Evaluation of the safety and acute toxicity of strains of the medicinal *Schizophyllum commune fr.* fungus

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Abstract. In this article, the safety grading of local strains JE4, JE5 and JE6 of the medicinal fungus S. commune and the study of the effect of acute toxicity in laboratory conditions are analyzed. The mycelial liquid of the strains was given to white mice in the same amount in the mouth, vein, and abdominal cavity. Experimental tasts were observed for 14 days. Permanent signs of dangerous toxicity in animals: changes in the color of the skin, fur, eyes and mucous membranes, activity towards food, behavior, changes in height, movement, sleep, tremors, seizures, salivation cases of separation, diarrhea and death were monitored. It is noted that the strains of Schizophyllum commune are harmless and not acutely toxic.

1 Introduction

Currently, the main methods of macrofungi mycotoxin analysis include thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography, mass spectrometry (GC-MS). These methods are time-consuming and often lack toxicological evaluation [11-13]. In contrast, methods for toxicity assays of mycotoxins in various animals have been informed. Mycotoxins of macrofungi are listed as the most dangerous toxins in the food industry [1]. However, in the pharmaceutical industry, as a result of the processing and synthesis of mycotoxins produced by fungi, they are used as a means of enhancing the mechanism of action of various drugs [2]. According to many scientists, plants are the main key in the ecological chain relating all objects of the biosphere [3]. Among the fungi growing in the forests of tropical countries, the medicinal mushroom *Schizophyllum* commune Fr was collected in large quantities by Japanese scientists and various mycotoxic properties were studied [4].

Four different methods can be used to determine the harmfulness of fungi and early assessment of the toxicity of mycotoxins. The first method is chemical detection, in which the isolated mycotoxin is studied for a long time, and most of the fungal mycotoxins are unstable and undergo rapid chemical changes [5]. The second method is to conduct experiments on animals, in which experiments are carried out in laboratory conditions with white mice, rats, guinea pigs or rabbits [6]. The third method focuses on determining their toxicity based on the classification of these fungi [7]. The fourth method is a non-scientific

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method that has been researched by people for many years and is considered a folk experience [8].

2 Materials and methods

In the course of our scientific research, samples of the fruit bodies of the medicinal fungus S commune were collected in different regions of our region. A separate strain was isolated from each of the collected samples [9]. The strains were isolated in the Laboratory of Microbiology, Karshi State University, and are currently kept in the collection of the department (JE4, JE5 and JE6). The safety assessment and acute toxicity of S. commune fungus (JE4) #1 and (JE5) #2 strains were studied. The researches were carried out together with scientific staff of the Pharmaco-toxicological Laboratory of Coordination Compounds and Bioactive Substances established at the Karshi State University, Microbiology and Tashkent Pharmaceutical Institute. Strains isolated from S.commune fungus were named (JE4) #1 and (JE5) #2 (Figure 1) MUK 4.2.2602-10. Control methods were carried out in full compliance with the use of biological and microbiological factors [10].

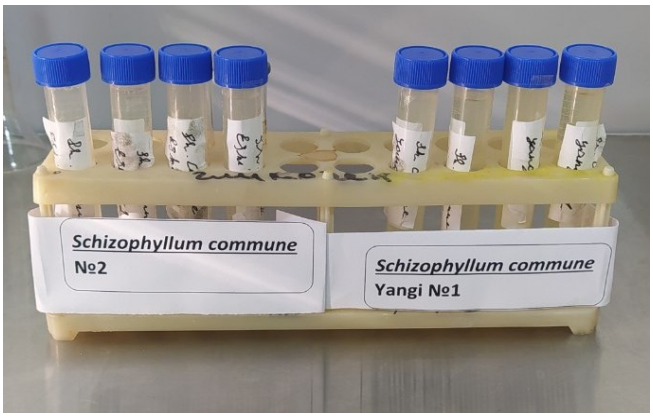

Fig. 1. Strains isolated from the medicinal fungus S commune. Mycelial liquid called #1 and #2.

