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Patulin: A potentially harmful food contaminant

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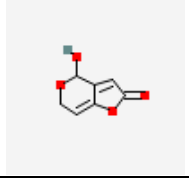
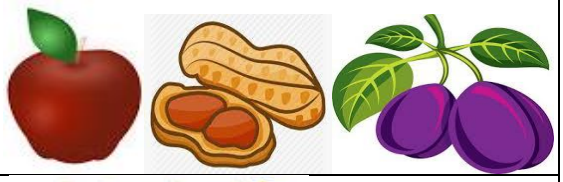
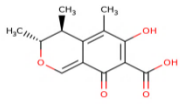
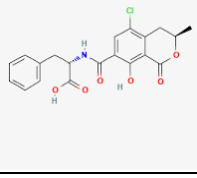
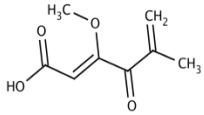
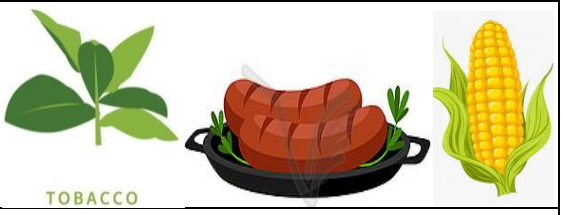
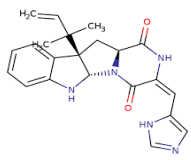
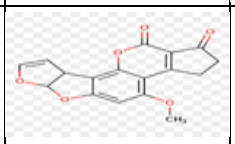
Abstract
Patulin is a mycotoxin generated by mold species such as *Penicillium*, *Aspergillus*, and *Byssochlamys*, being the most important microbes responsible. It is found naturally in a variety of fruits, juices, and other foods that are sold and consumed all over the world. PAT has been linked to a variety of negative health effects, and its widespread presence in commercial fruit juices, apple products, and various cereal grains raises public health concerns. It has a wide range of toxicity in animals and humans. As a result, determining these harmful compounds quickly and with high sensitivity is critical. Patulin's hazard needs its management and elimination, necessitating the development of food processing and handling strategies that can accomplish so. The physicochemical characteristics, variables affecting patulin contamination, PAT limits and regulations, detection and management methods, and toxicity and carcinogenicity are all discussed in this article.

Keywords: Patulin, mycotoxins, carcinogenic, neurotoxic, food contaminant

1. Introduction
Food safety has recently become a worldwide concern as a result of the alarming growth in chemical and biological toxins in the environment. Mycotoxins are a class of highly poisonous, carcinogenic, and mutagenic secondary metabolites produced by a specific species of fungi that have the potential to damage numerous physiological systems [1, 2]. "Mycotoxins in the food chain are an inescapable and important problem that the world is dealing with [3]. The contamination of food products is caused by the secondary metabolites of tiny filamentous fungus [4, 5]. Aflatoxin A, patulin, fumonisins, zearalenone, and deoxynivalenol are the most dangerous mycotoxins. Multiple mycotoxins may appear in food at the same time, raising the amount of worry [6].
Patulin (4-hydroxy-4H-furo [3, 2-c]) pyran-2(6H)-one) is a protein that contains four hydroxyl groups. It is a water-soluble polyketide lactone that is produced by fungal species such as *Aspergillus*, *Byssochlamys*, and *Penicillium* as a secondary metabolite and mycotoxin [7]. PAT is a crystalline and colourless molecule [8] that has been classified as low molecular weight and hazardous compound [9]. Harold Raistrick was the first to isolate patulin from *Penicillium griseofulvin* in 1943 [10].
Penicillium patulum and *P. expansum* are considered to be the most common fungus that causes patulin contamination. An individual strain of *P. expansum* usually produces several secondary metabolites, in addition to patulin. It causes blue mold, a mushy, brown fruit rot that becomes greenish to blue over time. As a result of the fungal infection, patulin accumulates in the affected fruit [11]. Susceptible items may be attacked by PAT-producing fungi during growth, harvest, food processing, or storage [12, 13]. *P. expansum*, the leading cause for the production of patulin, thrives majorly on apples and apple derivatives [14]. Patulin has also been discovered in several vegetables, cereals, grapes, apricots, persimmons, strawberries, nectarines, peaches, plums, tomatoes, bananas, black currants, almonds, peanuts, hazelnuts, infant food, peanuts, legumes, meat, and seafood [5, 15].

