TOXIC AND HYGIENIC PROPERTIES OF BIOLOGICALLY ACTIVE FOOD ADDITIVES "STEVIAMAR", "SKVALEAMIN NEO", "SKVALEMARIN"

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ABSTRACT

The article presents the results of studying the toxicological and hygienic properties of biologically active food additives "Steviamar", "Skvaleamin Neo", and "Skvalemarin", developed for the first time in Uzbekistan.

KEYWORDS: *Biologically Active Additives (BAA), Amaranth, Food Additives, Toxicology, Allergy, Resorption, Absorption, Moderate Lethal Dose (LD_{50}).*

INTRODUCTION

"Steviamar", "Skvaleamin Neo", and "Skvalemarin" were developed for the first time in Uzbekistan and production was launched at SIFAT AGRO SERVIS LLC in Andijan, biologically active food supplements made from plant extracts rich in vitamins, and minerals and are intended for use in as a source of biologically active food supplements that are lacking in the body [1-3].

The corresponding samples were transferred to the Republican Center for State Sanitary and Epidemiological Surveillance of the Ministry of Health of the Republic of Uzbekistan to check the compliance of these biologically active additives with toxicological and hygienic requirements. The activities of the toxicological laboratory of the Center are carried out based on accreditation certificate No. UZ.AMT.07 MAI.086, registered in the State Register of the National Accreditation System of the Republic of Uzbekistan and issued on February 18, 2008, to the testing laboratory (centre).

Experimental Period: January 9-February 11, 2019 (head of the department and laboratory of toxicology Eshmuradov Sh.Kh., laboratory assistant Yakubova G.T., laboratory assistant Shoyusupova M.M.)

Information about biologically active food supplements:

- In one bottle of "Skvaleamin Neo" amaranth seeds – 57 g, milk thistle leaves – 2 g, safflower leaves – 2 g, safflower flowers – 0,5 g.
- One filter package of amaranth "Steviamar" contains amaranth flowers – 2,5 g, amaranth leaves – 2,5 g, stevia leaves – 2,5 g, and milk thistle leaves – 2,.5 g.
- "Skvalemarin" contains 20 ml of amaranth oil and 5 ml of milk thistle oil.

➢ Tests of biologically active food additives “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” were carried out in accordance with the Decree of the Cabinet of Ministers of the Republic of Uzbekistan №131 dated April 30, 2016 "On approval of the Regulations on the procedure for passing licensing procedures in the system of the sanitary and epidemiological service of the Republic of Uzbekistan", in order to confirm the recommendations for application, indications, contraindications, determination of side effects.

TOXICOLOGICAL TEST RESULTS [4-7]

2.1. Determination of the average mortality from biologically active food supplements administered orally into the stomach

Experiments to determine the acute toxicity of biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin”, i.e. the average lethal dose (LD_{50}), were carried out on white rats. The initial weight of the animals was 160-210 g.

Doses of 2000, 3000, 4000 and 5000 mg/kg BAA in tablet form were administered to rats in the stomach through an iron tube once and the animals were observed for 21 days. As a criterion for the action of biologically active food additives, the occurrence of symptoms of poisoning in animals and the occurrence of a lethal outcome were taken.

The results of the experiments showed that during the control period when testing the tested doses of biologically active food supplements in animals, there were no changes in behaviour, appearance, or attitude to food, or water, and there was no death. The table shows results for only the highest dose of 5000 mg/kg of the study dose of each dietary supplement tested (Table 1).

TABLE 1. THE RESULTS OF MONITORING MORTALITY IN THE INTRODUCTION OF BIOLOGICALLY ACTIVE FOOD SUPPLEMENTS INTO THE STOMACH OF ANIMALS IN A SINGLE DOSE OF 5000 MG/KG

Name of the biologically active food supplement	Dose, mg/kg	Days of observation and the ratio of the total number of animals to the number of dead animals								Total number of dead animals
		1-day	2-day	3-day	4-day	5-day	6-day	14-day	21-day	
“Skvaleamin Neo”	5000	6/0	6/0	6/0	6/0	6/0	6/0	6/0	6/0	0
“Steviamar”	5000	6/0	6/0	6/0	6/0	6/0	6/0	6/0	6/0	0
“Skvalemarin”	5000	6/0	6/0	6/0	6/0	6/0	6/0	6/0	6/0	0

Conclusions: the average lethal dose of biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” is more than 5000 mg/kg with a single injection into the stomach of white rats and according to the state standard (GOST 12.1.007-76) corresponds to class 4 low - hazard substances.

2.2. The results of the study of the local action of biologically active food supplements on the skin

To study the local action of biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” on the skin, tests were carried out on guinea pigs. The initial weight of the animals was 320-360 g.

Initially, the right and left sides of the animals were cleaned of hair 5x5 cm in size using electric scissors. In the dehydrated skin of the right side of the animals, the test samples of native food additives were applied once at a dose of 20 mg/cm^2 separately in groups and left for 4 hours. The left side of the animals was left for control. After the prescribed time, the skin surface was washed with warm water and wiped dry.

As a criterion for the action of biologically active food additives, attention was paid to the symptoms of redness, and swelling that may occur on the skin, and their severity was assessed in points.