3 The obtained results and their analysis

The experiments were conducted on 30 white mice of both sexes, weighing 13-15 g. White mice were acclimatized and adapted with attention to water and food ration in plastic containers (Figure 2).

Fig. 2. Acclimatization process of white mice in plastic containers with controlled water and feeding.

Seven days later, 1 ml of the mycelial liquid of the fungus was taken, diluted 109 times, and the suspension was administered orally to rodents using a 0.5 ml syringe. A low concentration of fungal mycelia of the test strain was injected into mouse cells (Figure 3).

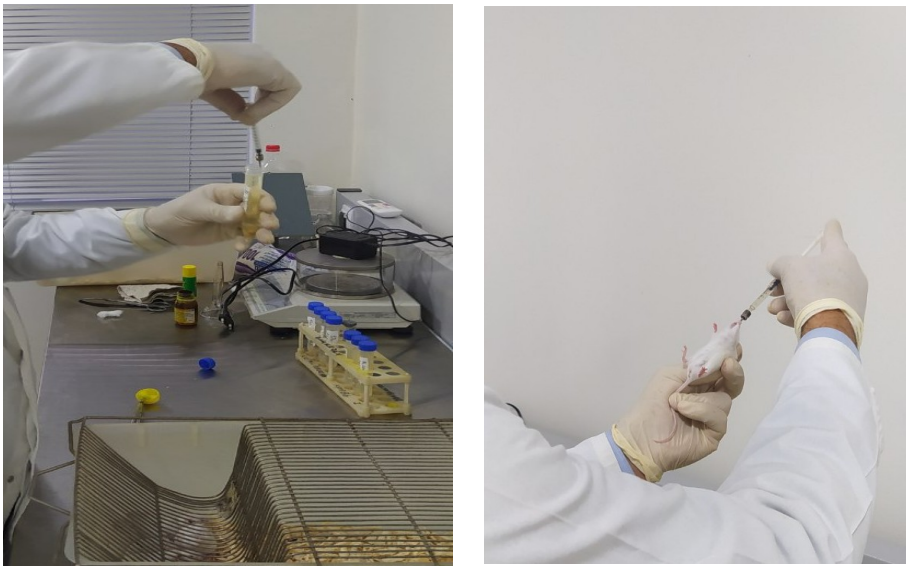

Fig. 3. The process of diluting 109 times the mycelial fluid of the fungus and injecting it orally to rodents using a 0.5 ml syringe.

A control group of mice (5 mice) was fed saline. Animal observation is carried out for 5 days. The results of the experiment are presented in Table 1 below.

Table 1. Safety evaluation of test strains.

Name of strains	quantity, ml	Number of mice
		died/total
Schizophyllum commune.- 10^9 No. 1	0.5	0/5
Schizophyllum commune -10^9 No. 2	0.5	0/5
0.9 % NaCl	0.5	0/5

Animals did not lose weight during observation. No animals died during the experiment. As a result, it showed the safety of the strains.

An experiment was also conducted to determine the acute toxicity of the strains isolated from the *S.commune* fungus. Determination of acute toxicity was carried out in 30 white mice weighing 13-14 g. Rodents were selected out of 5 and divided into groups. Mycelial liquid of the tested local strains was given by mouth to mice, injected in vein, in the same amount. A 0.9% sodium chloride solution was sent to the control group by the same way. Animals were observed for 14 days. Permanent signs of dangerous toxicity: changes in the color of the skin, fur, eyes and mucous membranes, activity towards food, behavior, changes in height, movement, sleep, tremors, seizures, salivation, diarrhea and death cases were monitored. During the experiment, none of the above symptoms of acute poisoning and death were noted. The results of the experiment are presented in Table 2 below.

Table 2. Evaluation of the acute toxicity of the investigated local strains.

