Central Asian Journal of Medicine

ON THE ISSUE OF ASSESSING THE TOXICITY RISK OF FORTIFICANT FOR STAPLE FOODS

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ABSTRACT

The most promising solution to the problem of micronutrient deficiency is the enrichment of mass consumption products with missing vital micronutrients, in particular fortifiers. The aim of the research was to assess the risk of toxicity of the Vitamin & Mineral Premix fortificant (vitamin and mineral premix), manufactured by AQC Chemlab Private Limited, India, when used on experimental animals after intragastric administration. The research was carried out in accordance with the legislative and regulatory and methodological documentation. Biochemical blood tests were performed on a semi-automatic biochemical analyzer "CYANSmart" with software (Cypress Diagnostics, Belgium) according to standard methods. A detailed analysis of peripheral blood was determined in the Goryaev chamber. Experimental tests were carried out in compliance with the rules adopted by the European Convention for the Protection of Vertebrates for Experiments or Other Scientific Purposes (ETS No. 123. Strasbourg, 1986). According to the results of toxicological studies, the tested fortifier "Vitamin & Mineral Premix" (vitamin and mineral premix), manufactured by AQC Chemlab Private Limited, India, does not have a negative effect on the health of experimental animals in the intragastric route of admission, according to the parameters of acute toxicity, it refers to low-hazard substances, does not have a cumulative, irritating effect on the mucous membranes of the eyes, sensitizing effect and does not cause pathological and structural changes in the internal organs of experimental animals. This fortifier meets the safety requirements in terms of toxicological indicators.

Key words: fortificant, vitamin and mineral premix, toxicological assessment, safety.

INTRODUCTION

It is proved that the deficiency of certain vitamins and trace elements contributes to the development of alimentary-dependent diseases, and thereby causes significant damage to health, expressed in a decrease in resistance to various diseases, the effect of harmful factors of the production environment and environmentally unfavorable environmental factors, a reduction in professional longevity and life expectancy of the population [1, 2, 6,]. The most promising solution to the problem of micronutrient deficiency is the enrichment of mass consumption products with missing vital micronutrients [3,4,5]. Fortification of staple foods that are widely distributed and widely consumed will help improve the nutritional status of a significant part of the population and does not require any changes to the diet or an individual decision to comply with the regime [7,]. In accordance with the requirements of the Law of the Republic of Uzbekistan "On sanitary and epidemiological welfare of the population", all new additives, including fortifiers for staple food imported or produced in the country, are subject to mandatory toxicological assessment in order to determine their medical and biological safety for public health when used. Presented for toxicological evaluation fortificant "Vitamin & Mineral Premix" (vitamin and mineral premix), manufactured by AQC Chemlab Private Limited, India for food fortification "Vitamin & Mineral Premix" contains 200 g of: vitamin B1 – 1,4; vitamin B2 – 1,9; vitamin B3 – 10,69; vitamin B9 – 0.9; vitamin B12 – 0.002; iron – 16.3; zinc – 26.78.

The methodological approach to assessing the safety of the fortifier "Vitamin & Mineral Premix" included the following research areas:

- determination of the oral medium-lethal dose;
- determination of cumulative (subacute) action;
- study of the irritating effect on the mucous membranes;
-study of the sensitizing properties of the composition of the fortifier.

During the research, there were no extraordinary circumstances that could affect the quality and reliability of the results obtained.

The aim of the research was to assess the risk of toxicity of Vitamin & Mineral Premix fortificant when used on experimental animals after intragastric administration.