2. Physicochemical Characteristics
Patulin ($C_7H_6O_4$) is a water-soluble white powder that is a heat-stable polyketide lactone. Patulin has a molar mass of 154.12 g/mol, a mono-isotopic mass of 154.026, and a melting point of 110 ^oC [7]. Patulin was found to bind to the solid section of the apple in a study because of its electrophilic properties [16]. PAT contains absorption bands of 5.6, 5.9, and 6.1 microns in the double bond area of an infrared scan [17]. Ether, chloroform, ethyl acetate, and ethanol are all soluble in it [18]. It is a highly reactive chemical that prefers to interact with sulfhydrylated substances. With electrophilic compounds, it can form covalent adducts [19]. Patulin binds to cysteine and depletes it within the cell. It can also react with proteins that include lysine and histidine.

Table 1: Occurrence of mycotoxins in different foods produced by fungal species

S.No	Fungal Species	Compound	Structure	Occurrence
1.	*P. expansum* *P. patulum* *A. terreus* *A. clavatus*	Patulin		
2.	*P. citrinum* *P. verrucosum* *P. nordicum*	Citrin		
3.	*P. verrucosum* *P. nordicum* *A. ocharaceus* *P. viridicatum* *P. cyclopium*	Ochratoxin-A		
4.	*P. aurantiogriseum* *P. alli*	Penicillic acid		
5.	*P. chrysogenum* *P. crustosum* *P. expansum* *P. flavigenum* *P. roqueforti*	Roquefortine C		
6.	*A. flavus* *A. parasiticus*	Aflatoxin		

3. Factors Affecting Patulin Contamination

Patulin is primarily transmitted to humans through contaminated food. Foods become contaminated with chemical poison as a result of fungal diseases. Patulin is mostly obtained by humans through fruits, particularly apples and their derivatives [20]. Temperature, humidity, chemical availability, pH, and other factors like fruit type influence food fungal deterioration. Not only do chemical and physical factors influence fungal development, but they also impact mycotoxin generation. However, no comprehensive analysis of all host modifications has been undertaken [21].

Fruits are infected by *P. expansum* mostly when the skin is broken. Other sources of fruit skin damage include chill bites, physical injury, and rodent and insect attacks [22]. Patulin build-up correlates with apparent blue mold symptoms and is commonly discovered in the rotting tissues of apples. The invasion of *P. expansum* spores on the wounds of fresh apples is the most common cause of blue mold. Such stem punctures, insect injuries, and bruises occur during the picking and handling of apples in the orchard, as well as during the last stages of product preparation [23].

4. Limits and Regulations

Patulin is now one of a small number of mycotoxins (Aflatoxins, Ochratoxin A, Zearalenone, Fumonisins, and Trichothecenes) whose levels in food are restricted in several nations around the world, with European countries among the first to suggest limits. A sub-acute rodent NOAEL of 43 µg/kg body weight, as well as genotoxicity studies, were primarily the cause for setting limits for patulin exposure, although a range of other types of toxicity also exists. Since 2003, European regulation 1425/3003 has set a limit level of 50 µg/L for fruit juices and derivatives, 25 µg/L for solid apple products, and 10 µg/L for juices and foods intended for children [10]. The provisional maximum tolerated daily intake (PMTDI) for patulin has been set at 0.4 g/kg body weight by the Scientific Committee on Foods [24].

The content of patulin in some foods is subject to the regulation set by authorities. In the EU, the limit is set to 50 mg/kg for apple juice and cider, 25 mg/kg in solid apple products, and 10 mg/kg in infant and baby food. Patulin is limited to 50 g/L by the US Food and Drug Administration (FDA) [7]. Apple juice should have a maximum concentration of 50 µg/L [25]. The level of Patulin in apple-based foods for

children has been established to be 5 times lower than the allowed level for adults, indicating that children under the age of 12 are considered a vulnerable demographic [26]. In addition to having a larger exposure per kilogram of body weight, children's physiology differs from that of adults, making them more vulnerable [27, 28].

