# Use of HT-29 Cell Line to Investigate Toxicological Effects of Mycotoxins: a Mini Review

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**Abstract**

HT-29 is the human colon adenocarcinoma cell line mostly used to study human colon cancers. Because of its ability to resemble mature intestinal cells, its use is wide. HT-29 cell line is a suitable *in vitro* model for intestinal cells to monitor the toxicity of mycotoxins, because these cells correspond to one of the main organs of action of mycotoxins. The main studied parameters of the effects of mycotoxins on HT-29 cell line include cell viability, cell apoptosis or necrosis, oxidative damage, immunotoxicity and macromolecule synthesis. In this review we provide an introduction to HT-29 cell line and its use for research of toxicological effects of mycotoxins, which represents an important food contaminant.

**Keywords**: cell viability, HT-29, mycotoxins, oxidative stress

## 1. Introduction

Mycotoxins, secondary metabolites of microscopic fungi, pose a problem for a food safety. The prevalence of these naturally occurring toxins is an issue for animal and above all human health preservation. The danger of mycotoxins is that the absence of visible fungi does not mean the absence of the toxins [1]. Mechanism of synergism, antagonism, but also individual interactions of mycotoxins is still not well explained. Because of this, the use of cell lines seems to be a suitable choice [2, 3]. The cell lines from colorectal cancer are widely used as pre-clinical model systems. Comprehensive insights into cell lines molecular characteristics could improve model selection for biomedical studies [4]. The gastrointestinal tract (GIT) is the first place to interact, where mycotoxins are introduced into the organism from food or feed even at higher doses compared to the other tissues. Proliferating intestinal HT-29 cell line may be a useful model for studying the influence of mycotoxin toxicity because intestinal epithelium could be the primary target of mycotoxins [5, 6].

## 2. Mycotoxins and their effects on HT-29 cell line

Definition of mycotoxins is that they are natural products produced by fungi that evoke a toxic response even at low concentration to higher vertebrates and other animals by a natural way [7]. In addition to animals, they can also be phytotoxic or have antimicrobial effects. The most common producers of mycotoxins are *Aspergillus*, *Fusarium* and *Penicillium* species, which produce the most important classes of mycotoxins including aflatoxins, trichothecenes (deoxynivalenol), fumonisins, ochratoxin A and zearalenone [8, 9]. The effects of mycotoxins are different and the most dangerous of them are carcinogenic, teratogenic and mutagenic effects [10]. An overview of the most common mycotoxins is shown in Table 1. The diseases that mycotoxins cause are called mycotoxicosis. The

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**Table 1.** Overview of mycotoxins and their producers and occurrence

| Mycotoxins | Producers | Occurrence |
|---|---|---|
| Aflatoxin ($B_1$, $B_2$, $G_1$,$G_2$,$M_1$) | *Aspergillus flavus, A.parasiticus* | peanuts, corn, rice, milk, wheat, cheese, pepper |
| Deoxynivalenol | *Fusarium graminearum, F. culmorum* | oat, barley, wheat, corn |
| Fumonisin | *F. verticillioides, F. proliferatum* | corn, wheat |
| Ochratoxin A | *A. ochraceus, A. flavus, Penicillium verrucosum* | peanuts, coffee, bean, cheese, grape |
| Patulin | *P. patulum, P. expansum* | apple, pear |
| T-2 toxin, HT-2 toxin | *F. sporotrichoides, F. poae* | oat, wheat, barley, corn |
| Zearalenone | *F. graminearum, F. tricinctum, F. culmorum* | corn, hay |