Materials and methods of research. The studies were conducted in accordance with the following regulatory and methodological documentation: GOST 32644 "Testing methods for the effects of chemical products on the human body. Acute oral toxicity - a method for determining the acute toxicity class", MR

No. 012-3/0244 "Procedure and methodology of pre-registration toxicological and hygienic examination of food additives" and GOST 32641 "Test methods for the effects of chemical products on the human body. Determination of toxicity with repeated / repeated oral administration of the substance in rodents. 28-day test". Biochemical blood tests were performed on a semi-automatic biochemical analyzer "CYANSmart" with software (Cypress Diagnostics, Belgium) according to standard methods (AST, ALT, alkaline phosphotase, total protein- kits of Cypress Diagnostics reagents, Belgium), hematocrit was determined on a hematocrit centrifuge (Cypress Diagnostics, Belgium), a detailed analysis of peripheral blood was determined in Goryaev's cell.

Experimental studies were carried out on small laboratory animals (white rats and mice) in accordance with the current regulatory and methodological framework. When extrapolating the obtained toxicological data from animals to humans, interspecific differences in the toxic effect in experimental animals, the degree of toxicity and danger of the additive, the specifics of a particular experiment (methods and methods of introducing the substance into the body, seasonal and circadian rhythms, etc.), uncertainty factors were taken into account.

When assessing the irritating effect on the skin and mucous membranes of the eyes, the sensitizing effect was used by direct transfer of experimental results to humans. Experimental tests were carried out in compliance with the rules adopted by the European Convention for the Protection of Vertebrates for Experiments or Other Scientific Purposes (ETS No. 123. Strasbourg, 1986).

For the experiment, the animals received the fortifier "Vitamin & Mineral Premix" in terms of 100 g of animal weight. The dose was administered as an aqueous solution (99%), the solvent was distilled water. A statistical method of variation series analysis was used to quantify the primary experimental data. The reliability of the difference between the data of the experimental and control groups of animals was assessed according to the Student's t –criterion, guided by a 5% ($p<0.05$) significance level, taking into account the number of animals participating in each experiment in accordance with the requirements of O'ZDST 8.072:2018 for conducting experimental studies.

Results and discussion

Under experimental conditions, the acute toxicity of the studied fortificant "Vitamin & Mineral Premix" was established on 2 types of laboratory animals (white mongrel rats and mice) with a single intragastric intake of each drug name in doses of 2000, 3500 and 5000 mg/kg of animal weight (Table 1).

Table 1

Lethal effects of the studied premix in intragastric administration to laboratory animals of both sexes

Name	Dose mg/kg	number of animals in the group/ number of dead animals	Clinical picture of intoxication	LD_{50}, mg/kg
Vitamin & Mineral Premix	2000	6/0	missing	> 5000
	3500	6/0	missing	
	5000	6/0	missing	

During the next day of observation, the animals maintained a normal reaction to external stimuli, the general condition and behavior of the animals was satisfactory. All the animals were active and willingly ate food, woolly coats and visible mucous membranes did not change. Thus, the results of observations of experimental animals in acute experience make it possible to attribute the fortifier "Vitamin & Mineral Premix" to hazard class IV (GOST 12.1.007), and according to the hygienic classification to hazard class V (low-hazard substance). To study the effect on the mucous membranes, a single inoculation of 0.05 ml of an aqueous solution (50%) of Vitamin & Mineral Premix fortifier was carried out into the conjunctival sac of the right eye of a guinea pig, the left served as a control (in group 3 individuals). Under the influence of a dietary supplement, there was no hyperemia, edema or lacrimation. The average group total score of mucosal irritation severity (Iir) after termination of contact was 0 points (Table 2).

Table 2

Evaluation of the irritating effect of the studied fortificant on the mucous membranes of the eyes

Product Name	Hyperemia conjunctiva	Edema of the eyelids	ptosis or blephorospasm	discharge from the eye	Iir, points
Vitamin & Mineral Premix	0/3	0/3	0/3	0/3	0

Consequently, the obtained research data showed that Vitamin & Mineral Premix fortifier has no irritating effect on the mucous membranes of the eyes (Iir=0). The cumulative ability of the investigated fortifier "Vitamin & Mineral Premix" was evaluated in a subacute experiment by the method of "subchronic toxicity" on white rats weighing up to 120 g.