5. Population at Risk

In geographic locations where basic hygienic procedures during food production are not implemented, mycotoxin-related dangers affect the entire population. Patulin levels above a certain threshold impact people of all races, genders, and ages. When it comes to toxicant exposure, some age groups are more vulnerable than others. Nursing infants, for example, are a vulnerable category; toxicant consumption by the mother may result in intoxicant levels in the breast milk that exceeds the tolerated daily intake (TDI) for this age group, even if the mother is only exposed to the adults' TDI. The level of Patulin in breast milk following a single dosage exposure of the mother and after several exposures were not at concern levels, according to a 2017 study [29].

6. Detection of Patulin

Patulin (PAT) is a mycotoxin that can be found in a variety of foods, including fruits and fruit-based products. Its natural prevalence in numerous food commodities, as well as recent toxicological assessments, has underlined the need for validated testing procedures. As a result, precise and effective testing is required for manufacturers to comply with rules and ensure food safety. Rapid and simple identification aids in the avoidance of contaminated items being imported, as well as the use of only high-quality fruits in the creation of fruit-based foods such as jams and juices [30]. For the early detection of Patulin-producing fungus, a range of modern molecular approaches have been developed. Until far, traditional procedures including the use of chemical compounds or physical treatments in food has given viable solutions.

6.1 Chromatographic Methods: Numerous chromatographic methods can be used for detecting PAT in food items [31]. These methods are delicate and targeted but require expensive tools and highly competent operators [32]. The first approach for identifying and quantifying patulin was thin layer chromatography (TLC), which has the advantage of being a simple and low-cost technology [33]. The most popular chromatographic method for patulin analysis is HPLC with UV or photodiode array detectors [34]. Patulin may be easily identified and quantified using this technique because of its distinctive absorption spectra. It achieves a detection limit of roughly 5 mg/L. Liquid chromatography coupled to mass spectrometry may also be used to determine PAT [35]. This approach gives greater selectivity and sensitivity, as well as the ability to analyse numerous analytes at the same time, but it has a comparatively low detection limit of 5.8 mg/ kg [36].

6.2 PCR Techniques: In the detection and identification of certain toxigenic melds in food, polymerase chain reaction (PCR) has been used as an alternate technique to microbiological and chemical procedures [37]. Several traditional PCR techniques based on the dehydrogenase gene have been established to identify patulin-producing melds. In the *Penicillium* and *Aspergillus* species, this enzyme is involved in the biosynthesis of patulin. Some of these PCR procedures were created to detect just certain *Penicillium* species [7, 38-40].

6.3 Biosensors: These are coupled to a transducing system and employ biological tools such as enzymes, aptamers, and antibodies for recognition. PAT in food may be investigated using a competitive immunoassay. A novel technique combining immunological recognition of PAT with a surface Plasmon resonance optical procedure was devised. A laser beam triggers interactions between tests and targets molecular particles on the biochip's surface, which causes a shift in resonance conditions and a noticeable change in reflectivity. This method was described as a cost-effective and efficient immunoassay for determining PAT. This test's limit of detection (LOD) in apple juice was 1.54 102 g/L [41].

Pennacchio *et al*. 2015 developed a novel fluorescence polarization technique that uses promising near-infrared (NIR) fluorescence sensors to detect PAT in food without preparation of the sample. On binding to particular antibodies, it is characterized by a rise in fluorescence polarisation emission of a PAT derivative tagged by fluorescence. PAT competes with the fluorescence labelled PAT derivative, allowing for a LOD of 6 102 g/L identification [32].

To immobilize tethered directed antibodies on the gold-plated surface of a quartz-equipped microbalance, [42] employed a photonics immobilization approach. The PAT LOD for this biosensor is 21.56 g/L. The micro-balanced Nano-sized analytes were weighed down by a "sandwich procedure" utilizing an extra antibody to make them detectable.

