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Patulin: its toxicity and possible contamination of products used in baby nutrition

Gönül Şahin, Songül Ünüvar*, Terken Baydar

Hacettepe University, Pharmaceutical Faculty, Ankara, Turkey

**İnönü University Medical Faculty, Department of Toxicology, Malatya, Turkey*

Summary

Patulin is a secondary metabolite produced by molds such as Penicillium, Aspergillus and Byssochlamys. Mainly patulin may be present in many foods including baby foods, fruits (especially apple) and apple-based products. Severity of patulin toxicity is correlated with the amount of consumed fruit products. Therefore, daily amount of consumption of these products is very important in infants and children who are vulnerable to toxic effects. In recent years, many studies on this field showed that patulin levels in fruit-based products were higher than the limit of provisional maximum tolerable daily intake. In this review, the major toxic effects of patulin, the limits in baby foods, and the approaches for prevention of formation of patulin have been summarized in the light of current literature knowledge. *(Turk Arch Ped 2011; 46: 266-70)*

Key words: Patulin, contamination in baby foods

Introduction

Mycotoxins are toxic metabolites formed by moulds including *Aspergillus, Penicillium, Alternaria* and *Fusarium* in certain humidity and temperature conditions. These toxins which can grow in different food products reach the humans by contaminating foods (1). These metabolites which are contacted frequently in daily life currently threaten the public health and cause economical losses because of refusal of exported products from custom gates. These moulds grow in unprocessed foods in appropriate conditions and cause the products to decay by changing their quantity and quality and affect the human health negatively (2). The most commonly confronted mycotoxins include aflatoxins, ochratoxin, trichothecenes, zearalenone, patulin and fumonisin. Among these toxic metabolites, patulin has been reported to form mostly in apple and apple products, in fruits including pear, apricot, peach and grape and less commonly in different foods including cheese and meat (3-6). Patulin contact generally occurs as a result of consumption of contaminated apple juices and other products containing apple. In recent years, warnings have been made worldwide about patulin containing foods and especially about apple containing newborn or infant formulas (7).

The mould responsible of the formation of patulin is mainly Penicillium expansum which is known as the apple pathogen (8). The secondary metabolites of *Penicillium* expansum including patulin are present in many vegetables and fruits as an indication of contamination. P. Expansum cause decaying of fruits during storage and thus patulin contamination in fruits. It causes decays in apples which is called “blue mould rot”. In 1940’s, this compound was started to be used as an antibiotic in treatment for common cold and it was recognized in the following years that it was a mycotoxin contaminating apples (9-11). Patulin is a low-molecular- weight cyclic tetraketide with a structure of unsaturated lactone. 6-methylsalycylic acid synthase and polyketide synthase enzymes are involved in patulin biosythesis (2,12-15). In this review, patulin toxicity, dimensions of possible risks in infant formulas and approaches to prevent patulin formation are evaluated.

Factors affecting patulin formation

Especially fruits with a content of high density water and sugar and a low pH due to organic acid content predispose to production of these moulds including mainly patulin (9,15). Optimum temperature for growth of P. expansum which is mainly responsible of patulin formation in fruits is 25°C. As the

Address for Correspondence: Songül Ünüvar MD, İnönü University Medical Faculty, Department of Toxicology, Malatya, Turkey
E-mail: songul.unuvar@inonu.edu.tr /**Received:** 02.17.2011 **Accepted:** 03.17.2011

temperature decreases, formation of patulin decreases. However, patulin can be formed at low temperatures like 0-4°C. Keeping fruits in the refrigerator generally does not inhibit patulin formation. It needs little oxygen and grows with a rate of lower than 2% in oxygen in the atmosphere. In conditions where the level of carbondioxide is higher than 15%, the microorganism grows faster. Therefore, it has been reported that the formation of the toxin can be decreased or prevented by arranging storage conditions of the fruits (9).

Patulin is found in the apple with a higher rate compared to other fruits. The toxin has been found especially in unprocessed apples, apple juice, apple wine, apple sauce and pulp. Most exposure to patulin occurs by consuming apple wine and apple juice prepared using decayed apples. Some investigators have suggested that patulin content of the apple is not affected by growth of the apple using traditional or organic methods. However, it has also been reported that provisional maximum tolerable daily intake can be exceeded in children who consume large amounts of organic fruit juice (6,9,15).

Patulin formation in apples is affected by geographical localization, the place of growth of the fruit, harvesting, pre-harvesting practices, method of harvesting, damage formed on the fruit, post-harvesting practices and storage conditions. It is not known which of these factors is mainly involved in the formation of patulin and it is not clear which factors in the growth of apples should be changed and in which way should they be changed to decresase patulin level (16). The properties of the fruit and mechanical demage in the fruit also effect patulin formation. Increase in pH in the tissues of the fruit, increase in sugar level, thinning of the rind of the fruit and thus weakening of defense barrier during the maturing stage of the fruit predispose to spread of moulds. Rainy weather during harvesting is another reason which increases fruit infections related to moulds and contamination. Therefore, the fruits should be harvested at dry air and rapidly carried to cold stores. In addition, fruits which are damaged mechanically and have been fallen on the ground should not be offered. Keeping the containers used during transport clean is another efficient method to prevent formation of spores (15,16).