The studied fortificant "Vitamin & Mineral Premix" was received by experimental animals for 28 days in the form of an aqueous solution. The initial dose was 1/10 of the maximum tolerated in terms of animal weight (500 mg / kg), followed by an increase of 1.5 times every 4 days. Control animals received

distilled water in an equivalent volume. The experimental animals were monitored throughout the experiment according to the following indicators: survival during the experiment, general condition, animal activity, feed intake, water consumption, body weight dynamics, morphological and biochemical composition of blood. No behavioral abnormalities were observed in the animals taken in the experiment during the entire observation period. Similarly to the control animals, they were active, neat, ate food well and responded adequately to external stimuli. There were no signs of intoxication and fatal outcomes. The determination of the body weight of the animals was made before the start of administration and at the end of the introduction of the fortifier "Vitamin & Mineral Premix" (Fig. 1).

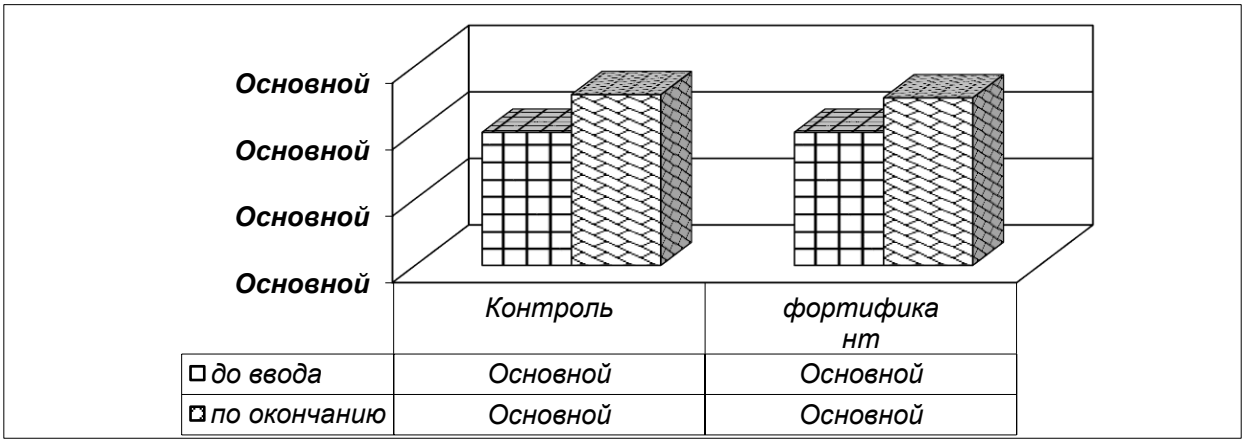

Fig. 1. Dynamics of body weight gain (%)

At the same time, there were no significant differences between the control and experimental groups.

In the study of hematological parameters of peripheral blood of experimental animals receiving the studied complex dietary supplement, no significant changes were found in any of the studied parameters (number of erythrocytes, 1012 / l; hemoglobin, g / l; leukocytes, 109 / l) (Table 3).

Table 3.

Averaged indicators of the morphological composition of rat blood under the subchronic effect of the studied fortifier

Groups	observation period	Гематологические показатели				
		hematocrit, %	hemoglobin concentration, g/l	thrombocrit, %	leukocytes, $\cdot 10^9$/л	red blood cells, $\cdot 10^{12}$/л
Control, distillation. water	before administration	35,4±1,1	132,6±4,0	0,461±0,02	14,71±2,23	6,76±1,39
	at the end	33,8±1,5	135,1±3,2	0,459±0,01	14,65±3,60	6,80±1,81
Vitamin & Mineral Premix	before administration	34,5±1,2	133,1±2,5	0,446±0,03	14,68±2,43	6,66±1,25
	at the end	35,2±1,8	135,2±4,1	0,448±0,03	14,70±2,66	6,72±1,21

The total number of erythrocytes, leukocytes, hemoglobin content, hematocrit and thrombocrit in all experimental animals did not differ statistically significantly from the control. As the results of studying the biochemical parameters of the blood serum of experimental and control animals showed, the activity of transaminase enzymes (AsT, AlT) and alkaline phosphatase (ALP) of experimental animals did not significantly differ from those of the control group. The indicators of total protein (TP) content of the control and experimental groups were also significantly the same (Table 4).

