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Original Article

# Patulin Effects on Semen Parameters and DNA Fragmentation in Men (In vitro Study)

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## Abstract

**Objective:** Approximately 25% of infertility in couples is due to poor quality of semen. Men exposure to toxins and chemicals can affect fertility. The most famous of mycotoxins, patulin that produced by a variety of molds in particular Aspergillus and penicillium and Byssochlamys is in rotting apples and apple juice and canned products from them as to the upper limit to be found. Patulin is teratogen, Mutagen and carcinogen. The effects of this toxin have not been studied in human sperm.

**Materials and Methods:** So, this study has been done on 40 semen samples taken from normal patients referring to Rooyan Institute. Motility, pH, morphology, viability and sperm DNA fragmentation (SCD test) in accordance with WHO standards were studied before and after (1 hour) the use of Patulin with 5 doses of 0.02, 0.05, 0.16, 0.33 and 0.5 micrograms per milliliter that was diluted with Ham'sF10.

**Results:** The results showed that concentrations of 0.33, 0.5 and 0.05 caused a significant decrease in sperm motility, pH and viability. The concentrations of 0.33 and 0.5 caused a significant damage on sperm DNA but there is no effect on morphology.

**Conclusion:** So it can be concluded that Patulin has destructive and harmful effects on human sperm DNA and its excessive amounts in fruit juices can reduce fertility.

**Key words:** Patulin, Sperm, Fertility, DNA fragmentation

## INTRODUCTION

About 25% of infertility in couples is due to low quality semen fluid. Exposing men with toxins and chemicals can affect their fertility. One of these toxins is patulin.

Patulin is one of the mycotoxins found in mildew fruits, especially apples and products derived from these apples, such as fruit juices and compotes. It is produced by several types of mildew, especially Aspergillus, Penicillium and Bissochlamys. The contamination of foods and juices with patulin is an important issue of food safety, given the high consumption of these items around the world. Studies show that in many apple juices produced in factories exceeds its limit value (50 µg/L, according to WHO) and sometimes doubles as much as it can (2). Exposure to this mycotoxin causes immunologic damage, particularly damage to macrophages (6, 12, 14, 25), neurological damage such as seizure, tremor, and digestive trauma (7, 8, 18). Patulin also has a genotoxic effect, causing damage to DNA and its carcinogenicity in mice and rats has proven. Recently, it has been discovered in a study that even patulin can be absorbed through the skin (10). Some studies have shown that patulin damages DNA synthesis. These genotoxic effects may be associated with the ability to react with sulfidryl groups and induce oxidative damage (13, 19). Further, the effects of this venom on thyroid and testis and the level of hormones in male rats were studied (20, 21). Chromosomal abnormalities by patulin in mammalian cells have also been proven (4, 22, 23). So far, there has been no study on the effect of this poison on human sperm. Therefore, this study attempted to first investigate the effects of this toxin on the laboratory on human sperm cells, especially on its DNA.

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## MATERIALS AND METHODS

### Sample Tested

Semen fluid samples of individuals referred to Royan Institute of Infertility Treatment Center (Tehran-Resalat-Bani Hashem) were studied. After Liquefaction for 30 minutes, the samples were counted according to WHO (2010) standards and samples from 75 to 110 million per ml were selected.

### Preparation of Concentrations

To evaluate the effect of patulin with different concentrations, a stock solution of patulin with a concentration of 1 ppm (prepared by Ham'sF10 medium) was prepared. This stock solution was diluted serially with Ham'sF10 medium and 100 mL of semen fluid was added to each to obtain concentrations of 0.02 µg/ml, 0.05 µg/ml, 0.016 µg/ml, 0.33 µg/ml and 0.5 µg/ml. The specimens were then incubated for 1 hour. The next stage of each sample was examined for mobility, Viability, DNA fragmentation and morphology. Sperm parameters were measured for at least 30 specimens before and after adding different concentrations of patulin. The control group was only added with Ham'sF10 environment.

### Viability

To determine the Viability rate of the sperm, they were mixed equally between trypan blue and semen fluid and after 3 to 5 minutes the live sperm (colorless) ratio was calculated to dead sperm (blue) in 100 counts of sperm.

