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Date Palm Fruit Do Not Induce Indirect Anthelmintic Activity

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In the wake of anthelmintic resistance many researchers screen natural plant products for any anthelmintic activity. Fruits such as papaya, pineapple and figs have shown promising results. Date palm fruit (*Phoenix dactylifera*) is a very popular food in the Middle East but little is known about its anthelmintic activity. Some researchers have shown that date palm fruit extract directly affect murine roundworm and whipworm *in vitro* and *in vivo*. Some researchers have shown that IL-22 is a key component in worm expulsion by increased mucin secretion in the intestine. We wanted to explore the possibility of an indirect anthelmintic effect mediated by date palm fruit. Using human LS 174T intestinal cells treated with IL-22 and date palm fruit aqueous extract, we were unable to induce mucin-4 expression detected by ELISA. Our results indicate that date palm fruit may not have indirect anthelmintic activity.

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Introduction

Parasitic helminth infection remains an important health burden worldwide. Control is principally attained through limited available classes of chemotherapy. The problem is also compounded by the development of resistance in livestock and loss of efficacy in humans (Lanusse et al., 2015). This has led many to screen natural plant-based products for anthelmintic activity. Enzyme from fruits such as papaya, pineapple and figs have shown great potential to be developed into novel anthelmintic (Mansur et al., 2014). Another plant compound which have gained interest is tannin. Tannin from various plant extracts have been shown to affect larval and adult helminths in vitro (Papachristou et al., 2009). The role of tannin in mediating anthelmintic effect have also been factorially demonstrated. Date palm fruit or its scientific name *Phoenix dactylifera* is a tannin rich fruit popular in the Middle East. Apart from being wholesome and nutritious date palm fruit is revered for its nutraceutical potential. It has been shown to be good in the control of diabetes and cardiovascular diseases as well as possessing anticancer, antimicrobial, antifungal and anthelmintic activity (Khalid et al., 2017). Conventional anthelmintic agents affect worm directly either by compromising worm cellular structure (benzimidazole) or acts as a depolarizing neuromuscular blocker resulting in spastic paralysis (pyrantel). Killed or paralysed worms are then easily expelled by peristalsis. Secretion of mucus by goblet cells is thought to play an important role in worm expulsion (Turner et al., 2013). Regulation of the immune response in helmintic infection is by Th2 type cytokines (Moreau and Chauvin 2010). IL-22 has been shown to be the key cytokine in worm expulsion by inducing upregulation of mucin genes (Behnsen et al., 2014).

Materials and Methods

Human colonic adenocarcinoma LS174T cells

LS174T colorectal adenocarcinoma cells (was purchased from Addexbio Technologies®) were cultured in Eagle's Minimum Essential Medium (was purchased from Addexbio Technologies®) containing 10% fetal bovine serum and 1% penicillin streptomycin 10,000U (all were purchased from Thermo Fisher Scientific), 37°C in the presence of 5% CO_2. All experiments were carried out in cells with more than 80% confluence.

Preparation of date palm fruit extract

100g of Ajwa date palm fruit (was purchased from SAG Retail Sdn Bhd (Malaysia) were ground using mortar and pestle before extracted with 300ml of distilled water at room temperature. The mixture was filtered and stored in -80°C before freeze-dried. The lyophilized extracts were kept in -80°C (Vayalil, 2002).

Supplementation of LS174T cells with date palm fruit extract

Confluent LS174T cells were incubated with culture medium containing various concentrations of date palm fruit extracts (25, 12.5, 6.25, 3.125 mg/ml for 24 and 48 hours at 37°C in the presence of 5% CO2. Media

containing extracts were filtered through 0.22µ membrane filter.

IL-22 exposure of LS174T cells

After removing the supplementation media (as described above) from LS174T cells, the cells were treated with 10 and 100 ng/ml of IL-22 (was purchased from RandD System). The IL-22 was dissolved in supplemented EMEM media. The cultured cells were incubated for 24 and 48 hours at 37°C in the presence of 5% CO_2.

Cell viability assay

LS174T cells were seeded at $1x10^5$ cells per well in 96-well plate. A 20µl of CellTiter 96® Aqueous One Solution reagent (was purchased from Promega, USA) was added into each well of the 96-well assay plate containing cells in 100µl of culture medium. The plates were incubated for 3 hours at 37°C in the presence of 5% CO_2. The absorbance of each well was read at 490nm using 96-well plate reader (Tecan Infinite Pro 200, Switzerland).