Wu *et al*. 2016, have created and characterized aptamers [43]. A monocatenary DNA sequence is referred to as an oligonucleotide aptamer. SELEX is a well-known approach for selecting aptamers (systematic evolution of ligands by exponential enrichment). SsDNA aptamers have a high affinity for PAT and have attractive properties like easy synthesis and labelling, non-immunogenicity, low-cost manufacturing, high stability, affinity, and specificity in target binding. However, labelling aptamers is costly, and labelling might reduce the activity of modified biomolecules, resulting in a more complicated, and time-consuming and time-consuming assay design [44].

A Nano-sensor based on manganese-doped ZnS quantum dots to selectively differentiate PAT through phosphorescence to design a simple luminescent sensor to detect PAT was created. This Nano-sensor can distinguish PAT from a variety of mycotoxins and can detect PAT in the range of 66.22 to 1,001 g/L with a LOD of 49.31 g/L [45].

To monitor PAT inhibitory action conduct metric urease-based biosensor was developed. This biosensor is ideal for assessing PAT concentrations beyond 50 g/L in apple juices and has a reasonably high PAT sensitivity, strong selectivity, and excellent signal repeatability [46].

A field-portable colorimetric apta sensor was developed for the selective, sensitive, and label-free detection of PAT, a carcinogenic mycotoxin. The diazonium chemistry and bi-functional polyethylene glycol as separators were used in electrochemical impedimetric apta sensing for label-free detection of PAT. The carboxyl-amine PEG proved to be an efficient separator, allowing electrons to pass from the redox probe to the electrode surface through a tunnel [47].

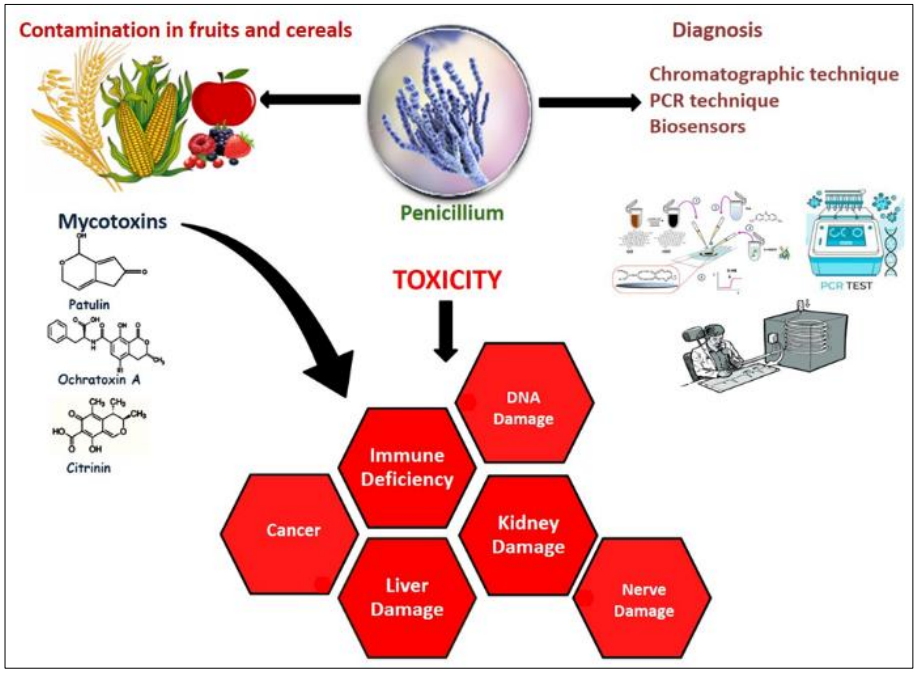


Fig 1: Impact of mycotoxins on human health

7. Toxicity and Carcinogenicity of Patulin

Concerns have been raised about the toxicological consequences of PAT on people and animals as a result of its great prevalence [48]. Patulin, which is generated by *P. expansum*, is a serious health issue since it can lead to serious acute and chronic toxicity, as well as carcinogenic and mutagenic consequences. PAT has been found to be teratogenic and genotoxic in a few investigations [49]. The interaction of patulin with sulfhydryl groups, proteins, and amino acids in the plasma membrane is considered to be the cause of patulin toxicity [50]. PAT's oral LD50 in mice and rats ranges from 20 to 100 mg per kg body weight. This figure is far greater than the actual PAT concentration to which human beings are exposed [51].