### DNA Damage

Sperm chromatin dispertion (SCD) and Wright staining (according to the protocols of the Royan Institute) were used to determine the DNA degradation. To calculate the percentage of DNA fragmentation, the SCD slides were observed under an optical microscope with a magnification of 100 ×.

### Papanicolaou Stain (Dif-quick)

To evaluate the morphology of the sperm before and after the addition of patulin toxin to the specimens via *Papanicolaou stain* (Dif-quick), a variety of sperm morphology were examined and percentage of sperm was calculated with specific morphology.

### Sperm Mobility

Before and after the addition of the sperm, sperm motility was evaluated and the sperms were divided into four groups: Group A: Progressive forward motility, Group B: Group returning forwards, Group C: Group by moving in place and Group D: immobility. Percentage of mobility in each group was calculated from 100 counts of sperm.

### Statistical Analysis

Statistical analyzes were performed using one-way ANOVA and t-test. Data comparison was expressed in terms of mean and significance level of 0/05 was considered as a significant level. Mobility and viability percent were determined according to WHO standards.

## RESULTS

The effects of patulin on normal semen samples collected from the Royan Institute were evaluated and the parameters of sperm motility, sperm viability, and DNA damage and morphology were evaluated.

### Sperm Motility

Sperm motility was evaluated before and after adding patulin to the specimens and compared to normal specimens. Results showed that in all concentrations after adding patulin to Saman sample, all types of mobility of A, B and C in sperms decreased compared to control group. Further, after adding patulin poison at higher concentrations, the amount of immotile sperms increase. At 0.5 concentration, this increase is quite significant (Figure 1).

### Sperm Viability

The Viability rate of the sperms was similarly tested in laboratory environment before and after semen specimens were exposed to different concentrations of the patulin. In all concentrations after the addition of patulin poison to semen samples, the Viability rate of sperms decreased significantly. This rate at concentrations (0.5) and (0.33) had more difference with control group (Figure 2).

### DNA Degradation (SCD Test)

DNA degradation was evaluated only at three concentrations of 0.5, 0.33 and 0.05. At each concentration, 3 Semen

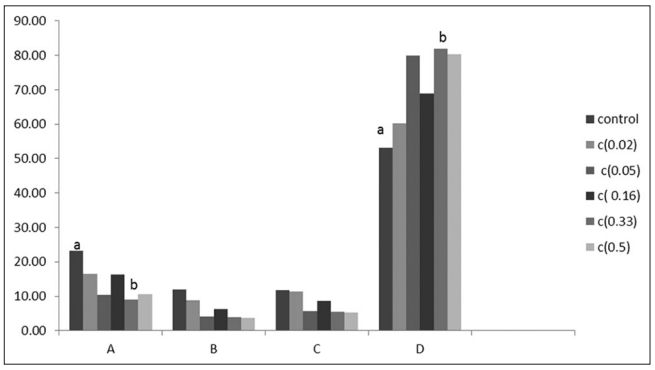


**Figure 1: Comparison of sperm motility in different concentrations with control group A: Direct forward, B: Move forward and then change direction, C: Move in place, D: motionless. The difference between a and b is significant (p≤0.05)**

samples were examined and evaluated (n=3). In the two concentrations of 0.5 and 0.33, DNA degradation was significantly different with the control group, with the highest degradation observed with increasing concentrations (0.5) (Figures 1-3).

