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ScienceDirect
Food and Chemical Toxicology 44 (2006) 2019–2024
www.elsevier.com/locate/foodchemtox

Evaluation of the reproductive toxicity of patulin in growing male rats

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Received 28 September 2005; accepted 30 June 2006

Abstract

Patulin is a mycotoxin produced by several *Penicillium*, *Aspergillus* and *Byssachlamys* species. Patulin can be produced on different food products including fruits, grains, cheese, cured meats, but in natural situations patulin is exclusively found in apple and apple products. Patulin, at dose of 0.1 mg/kg bw/day, was administered by gavage to the growing male rats aged 5–6 week for 60 or 90 days. At the end of the experiment, sperm counts and morphology were investigated. Also, effects of patulin on the epididymis, seminal vesicle and prostate tissues were examined histopathologically and morphologically.

While sperm counts increased in patulin-treated rats for 60 days, sperm counts in patulin-treated rats for 90 days decreased compared to the corresponding control group. Patulin affected sperm morphology of growing male rats. Tail abnormalities like bent and/or coiled tails, and sticking of sperm tails were observed. A significant change was not determined in absolute and relative weights of the seminal vesicle and prostate of patulin-treated rats. While absolute cauda epididymal weights increased in rats treated with patulin for 60 days, absolute and relative cauda epididymal weights reduced in rats treated with patulin for 90 days. In histologic examination, some histopathological changes were observed in the epididymis and prostate tissues of rats in patulin treatment groups.

Keywords: Patulin; Mycotoxin; Reproductive toxicology; Sperm count; Sperm morphology

1. Introduction

Patulin is a mycotoxin produced by several *Penicillium*, *Aspergillus* and *Byssachlamys* species, but *Penicillium expansum* is the most commonly encountered species. Patulin can be produced on different food products including fruits, grains, cheese, cured meats, but in natural situations, patulin is almost exclusively found in apple and apple products and sometimes also in peach, pear, apricot and grape products (Mortimer et al., 1985; Harrison, 1989; Paster et al., 1995).

Gökmen and Acar (1998) analysed 215 samples of apple juice concentrate for patulin in Turkey. Their study detected patulin in all 215 samples at concentrations ranging from 7 to 376 μg/L. The patulin levels exceed 50 μg/L in 46% of all analysed samples. Another study by Yurdun et al. (2001) reported that patulin was present at concentration ranging from 19.1 to 732.8 μg/L in 60% of apple juice consumption by the Turkish population. In addition, 44% of the analysed apple juice samples exceeded the 50 μg/L of recommended limit set by the World Health Organization (WHO). Beretta et al. (2000) detected patulin in apple-based baby-foods.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) decided to establish a maximum level for patulin of 50 μg/kg in apple juice and ready made soft drinks containing apple juice (WHO, 1998). Patulin is classified as Group 3 by the IARC (International Agency for Research on Cancer) because there is insufficient evidence of carcinogenicity in experimental animals and humans (Alves et al., 2000). There are a number of research studies related to reproductive and developmental toxicity, carcinogenicity, mutagenecity and immunotoxicity of patulin (Becci et al., 1981; Choudhary et al., 1992; Smith et al., 1993; Llewellyn et al., 1998; Alves et al., 2000). Patulin

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doi:10.1016/j.fct.2006.06.022

has a strong affinity for sulfhydryl group, which explains why it inhibits the activity of many enzymes (Askar, 1999). In addition, it was reported that patulin, at a concentration of 295 mg/L in drinking water for 4 weeks, caused effects on the gastro-intestinal tract such as fundic ulcer in rats (Speijers et al., 1988). In short-term (14-day treatment) studies, patulin produced histopathological lesions in the gastro-intestinal tract, including epithelial degeneration, hemorrhage, ulceration of gastric mucosa, neutrophil and mononuclear cell infiltration at doses of 24 and 36 mg/kg bw in mice (McKinley and Carlton, 1980) and at the doses of 28 or 41 mg/kg bw in rats (McKinley et al., 1982).

When the recommended level of patulin is exceeded in fruit juices and other fruit products, it may cause potential risk in humans, particularly in children. Although there are some researches about reproductive toxicity (Becci et al., 1981; Choudhary et al., 1992), the effects of patulin on sexual development and reproductive parameters such as sperm count and morphology have not been studied. Therefore, this study was designed to evaluate the subchronic effect of patulin on reproductive system of growing male rats. In a general subchronic toxicity test, dosing is continued for 60–90 days (Hayes, 1994). For adverse effects of an agent on the reproductive system, treatment should be continued for a minimum of six cycles of the seminiferous epithelium to ensure that all possible adverse effects are expressed in each end point observed (Hayes, 1994). This day for rat is about 70 days. Therefore, two different experiment durations were chosen as 60 and 90 days.