The organic structure of the fruit can act as a depot for mould spores. After harvesting apples are usually stored in stores at 1-3 °C. Apples can remain under these conditions for 9-28 weeks depending on the species. During preperation of apple juice, practices including filtration, centrifuging and fermentation may decrease patulin levels (6,16,17).

Patulin toxicity

Patulin has an electrophylic structure due to conjugated double bonds. It interacts with nucleophylic groups irreversibly because of this property and leads to structural toxicity by changing the structure of DNA and various proteins including glutathione (18,19).

Although patulin accumulates mainly in erythrocytes, it tends to accumulate also in organs which are supplied with large amount of blood including the spleen, kidney, lung and liver. Acute symptoms of patulin toxicity include agitation, convulsion, edema, ulceration and vomiting. In experimental animals, patulin has been shown to cause epithelial degeneration, bleeding, gastric mucosal ulceration and histopathological lesions including presence of neutrophyle and mononuclear cells in the digestive system canal (20). Its chronic effects have not been elucidated in humans, but it has been documented that is is immunotoxic and genotoxic in experimental animals (21). In addition, it has been reported to cause neurotoxic effects including sympathetic nervous system palsy, involuntary movements in the muscles, tremors and cerebral bleedings (8).

Patulin which was shown to cause teratogenicity and toxicity in reproduction was classified as Group III carcinogene by the IARC (International Agency for Research on Cancer) (8,9,13,22). In addition it was shown to increase the risk of allergy and induce DNA damage (12,23). It was shown to cause persistent breaking of single and double strands of the DNA of Escherichia coli, oxidative damage in human cells and decrease glutathione levels. Patulin causes cytotoxicity with its effect of stimulating reactive oxygen compounds (ROC) and with its ability to attack thiols (24). Because of its high affinity for sulphydryle groups it inhibits the function of many enzymes including ATP-ase, alkalen phosphatase, aldolase and hexokinase (12,9). In vitro, it was found that a specific protein kinase is involved in patulin toxicity. It was found that patulin caused activation of ornitine decarboxylase which is an indicator of cell growth and acts as a stress signal in production of p53 protein which guides apopytosis (24).

The median lethal dose of patulin (LD50) was found to be 5 mg/kg, when it was given intraperitoneally in mice. The level which showed no toxic effect (NOEL, no-observed effect level) was found to be 43 µg/kg. Provisional Maximum Tolerable Daily Intake (PMTDI) and the highest daily allowable level for patulin according to JECFA (Joint Expert Committee on Food additives) is 0,4 mg/kg body weight/day (12,25).

Provisional limits for patulin and the status in Turkey

Many countries have put limitations for food products which are convenient for mycotoxin growth because of negative effects on human and animal health. Follow up and control of patulin levels in foods is important in terms of protecting the consumers. FDA reported the allowable highest level as 50 µg/kg for apple juices and other food products containing apple in Codex Alimentarius. The European Union reported a warning for patulin with the number 2003/78/EC (26). Although the EU determined this highest limit to be 50 µg/kg in apple juice products, 25 µg/kg in solid apple products and 10 µg/kg in infant formulas, there are differences in the amounts found in similar products in different countries in EU (7,27,28). The amount of patulin has been determined in apple juices in many

countries including South Africa, Brazil, Cuba, Turkey, Belgium, Iran and Italy. In most of these investigations, patulin levels have been reported to be below the limits stated in Codex Alimentarius. Only the patulin levels determined in Turkey and Iran have been found to be higher than the limits (22,29). Although adjustments about patulin are beneficial for consumers, patulin levels outside these limits can be found in countries outside EU. Patulin levels in Australia, Iran and Turkey have been found to exceed the EU limits (by 30-40%) (30,31).

The limits for fruits and fruit juices have been determined in many countries (Table 1) (6). Since newborns and young children consume fruit-based products frequently, the highest limit for them have been determined to be 10 µg/kg (32).

In many studies performed in our country, patulin content of apple juice and apple concentrates has been found to occasionally exceed international standard limits (33). Patulin content was found to exceed the limit of 50 µg/kg in 98 of 215 apple juice samples produced in different periods by different companies in 1994 in Turkey (29). In 2001, patulin level was found to exceed 50 µg/kg in 42 of 45 apple juice samples found in the market (34). The limits defined in the notification with the

Table 1. Patulin content in apple products (6).