Table 4.

Biochemical blood parameters of white rats under subchronic exposure to fortificant (Mch±m)

Groups	Observation period	Biochemical parameters			
		AlT, E/l	AsT, E/l	AlT, E/l	TP,g/l
Контроль, дистил. вода	before administration	55,3±2,8	115,2±6,1	350,9±21,8	66,2±2,7
	at the end	57,2±3,5	114,9±6,2	340,9±22,3	66,1±2,3
Vitamin & Mineral Premix	before administration	54,3±3,2	113,8±6,2	330,8±21,8	64,8±2,6
	at the end	55,7±2,8	114,2±5,6	340,5±23,4	66,3±2,7

At the end of the experiment, control group rats and animals treated with Vitamin & Mineral Premix fortificant were euthanized by administering ether to anesthesia and the condition of internal organs was assessed visually during autopsy. According to the results of microscopic examination of organs, there were no differences between the experimental and control groups. The determination of the relative mass coefficients of the internal organs showed that the fortificant did not cause degenerative changes in the lymphoid and most important internal organs. Studies have shown that daily intragastric administration to rats in an increasing dose of Vitamin & Mineral Premix fortificant for 28 days does not cause lethal effects, does not lead to changes in physiological parameters, does not cause dystrophic or destructive changes in parenchymal organs and is not accompanied by irritation of mucous membranes. Thus, the conducted subchronic experiment showed that Vitamin & Mineral Premix fortifier does not have cumulative properties. When studying the sensitizing effect in experimental animals, 5 days after the subchronic experiment, sensitization was detected by setting a skin scarification test with a drop of minimal dilution of the fortifier

"Vitamin & Mineral Premix" (test - antigen), which does not cause a visible reaction in animals of the control group (50% dilution), to animals of the control

group, the permissive dose was administered in the same way as to experimental animals: a drop of the additive was applied to a section of the lateral surface of the trunk, then an incision was made with a scarifier through a drop 1-1.5 cm long. Detection of sensitization is carried out 4-24-48 hours after scarification of the studied complex additive. The skin reaction at the scarification site is taken into account according to the appropriate scale (Table 5).

Table 5.

The results of the evaluation of the sensitizing effect of the studied fortifier

Tested concentration	Hyperemia	Hyperemia and seal Blister	Blister up to 5 mm, hyperemia around	Blister up to 10 mm, lichenification	Is, ponts
Control, distil. water	0/6	0/6	0/6	0/6	0
Vitamin & Mineral Premix	0/6	0/6	0/6	0/6	0

Testing carried out after the scarification test did not reveal sensitizing properties in the Vitamin & Mineral Premix fortifier, as a result of which there was no need for a detailed allergy study (Is =0).

Conclusion. Based on the results of toxicological studies, it was found that the tested fortificant "Vitamin & Mineral Premix" in the intragastric route of admission does not have a negative effect on the health of experimental animals, according to the parameters of acute toxicity refers to low-hazard substances, does not have a cumulative, irritating effect on the mucous membranes of the eyes and sensitizing effect. Dystrophic, necrotic and inflammatory changes in animals observed in the experiment, as well as differences in the structure of their internal organs were not found. Consequently, the results obtained allow us to conclude that with repeated oral intake into the body, the fortificant "Vitamin & Mineral Premix", manufactured by AQC Chemlab Private Limited, India, meets the safety requirements according to toxicological indicators.

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