Human Mucin 4 Enzyme-linked Immunosorbent Assay (ELISA)

Mucin 4 (MUC4) concentration of medium was measured by ELISA (was purchased from Elabscience) according to manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed using GraphPad PRISM software (version 12.0). The results are presented as medians and interquartile range. Differences between group were analyzed using Kruskal-Wallis test. *P*-value of 0.05 or less was considered statistically significant.

Results and Discussions

Cell viability testing using MTS assay in Fig. 1 (refer to Appendix I) showed that LS174T cells remain viable after 24 and 48 hours of incubation with various concentrations of Ajwa date palm fruit extract. There was also no significant difference in cell viability between the different concentrations used. Two concentrations (25mg/ml, 12.5mg/ml) which showed optimum cells viability were chosen for the actual experiment. Our experiment did not result in the detection of mucin 4 protein by ELISA. In the presence of 10ul of IL-22, the treatment of both 25mg/ml and 12.5mg/ml failed to induce LS174T cells to secrete mucin 4. Turner et al., in 2013 who used 10ul of IL-22 did not measure protein expression but instead measured gene expression of several mucin genes.

Infection with helminths have been established to be the T-helper 2 (Th2)-mediated protective immunity that is mediated by Th2 cells and cytokines they produced such as IL-4, IL-5, IL-9, IL-10 and IL-13 (Finlay CM et al., 2014). Typically, helminth infection is also associated with hypereosinophilia, considerable IgE production, mucus mastocytosis and goblet cell hyperplasia (Moreau and Chauvin, 2010).

On the other hand, IL-22 belongs to the IL-10 subfamily which is known as an anti-inflammatory and immunosuppressive cytokine. IL-22 is undetected in normal colonic mucosa but are abundant during severe colitis (Andoh et al., 2005). Study by Turner et al., in 2013 proved that interleukin 22 (IL-22) plays a pivotal role in goblet cell hyperplasia and able to increase mucin production by intestinal epithelium in certain settings. In present study, IL-22 was used to induce human mucin 4 (MUC4) production, but it is found that no mucin 4 protein detected via enzyme-linked immunosorbent assay (ELISA) test. This suggests that although there were no detectable proteins like mucin 4 genetic level upregulation may still be happening. In conclusion, rigorous *in-vitro* and *in-vivo* study regarding mechanism on helminth infection and expulsion need to be carried out to provide better understanding and best solution to cater this health problem worldwide.

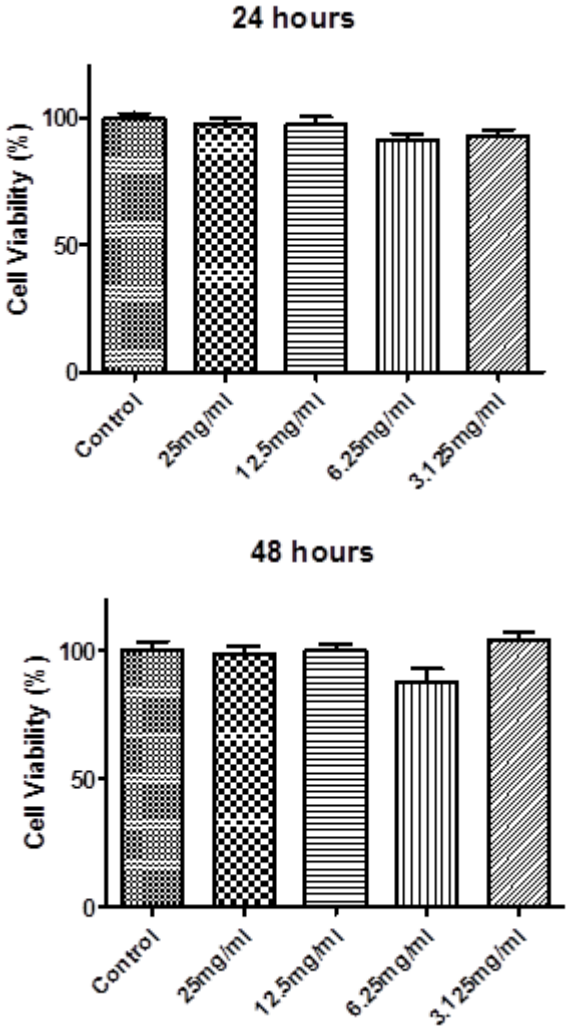

Fig.**1** The effect of Ajwa date palm fruit extracts on the rate of LS174T cells viability at 24 and 48 hours of incubation.

Conclusions

Our experiments showed that treatment of date palm fruit extract with IL-22, a key component in worm expulsion to LS174T cells did not induce enough mucin as detected by ELISA which may indicate the absence of an indirect anthelmintic property of date palm fruit.

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Conflict of Interest

All the authors declare that they have no conflict of interest.

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