Apple product	Country	Patulin found/ total number of samples	The lowest level found (LOL)	Range (µg/L or µg/kg)
Apple juice, open or closed fruit juices and nectars	South Africa	4/17	5	5–45
	Sweden	5/39	2	2–50
	Turkey	27/45	5	19.1–732.8
	EU	35/43	0.67	2.5–38.8
	Italy	3/8	5	5.8–56.4
	Japan	15/76	1	1.4–45.6
	Iran	13/42	15	15–285.3
	Italy	16/33	0.5	0.5–53.4
	Spain	5/17	0.3	1.5–50.9
	Italy	19/32	1.57	1.57–44.89
	Brazil	4/100	3	3–7
	Greece	66/66	0.23	0.9–36.8
	Spain	66/100	0.7	0.7–118.7
	Spain	30/71	2,08	2.08–15.0
	Portugal	?/49	1.2	1.2–42
	Iran	150/150	3	6.0–106.0
Organic apple juice, organic open or closed fruit juice	Italy	6/21	1,57	1.57–47.91
	Italy	3/7	5	30.4–33.2
	Italy	12/24	0,5	0.5–69.3
	Portugal	?/19	1,2	0.5–9.2
Apple puree	Italy	2/4	5	15.9–16.7
	Italy	0/4	0.5	
	Argentina	4/8	3.8	22–221
	Spain	6/18	0.3	7.7–28.4
	Spain	4/77	2.08	2.08–17.6
Organic apple puree	1/2	5	74.2	
	Italy	7/13	0.5	>0.5
Infant Formula	South Africa	6/17	5	5–20
	Italy	0/6	5	
	Italy	1/11	0.5	0.7
	Spain	42/124	2.08	2.08–9.6
	Portugal	?/70	1.2	1.2–5.7
Organic infant formula	Italy	2/4	5	13.1–17.7
	Italy	2/12	0.5	>0.5
	Portugal	?/70	1.2	
Marmalade	Argentina	6/26	2.8	17–39
Apple wine	South Africa	2/8	5	5–10
	European Union	3/7	0.67	2.8–6.1

Table 2. The highest limits for patulin in foods specified in the Turkish Alimentary Codex (35).

Food substance	The highest limit (μg/kg)
Fruit juices, fruit juice concentrate and fruit nectars	50
Distilled alcoholic drinks, apple wine, other fermented products which are produced from apple or which contain apple juice	50
Solid apple products (including apple composte and apple puree presented for consumption directly)	25
Apple juice and solid apple products produced for infants and young children and marketed/labeled for this purpose (including apple composte and apple puree)	10
Non-cereal based solid foods	10

number 2008/26 published in the official paper (1705.2008-26879) about Turkish Alimentary Codex Maximum Limits of Contaminants in Food Products are given in Table 2 (35).

Prevention of patulin formation

Chemical, biological and physical agents are used to inhibit and/or decrease mould growth and formation of mycotoxins in foods. With this objective, use of ascorbic acid, gamma-radiation, ozone, heat treatment and microorganisms acting as antifungal agents against P. Expansum has been tried. However, the cost and inadequacy of these practices have limited their use. Therefore, inexpensive methods including discarding damaged fruits, washing fruits and vegetables and removing the decayed parts before patulin formation starts have been tried (5,10).

Even though post-harvesting factors are more effective in formation of patulin, many factors predispose to formation of mycotoxins. Codex Alimentarius Commission (2002) suggested that mould infections in fruits and vegetables could be decreased by measures defined in "Good Agricultural Practices (GAP)". It was proposed that foliar calcium sprays can be used during growth period and apples can be protected against mould infections by using low rates of nitrogen. As a result of amonnium molibdate application to apple trees before harvesting, a marked decrease in formation of blue mould caused by patulin was observed in apples kept in cold stores three months later (9). The compound named trans-2-hexenal is a smelling substance found in the structure of many fruits and vegetables and it was found to be a good inhibitor against P. Expansum (36). Another method is use of microorganisms including Rhodotorula glutinis isolated from the outer surface of the fruit against growth of moulds (10). In addition, cleaning of cases used for transportation of apples with chlor compounds (for example, o-phenylphenate and quarternary amonnium compounds) prevent spor contamination (9,15). Use of antifungal agents is not a very effective method against patulin formation, because resistance to these substances develops. In addition, use of antifungal agents are considered to be inappropriate, because they are harmful for the environment and leave residues on the fruits and vegetables (37,38). 2-deoxy-glucose which was proposed to be used as another option in addition to antifungal agents was observed to increase patulin content (39).

Conclusively, patulin is a significant mycotoxin which is threatening for human health (mainly for the health of infants and children). Especially in our country, fruit juice and fruit-based products and infant formulas should be inspected regularly by the related ministry and licensed by sub-committees including related scientists such as pediatricians and toxicologists. All fruit-based products which will be imported from countries outside EU should be closely inspected by the national authorized committees in terms of patulin and other mycotoxins and following the determination of these levels the products which are appropriate in terms of these limits should be given sales licence. Paying the same attention for our products which will be exported will also prevent our economical losses. Raising awareness of the manufacturing company is primarily necessary to protect public health. It is also important that consumers are informed and educated by visual or written media. Healthcare workers and mainly physicians shoul be informed about possible undesirable effects and severe health problems arising form patulin exposure and should be aware of the fact that unexpected events originating from foods can be related to mycotoxins including patulin. Scientific studies directed to monitoring and prevention should be encouraged and supported.

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