## DISCUSSION

Patulin is one of the mycotoxins produced by molds in rotten fruits, especially apples and juices derived from them. Since this combination is carcinogenic, mutagenic, and teratogenic, which can weaken the immune system and complications of the digestive system, it can also affect sperm DNA [11]. The increase in infertility in men today has been considered. Unfortunately, in many apple juice factories, there is no precise control over the standard level of this substance in apple juice, so the high consumption of these products can lead to DNA degradation and abnormal sperm production, resulting in reduced fertility. Permissible levels of patulin in apple juice produced at factories is 50 ng/ml based on the national standard of Iran 5925 in 2001 or 50 µg/liter (50 ng/ml) based on WHO. Usually, the amount of this substance in some apple juices from Iran's factories is more than its permitted value of 76.45 and 88.36kg and even 125.25mg/lit [1], which is approximately twice what it is allowed. Therefore, the high consumption of these fruit juices can have an adverse effect on the health of people. It has been found that the patulin has a very strong stretch with sulfidryl groups. That's why it can stop many enzymes. Some studies have shown that patulin damages DNA synthesis. These Genotoxic effects may be related to the ability to react with sulfidryl and alpha oxidative damage (19, 13). However, WHO has so far not concluded that patulin has a genotoxic property. Concerning the effects of various elements such as copper (15), zinc (5), cobalt and chromium (16), or various pesticides such as pyridaben (3) hinazone and diazinon (2) Phenel-hydrogainone (26) and Aflatoxin B1 (17) on sperm parameters, various studies have been done. But the effect of patulin on fertility has been studied only in rats and mice, and it has been shown that this toxin reduces sperm (24) and affects granulosa cells (9). It also has direct effects on cell membrane, but so far, the direct effect of this toxin on human sperm cells is not available in a laboratory environment. Therefore, this paper attempts to investigate the effect of patulin on sperm parameters and in particular sperm fragmentation. Results of this study showed that this toxin can degrade the sperm DNA and also decrease the sperm motility and viability rate of the sperm, so it can affect male fertility.

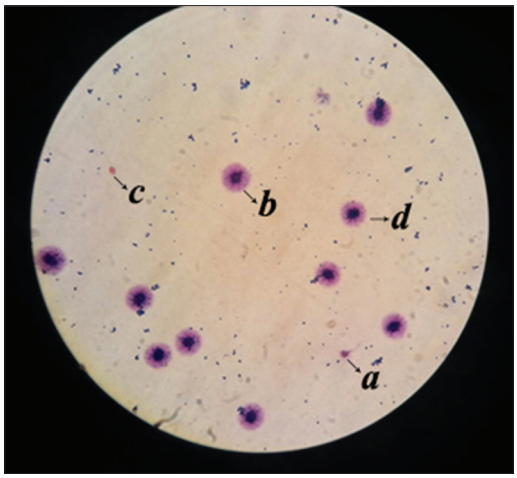


**Figure 1: a: sperm without Halo (sperm with DNA fragmentation) b: Large halo around the sperm's head (healthy DNA) c: sperm with decomposed DNA (pink sperm head) d: sperm with middle halo (Healthy DNA)**

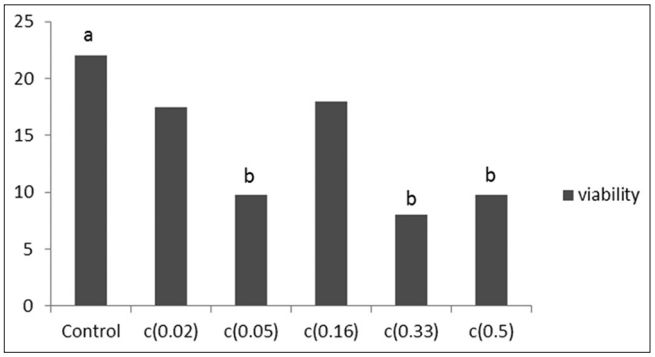


**Figure 2: Comparison of the Viability of sperm with different concentrations in the control group.The difference between a and b is significant (p≤0.05)**

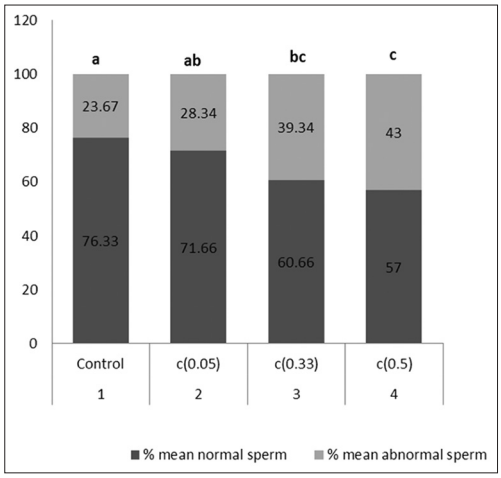


**Figure 3: Comparison of DNA degradation status at three concentrations of 0.5, 0.33 and 0.05 compared to the control group. As it can be seen, in the concentration of 0.33 and 0.5, there were significant differences in DNA fragmentation with control group and in these concentrations percent of abnormal sperms is more. The difference between a with b and c is significant (p≤0.05)**

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