Studies on the toxicity mechanism of Patulin have indicated that it is responsible for oxidative pathway damage. It entails the formation of sulfhydryl-containing molecules, which results in an increase in the concentration of reactive oxygen species (ROS) within the cell. When ROS interacts with bioactive chemicals, it produces systemic oxidative damage, which leads to cellular malformations and illnesses [7, 52]. Glutathione depletion has been observed as a side effect of the toxin, which might be linked to oxidative damage [53]. Patulin binds to cysteine and depletes it within the cell. It can also react with proteins that include lysine and histidine. Patulin's principal toxicity route is the production of adducts [54]. The numerous harmful effects of patulin appear to have a complex basis. Acute signs of clinical patulin intoxication include dyspnoea, pulmonary congestion, edema, ulcer, hyperemia, GI tract distension, intestinal inflammation and hemorrhage, epithelial cell degeneration, vomiting, and kidney damage [55-57]. At lower doses, PAT affects lymphocyte proliferation. Patulin consumption has been linked to neurotoxic, immunotoxic, genotoxic, teratogenic, and carcinogenic effects in the long term [58-62]. Chronic exposure to Patulin may also alter the regular microflora balance of the intestine [63]. Patulin is a genotoxic, reprotoxic, embryo toxic, and immunosuppressive toxin [64, 65]. Patulin toxicity analysis revealed harm to important organs and systems such as the gastrointestinal tract, liver, kidney, and others [66].

7.1 Intestinal Toxicity: The gastrointestinal (GI) tract is the major location of exposure to xenobiotic found in food, and also where they are found in the highest amounts [67]. Mycotoxins have been shown to affect a variety of GIT functions, including a decrease in surface area, a change in Teer etc [68]. PAT also causes damage to the intestine causing ulcers, inflammation and bleeding [69]. It induces a reduction in goblet cells and a rise in apoptosis in the gut, making it particularly hazardous [70].

7.2 Immunotoxicity: PAT, has long been known to influence immune responses [71]. It increases allergic immune response by causing airway hyperactivity and eosinophilic lung inflammation [72]. In human peripheral blood mononuclear cells, PAT exposure causes lower production of IL-4, IL-13, IFN-δ, and IL-10, as well as intracellular GSH depletion [73].

7.3 Neurotoxicity: PAT promotes ATP depletion as well as mitochondrial and lysosomal abnormalities in neuro-2a cells [74]. Mice exposed to PAT for 8 weeks had higher amounts of GSSG, ROs, thiobarbituric acid reactive compounds, and protein carbonyl, whereas protein thiol and total thiol groups were down-regulated. Glutathione peroxidase and glutathione reductase activity get reduced by PAT [54].

7.4 Renal Toxicity: Patulin promotes glomerular degeneration and bleeding in the tubules of the cortical zone of the kidney tissues [75]. PAT disrupts the configuration of renal cells and reduces the ability of Zebrafish embryos to clear dextran [76]. PAT inhibits the development of human embryonic kidney cells, implying that it produces higher oxidative stress in the cells, leading to apoptosis [77].

7.5 Carcinogenicity: Animal studies are typically regarded as adequate models for analysing potential detrimental effects in humans, according to FDA. As a result of the negative effects of PAT in animal studies, FDA considers that people may be at risk of damage at certain doses of PAT exposure [78, 79]. PAT is classified as Group 3 by the International Agency for Research on Cancer, which implies there is no evidence of carcinogenicity in humans and limited data on carcinogenesis

in experimental animals [80]. A NOEL of 43 mg/kg bw/day was established based on long-term toxicity/carcinogenicity investigations on patulin in rats. A PMTDI of 0.4 mg/kg bw and a safety factor of 100 has been established based on this NOEL [81].

8. Management and Control of Patulin

According to the Food and Agriculture Organization of the United Nations (FAO), mycotoxins impact around 25% of worldwide food and feed crop production [82]. Synthetic fungicides have been the standard strategy for reducing post-harvest losses caused by mycotoxin infections over the years [83]. But it causes many health risks connected with their usage. Physical treatments have also been used to successfully combat fungal development, but they do not give long-term protection against re-infection of the fruit following treatment [84]. Although each of the treatments can minimize decay when used alone, combining two or more approaches may be more effective against infection from post-harvest illness.