2. Materials and methods

2.1. Chemicals

Patulin (4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one) was obtained from Sigma Chemical Company (St. Louis, Missouri, USA).

2.2. Animal and experimental design

Wistar albino male rats aged 5–6 weeks were obtained from The Production Center of Experimental Animals in Hacettepe University, Ankara, Turkey. The rats were randomly divided into four groups of 10 animals each, housed 2 per cage and individually labelled. Two of the four groups were considered as treatment groups, and the other two were control groups. The rats in first treatment group (P-60) were given 0.1 mg/kg bw/day of patulin for 60 days, while the corresponding control group (C-60) was given sterile water. The rats in second treatment group (P-90) were given 0.1 mg/kg bw/day of patulin for 90 days. Again, the corresponding second control group (C-90) was given sterile water. Administration was made by gavage and the rats in control groups were given by same way sterile water, which is equal to the amount given to rats in treatment groups. 0.1 mg/kg bw was selected as the dose level for this study. Patulin was dissolved in sterile water.

Dose was chosen based on extrapolation from estimated human exposure level as described Llewellyn et al. (1998).

The animals were maintained under controlled conditions of temperature (22 ± 2 °C), relative humidity ($68 \pm 4\%$) and 12-h light and dark. Animals received standard laboratory diet and water *ad lib* during the experiment. Individual animal weights were recorded weekly during the experiment.

At the end of the study, the terminal body weight of each animal was recorded. The cauda epididymis, seminal vesicle and prostate tissues were dissected out and weighed. Then, relative organ weights were calculated.

2.3. Sperm count and morphology

The cauda epididymis was dissected and minced with scissors in 1 ml physiological NaCl-solution (0.9%). After sperm were allowed to swim out of the epididymis for 30 min, the epididymal content was diluted 10 times with normal saline (Pant et al., 1996). A drop of the sperm suspension was smeared onto a slide. Smear was dried, fixed in alcohol and stained with Giemsa's stain for observation of abnormalities (Akbarsha and Murugaian, 2000). One thousand sperms per animal were screened and classified into normal and different abnormal types as procedure of Narayana et al. (2002) and Akbarsha and Murugaian (2000) under a light microscope. A mean % abnormal sperm for a group was calculated by using a % abnormal sperm of each animal. The sperm suspension was also used for the sperm count using Neubauer's chamber. Sperm were counted at a magnification of ×420 under a light microscope. The sperm counting of each animal was made in duplicate and the mean was taken. A group mean was calculated by using a mean sperm count of each animal. Epididymal sperm counts were expressed as number of sperms per cauda epididymis.

2.4. Histopathological examination

Tissue samples ($n = 10$) of cauda epididymis, seminal vesicle and prostat were collected from all groups and fixed in Bouin's fixative, routinely processed and stained with Harris Hematoxylin and Eosin stain for histopathological examination by light microscope.

2.5. Statistical analysis

For each group, mean and SE were calculated and data were analysed by two tailed "Student *t*-test" using SPSS package program, version 9.0 and $p \leqslant 0.05$ was considered as level of significance. Histopathological findings were compared with the control using Fisher's exact test ($p \leqslant 0.05$).

3. Results

3.1. Effects on reproductive organ weights

There was no statistically significant difference in body weight gain between the treatment and control groups (Table 1). Also, it was not determined a significant change in absolute and relative the seminal vesicle and prostate weights of patulin-treated rats. While absolute cauda epididymal weights were increased in rats treated with patulin for 60 days, absolute and relative cauda epididymal weights were reduced in rats treated with patulin for 90 days compared to the corresponding controls (Table 1).

3.2. Sperm count and morphology

Sperm counts increased significantly in patulin-treated rats for 60 days. However, in patulin-treated rats for 90 days, sperm counts decreased significantly compared to the corresponding control group (Table 1).

Patulin affected slightly sperm morphology of growing male rats. The observed tail abnormalities were bent and/or coiled tails, and sticking of sperm tails, fragile tail (Figs. 1–3). Separated heads was also observed. The percentage of