Name of strain	Quantity, ml	Method of sending	Number of died mice/total
Schizophyllum commune - 10^9 No. 1	0.5	o; v/i; q/b;	0/5
Schizophyllum commune - 10^9 No. 2	0.5	o; v/i; q/b;	0/5
0.9 % NaCl	0.5	o; v/i; q/b;	0/5

The tested strains were not found to be acutely toxic even when the same amount was given to the abdominal cavity of mice by mouth, injected in vein.

4 Conclusion

As a result of our research, when assessing the safety of the tested strains, 1 ml of mycelial liquid was taken, diluted 109 times, given by mouth using a 0.5 ml syringe and observed for 5 days. The animals did not lose weight. No animals died during the experiment. The result showed that the strains were harmless. In the experiment to determine the acute toxicity of the strains, the mycelial liquid was not found to be acutely toxic even when the same amount was given to the mice's abdominal cavity by mouth and injected in vein. It was concluded that the tested strains of the *Schizophyllum commune* fungus were harmless and not acutely toxic during the experiment.

References

1. J.H. Tegzes, B. Puschner, Toxic mushrooms, Veterinary clinics of north America: Small Animal Practice, **32**, **2**, 397–407 (2002)
2. C. Lei, W. Tangkanakul, L. Lu, "Mushroom poisoning surveillance analysis" OSIR Journal, **1**, **1**, 8–11 (2006)
3. Z.Z. Uzakov, K.N. Karimov, Z.U. Uzakov, R.A. Eshonkulov, Accumulation of Heavy Metals in Agricultural Crops and Ecological Series of Crops Placement, Baghdad Science Journal, **20**, **1** (2023)
4. T. Fukuwatari, E. Sugimoto, K. Yokoyama, K. Shibata, "Establishment of animal model for elucidating the mechanism of intoxication by the poisonous mushroom Clitocybe acromelalga," Journal of the Food Hygienic Society of Japan (Shokuhin Eiseigaku Zasshi), **42**, **3**, 185–189 (2001)
5. J.H. Diaz, "Evolving global epidemiology, syndromic classification, general management, and prevention of unknown mushroom poisonings," Critical Care Medicine, **33**, **2**, 419–426 (2005)
6. T. Fukuwatari, E. Sugimoto, K. Shibata, Effect of feeding with a poisonous mushroom Clitocybe acromelalga on the metabolism of tryptophan-niacin in rats, Shokuhin Eiseigaku zasshi, Journal of the Food Hygienic Society of Japan, **42**, **3**, 190-196 (2001)
7. P. Wexler, B.D. Anderson, S.C. Gad, Encyclopedia of toxicology (Academic Press, Cambridge, MA, USA, 2005)
8. M. Lu, "Present status and future prospects of the mushroom industry in China" Acta Edulis Fungi, **13**, **1**, 1–5 (2006)
9. MUK 4.2.2602-10. 4.2. Control methods. Biological and microbiological factors. System of pre-registration preclinical study of drug safety. Selection, testing and storage of production strains used in the production of probiotics. Guidelines (approved

by Rospotrebnadzor on April 21, 2010), From the “Medicine and Pharmaceuticals” information bank (2010)

10. E. Erkin, S. Jamila, M. Dilmurod, *Schizophyllum communasi* Fr. on the territory of Uzbekistan isolation of pure culture of medicinal fungus, Universum: chemistry and biology, **6-4**, **96**, 4-7 (2022)
11. D. Flajs, A.M. Domijan, D. Ivić, B. Cvjetković, M. Peraica, ELISA and HPLC analysis of ochratoxin A in red wines of Croatia, Food Control, **20**, 590–592 (2009)
12. L. Gong, Y.M. Jiang, F. Chen, Molecular strategies for detection and quantification of mycotoxin-producing Fusarium species: A review, J. Sci. Food Agric, **95**, 1767–1776 (2015)
13. C.D. Liao, J.W. Wong, K. Zhang, D.G. Hayward, N.S. Lee, M.W. Trucksess, Multi-mycotoxin analysis of finished grain and nut products using high-performance liquid chromatography–triple-quadrupole mass spectrometry, J. Agric. Food Chem, **61**, 4771–4782 (2013)