The Joint FAO/WHO Food Standards Programme recommends many patulin control methods, many of which are based on careful fruit selection as part of good agricultural management [85]. According to previous research, cleaning fruits with high-pressure water and removing bad fruits before storage helps prevent fungal infestation and, as a result, Patulin formation [86]. Current approaches for avoiding mycotoxin contamination of foods include

(1) Precise moisture control,
(2) Good manufacturing processes,
(3) Effective quality assurance efforts, and
(4) Following the Hazard Analysis Critical Control Points principles [87, 88].

8.1 Chemical Methods: Exogenous potassium phosphide therapy has recently demonstrated promising benefits in limiting the development of Patulin-producing fungus [89]. *P. expansum* growth, spore germination, and Patulin synthesis were all completely inhibited by treatment including sodium hypochlorite, hydrogen peroxide, and copper sulfate [90]. In fruit samples and PDB media, chlorine dioxide showed antifungal action against *P. expansum* [91]. Propolis, a honeybee product, has been used as a natural antifungal drug to stop PAT-producing fungus from growing [92].

8.2 Physical Methods: Heat and pulsed light remediation [93], UV irradiation [94], gamma radiations with a 67 percent decrease in PAT at 1.0 kg dosage [95], and adsorption methods [96] are some of the successful physical treatments. PAT photo degradation and UV irradiation follow first-order kinetics, with more acidic solutions having a quicker reaction rate [97, 98]. However, these approaches have demonstrated limited degrading efficacy, as well as non-desired losses and unfavourable effects on product nutritional and organoleptic qualities [99]. High hydrostatic pressure (HHP) is another non-thermal approach that has proved to reduce PAT concentration significantly. It also keeps the food's organoleptic and nutritional properties [100, 101].

8.3 Enzymes for Pat Degradation: Due to the obvious selectivity and quickness with which enzymes safely degrade and detoxify PAT in contaminated fruit juices, they are a potential method that has received a lot of attention during the last decade [102]. As a]result, numerous techniques have been developed, such as pig pancreatic lipase (PPL) immobilized with calcium carbonate [103,104], which destroyed 99 percent of PAT in 3 hours at pH 5.0 and 30 degrees Celsius. When used under pH 6.0 circumstances for 42 hours at a temperature of 40°C, the method performed excellently [103].

Porcine pancreatic lipase enzyme may break down over 90% of PAT in an aqueous solution at pH 7.5, 40°C for 48 hours, according to [105], who identified the residual product as C7H11O4+. PAT degradation in apple juice was more than 70% from an initial concentration of 1x 103 g/L. For immobilized PPL at 3 104 g/L, the optimal condition was 40°C for 18 hours. Apple juice's sensory and nutritional qualities were not adversely affected.

8.4 Microbes for Pat Control: Several microorganisms, including bacteria, molds, and yeasts, have been proven to break down PAT and can be used safely in food preparation in recent years. Due to its tolerance to very high concentrations of PAT (1x105 g/L), *Lactobacillus plantarum* is a good prospective candidate for biodegradation. After incubation for 4 hours at 37°C with 1x1010 cells/mL, *Lactobacillus plantarum* cells were able to break down 80% of PAT [106]. *P. caribbica* was recently shown to be capable of decomposing PAT in response to being stressed by PAT [107]. In the case of molds recently discovered that *Byssochlamys nivea* FF1-2 is a filamentous fungus with excellent PAT biodegradation capacity [108].

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Ethical Matters: No animal model has been used for the present work.

10. Conclusion

Patulin is a harmful chemical food pollutant produced by a variety of mold species, including those of the genera *Aspergillus*, *Penicillium, and Byssochlamys*. It's the most prevalent mycotoxin discovered in apples, apple products, and a variety of other fruits and vegetables. Patulin exposure causes hepatotoxic, genotoxic, and immunotoxic. The HPLC method with UV detection is quick and accurate, and it's been used on a variety of matrices. Although TLC and GC procedures are available, it is the method of choice for regular determination and monitoring of patulin levels.

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