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Edulitin 2, a Ribotoxin-like protein from *Boletus edulis*: Assessment of its Cytotoxic Effects on Colon Adenocarcinoma Cell Lines

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INTRODUCTION & AIM

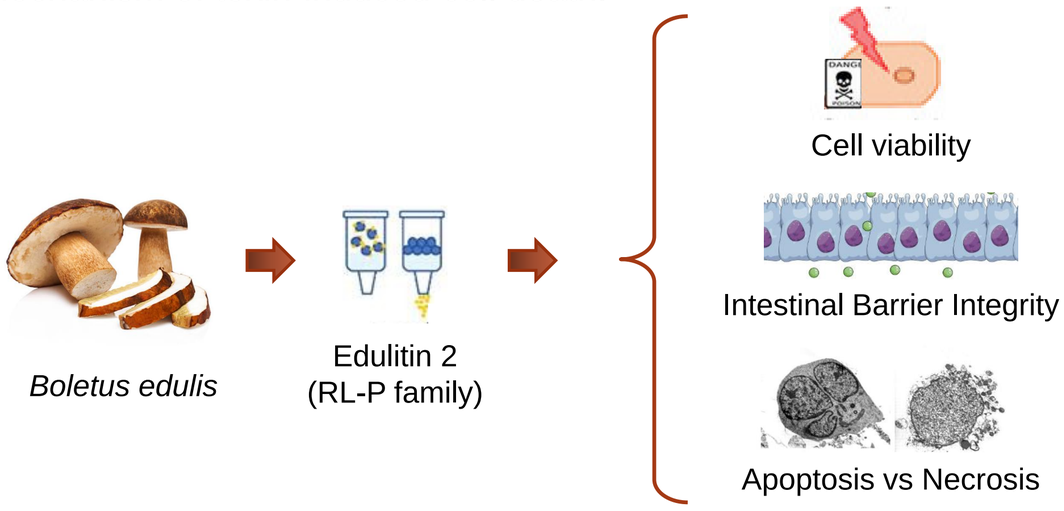
Edulitin 2 is a novel member of ribotoxin-like proteins (RL-P) from the prized edible mushroom *Boletus edulis* (known as 'Porcini') [1].

Research Context

RL-P, like ribosome-inactivating proteins (RIP), inhibit protein synthesis by targeting the sarcin-ricin loop of ribosomal RNA. Their cytotoxicity makes them attractive candidates for immunotoxins — engineered molecules that selectively destroy tumor cells [2]. However, immunotoxins based on non-self toxins may elicit neutralizing antibody responses, thus compromising therapeutic efficacy. RIP and RL-P derived from edible sources, coming into contact at low doses with our immune system through diet, could induce immunogenic tolerance, reducing the risk of adverse reaction and improving their clinical tolerability [3].

This study focuses on the effects of edulitin 2 on colon adenocarcinoma cell lines, Caco-2 and HT29, by assessing:

- Cell viability;
- Disruption of intestinal barrier integrity;
- Mechanism of toxin-induced cell death.



CONCLUSIONS

- Edulitin 2 induces a time-dependent reduction in cell viability, with significant cytotoxic effects observed only after prolonged exposure (48–72 h).
- Intestinal epithelial barrier integrity is compromised only after extended exposure, as evidenced by a decrease in transepithelial electrical resistance (TEER) values.
- Reduced immunofluorescence signal intensity of claudin-3 following 72 h of treatment confirms the disruption of tight junctions.
- Apoptosis appears to be the exclusive mechanism of cell death triggered by edulitin 2 treatment.
- Partial protection against edulitin 2-induced cytotoxicity is achieved using oxidative stress scavengers, suggesting a multifactorial involvement in cell death mechanisms.

REFERENCES & ACKNOWLEDGEMENTS

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METHODS

Cytotoxicity was assessed by an MTS reduction assay in Caco-2 and HT29 cell lines treated for 24, 48 and 72 h with increasing concentrations (10^{-10} – 3×10^{-5} M) of edulitin 2. Caco-2 cell monolayers integrity was evaluated by measuring Tran-Epithelial Electrical Resistance (TEER) at 0, 8, 24, 48 and 72 h post-treatment. Claudin-3 immunofluorescence was performed to assess tight junction integrity. Cell death was analyzed by Annexin V/PI flow cytometry. To investigate the role of oxidative stress, cells were pretreated with catalase (CAT) and sodium pyruvate (NaPyr) prior to edulitin 2 exposure.