most famous of these are ergotism, acute aflatoxicosis, alimentary toxic aleukia, Reye´s syndrome and cardiac beriberi. Despite modern technologies, mycotoxins still pose a threat to agricultural commodities and a risk to health, whether of animals or humans [11, 12]. An important intestinal barrier defence against natural toxins and pathogens is the epithelial monolayer in GIT. However, recent studies suggest that the mucus barrier and microbiota are the emerging targets of mycotoxins [13]. In 1964, HT-29 cell line was first time isolated from human colon adenocarcinoma. Initially, this cell line was used to study various aspects of human cancer biology. However, it was later found that these cells are able to express the characteristics of mature intestinal cells, such as enterocytes or mucus-producing cells. HT-29 cells are described by consuming high levels of glucose. For this reason, it is necessary to ensure a high concentration of glucose in the medium. Replacement of glucose in the culture medium with galactose results in reversible enterocyte differentiation. This finding led HT-29 cells to become a unique model for studying the molecular mechanisms of intestinal cell differentiation. Under suitable culture conditions, optionally after the addition of specific inducers, these cells can be modulated to express different enterocyte differentiation pathways. Because of this, HT-29 cells are considered a pluripotent intestinal cell line that can be used to investigate structural and molecular phenomena associated with cell differentiation. The use of HT-29 cell line as a model has several advantages, but also limitations. This cell line resembles small intestinal enterocytes in a differentiated phenotype in terms of its structure, the presence of hydrolases and the time course of the differentiation process. Nevertheless, HT-29 cells also have limitations because they are malignant cells with high glucose consumption, impaired glucose metabolism and although they have characteristics of small intestinal cell, they are colon cells. However, these cells cannot be compared to normal colon enterocytes because they contain hydrolases associated with microvilli that are not found in the colon [14]. The main studied parameters of the effect of mycotoxins on the cell line include cell viability, cell apoptosis or necrosis, DNA damage, oxidative damage, immunotoxicity and macromolecule synthesis (RNA, DNA, proteins) [15]. Over the last few years, researchers have used various methods to study the mechanisms of action of mycotoxins. Nevertheless, data on cytotoxic effects on mixtures of mycotoxins may vary depending on the type of cells exposed in a dose-effect ratio. In experiments with combinations of mycotoxins on cell lines, an enhanced cytotoxic effect was confirmed. It follows that the co-occurrence of mycotoxins in food or feed may increase the cytotoxic effect compared to the cytotoxic effect of mycotoxin alone [16]. HT-29 cell line is used to characterize the enteropathogenic effect of mycotoxins. In differentiated HT-29 cells, nutrient uptake is specifically affected depending on the nutrients studied and mycotoxin concentration [17]. Mycotoxins are known to affect oxidative stress in HT-29 cells. Mycotoxin triggers a rapid and concentration-dependent increase in reactive oxygen species (ROS) in HT-29 cells, which can be detected at a certain concentration. Mycotoxins have the potential to increase the proliferation of cancer cells, thereby increasing the total cellular protein content. It follows that ROS is normally a very rapid cellular response to toxic substances, but under appropriate conditions it can also

stimulate cell proliferation [18]. The study by [19], showed that the application of patulin caused significant decrease of viability and superoxide dismutase activity in HT-29 cell line. Decreased viability was observed in groups with concentrations from 0.39 to 100 μg.ml$^{-1}$. This study confirmed the cytotoxic effect of patulin on the HT-29 cell line due to the formation of oxidative stress and reduced viability. In the experimental studies conducted by [18], the effect of deoxynivalenol on the HT-29 cell line was investigated. Deoxynivalenol is able to participate in the development of oxidative stress and inhibition of proteosynthesis. Incubation of deoxynivalenol with HT-29 cells caused a significant increase in the transcription factor Nrf2, which is one of the key elements that control the antioxidant response in cells and thus play an important role in providing protection against toxic substances. As mycotoxins such as aflatoxin, zearalenone and ochratoxin A may co-exist in some foods, it is necessary to investigate these synergistic effects. The results of the combined toxic effects of these mycotoxins showed significantly increased epithelial permeability and modulated protein secretion in intestinal epithelial cells in HT-29 cell line [20]. The experiment performed by [21], showed the cytoprotective effect of epigallocatechin-3-gallate (EGCG) in combination with deoxynivalenol (DON) on the HT-29 cell line. EGCG is a polyphenolic substance found in green tea with significant anticancer effects. The results of their experiment showed that EGCG is involved in reducing mycotoxin-induced ROS. According to the study of [22], lutein has cytoprotective effect against DON, because application of 10 μM lutein with 250 ng/ml DON resulted in 95% viability in HT-29 cells. Antioxidant and free radical scavenging activity and immunomodulatory characteristics of lutein indicate that it has a potential as a treatment for DON-induced mycotoxicosis, however these results were taking place only *in vitro* and *in vivo* experiments are necessary to confirm this effect. The search for new natural substances can be helpful in developing new nutritional strategies to reduce the toxicity of mycotoxins and thus to improve food safety [21]. Other studies are dealing with mycotoxins as potential treatment against colorectal cancer. The study by [23] demonstrated that reduced-gliotoxin triggered rapid cell detachment and induced the apoptosis of HT-29 cells. Furthermore, gliotoxin induced excessive ROS production, resulting in the activation of endogenous and exogenous apoptopic pathways. Another mycotoxin, verrucosidin, induced selective cell death in microenvironmental conditions operating *via* GRP78 down-regulating activity in HT-29. This may be due to the fact that it inhibits the survival of HT-29 cells in the presence of glucose deficiency. Under normal growth conditions, verrucosidin did not show any cytotoxic effect as long as there was a sufficient supply of glucose in the culture medium [24].

## 3. Conclusions

The toxic effects of mycotoxins, their interactions with each other or with other substances, are still the subject of current studies to better understand the mechanism of action. The experiments of cell lines with combination of mycotoxins, which are used for the search of new medicines, natural substances or study of cytotoxicity, may ensure the quality and safety of food and feed. A cell line like HT-29 serves as a relatively fast and efficient biological model that reflects the physiological processes taking place in the organism. However, *in vitro* processes still need to be improved to emulate the actual conditions taking place in the body. Because of this, it is necessary to continue the studies by using cell lines and gradually reduce the risk of endangering animal or human health.

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