cells (magnification 20x)

Conclusion

The current investigation showed possible cytotoxic activities of different solvent extracts of *T. nivea* and *T. claveryi* fungal samples collected. The extracts were tested against HepG2 and L929 cells. Results showed that both hexane and ethyl acetate extracts showed promising cytotoxic activities in both cell lines, where the cell viability was greatly affected by the increase in extract concentration. Moreover, the treated cells also showed some morphological changes as affected by the extracts. The current results can be considered as preliminary investigations, which pave the road for further work aiming at identifying the active components in different truffle extracts.

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