RESULTS & DISCUSSION

Edulitin 2-induced Cytotoxicity in Colon Adenocarcinoma Cells

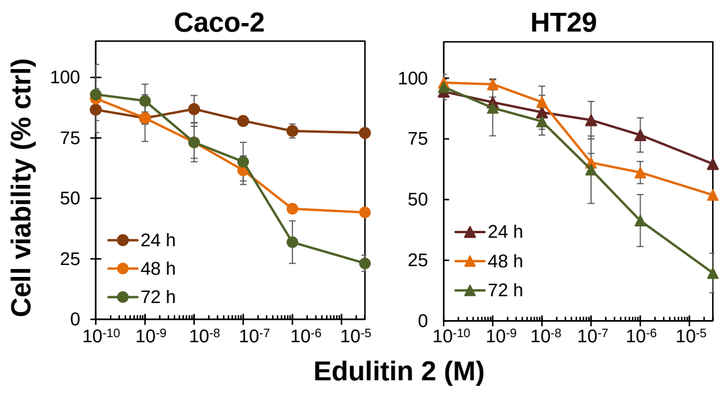

Figure 1. Edulitin 2 affected cell viability in dose– and time–dependent manner, reducing viability to 31,9% in Caco-2 cells and 41,4% in HT29 cells after 72 h of exposure to 10^{-6} M concentration.

Effects of Edulitin 2 on Epithelial Barrier Function

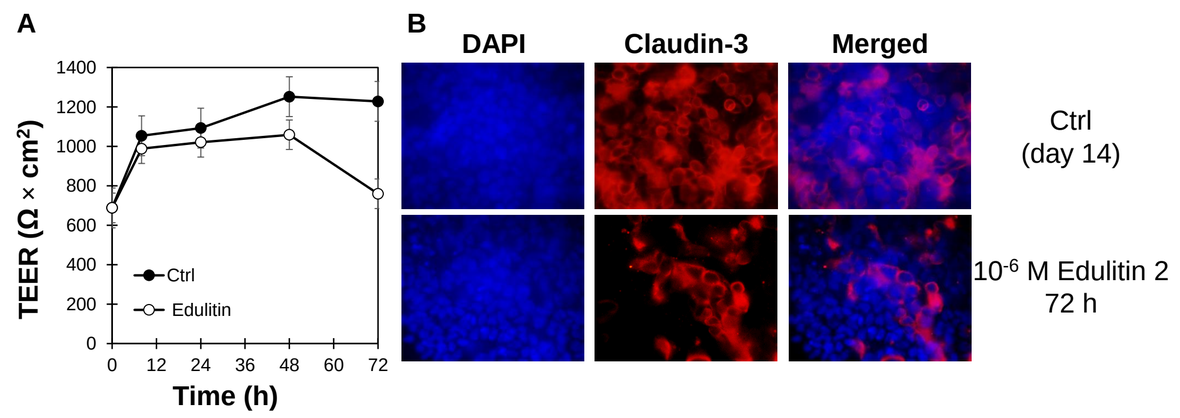

Figure 2. A. At 10^{-6} M concentration, edulitin 2 compromised epithelial barrier function, as evidenced by a reduction in TEER values by 1.2- and 1.6-fold in Caco-2 cell monolayers at 48 and 72 h post-intoxication, respectively. **B.** Exposure to 10^{-6} M of edulitin 2 also led to a decrease in claudin-3-associated immunofluorescence intensity, suggesting disruption of tight junctions.

Evaluation of Edulitin 2-induced Cell Death in Caco-2 and HT29 Cells

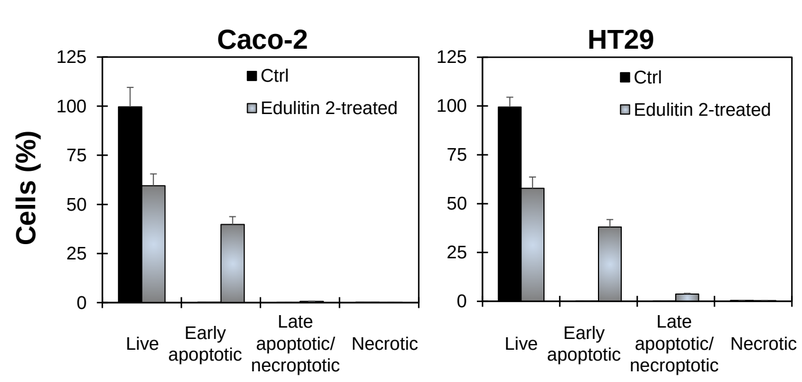

Figure 3. Edulitin 2 triggered apoptosis without the involvement of necrosis or necroptosis after 48 h at 10^{-6} M concentration.

Impact of ROS Scavengers on Edulitin 2-mediated Cytotoxicity in Caco-2 and HT29 Cells

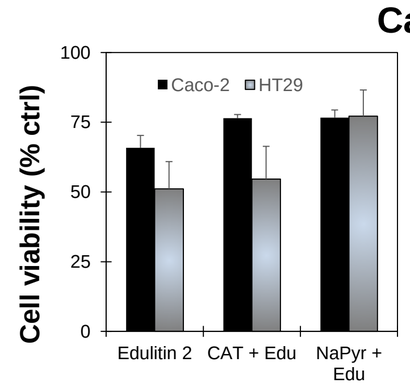

Figure 4. At 10^{-6} M concentration, the cytotoxicity induced by edulitin 2 was attenuated by preincubating with antioxidants, suggesting that multiple mechanisms may be involved in the pathogenesis of edulitin 2-mediated cytotoxicity.