Redness was visual and puffiness was detected by measuring skin thickness with an electronic micrometre. The observation period lasted 14 days.

The results of the experiment showed that the experiment, biologically active food supplements "Steviamar", "Skvaleamin Neo", and "Skvalemarin" in the test dose did not cause redness and swelling of the skin (Table 2).

TABLE 2. THE DEGREE OF REDNESS AND SWELLING OF THE SKIN OF GUINEA PIGS UNDER THE INFLUENCE OF BIOLOGICALLY ACTIVE FOOD ADDITIVES (IN POINTS)

Name of the biologically active food supplement	Observed indicators	Observation time and evaluation										
		Background	4 hours	1-day	2-day	3-day	4-day	5-day	6-day	7-day	10-day	14-day
"Skvaleamin Neo"	redness	0	0	0	0	0	0	0	0	0	0	0
	oedema	0	0	0	0	0	0	0	0	0	0	0
"Steviamar"	redness	0	0	0	0	0	0	0	0	0	0	0
	oedema	0	0	0	0	0	0	0	0	0	0	0
"Skvalemarin"	redness	0	0	0	0	0	0	0	0	0	0	0
	oedema	0	0	0	0	0	0	0	0	0	0	0

Conclusions: Biologically active food supplements "Steviamar", "Skvaleamin Neo", and "Skvalemarin" with a single application to the skin did not cause changes (redness 0 points, oedema 0 points) and according to a special classification belong to the category of substances that do not cause inflammation (irritation) on the skin, i.e., do not cause local skin changes.

2.3. Suction of biologically active additives to food through the skin

To study the absorption of biologically active food supplements "Steviamar", "Skvaleamin Neo", and "Skvalemarin" through the skin, tests were carried out on guinea pigs.

During the observation period, the impact criterion was changed in the behaviour of animals – changes in mobility, needs for food and water, external form, urine colour, increase in eye secretions increased heart rate and increased respiration.

Results showed that the subject's biological active food supplements did not produce any changes in the experimental animals, and their behaviour did not differ from the animals in the control groups.

Conclusions: Biologically active food supplements "Steviamar", "Skvaleamin Neo", and "Skvalemarin" we're not absorbed through the skin of animals, did not lead to any changes in the behaviour of animals and are not resorptive.

2.4. Influence of biologically active food supplements on the mucous membrane of the eye and cornea

The effect of biologically active food additives "Steviamar", "Skvaleamin Neo", and "And Skvalemarin" on the mucous membrane of the eye and cornea was studied on rabbits in groups of 3 individuals.

The samples were instilled into the conjunctival sac of the right eye of the animals, 2 drops separately, and the tear-nasal canal was pressed with a finger for one minute, while the left eye performed a control function (only 2 drops of water were instilled into the conjunctiva). The observation period was 14 days.

The results showed that when using the studied biologically active food supplements "Steviamar", "Skvaleamin Neo", and "Skvalemarin" in the conjunctiva of the eye, and eyelids, there is no increase in the secretion of the eye and changes in the cornea (damage to the cornea, the affected area of the cornea) (Table 3).

TABLE 3, THE RESULTS OF THE EVALUATION OF THE IMPACT OF THE TESTED FOOD ADDITIVES ON THE MUCOUS MEMBRANE OF THE EYE AND CORNEA

Name of the biologically active food supplement	Observed indicators	Observation time and evaluation										
		Background	4 hours	1-day	2-day	3-day	4-day	5-day	6-day	7-day	10-day	14-day
"Skvaleamin Neo"	redness of the conjunctiva	0	0	0	0	0	0	0	0	0	0	0
	swelling of the eyelids	0	0	0	0	0	0	0	0	0	0	0
	tearing	0	0	0	0	0	0	0	0	0	0	0
	corneal opacity	0	0	0	0	0	0	0	0	0	0	0
	affected area of the cornea	0	0	0	0	0	0	0	0	0	0	0
"Steviamar"	redness of the conjunctiva	0	0	0	0	0	0	0	0	0	0	0
	swelling of the eyelids	0	0	0	0	0	0	0	0	0	0	0
	tearing	0	0	0	0	0	0	0	0	0	0	0
	corneal opacity	0	0	0	0	0	0	0	0	0	0	0
	affected area of the cornea	0	0	0	0	0	0	0	0	0	0	0
"Skvalemarin"	redness of the conjunctiva	0	0	0	0	0	0	0	0	0	0	0
	swelling of the eyelids	0	0	0	0	0	0	0	0	0	0	0

	tearing	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	corneal opacity	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	affected area of the cornea	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Conclusions: Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” are substances that do not damage the eyes (mucosa and cornea) according to the degree of local action.

2.5. The study of the cumulative properties of biologically active food supplements

AT In the previous experiment, with the introduction of test samples through the stomach of white rats at a dose exceeding 5000 mg/kg, the absence of mortality of animals at the maximum tested dose did not allow us to calculate their average lethal dose and average time of death. Thus, the results of this experiment indicate that the tested biologically active food supplements do not accumulate in the body.

Conclusions: Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” belong to the class of substances, the cumulative property of which is functional.

2.6. Study of allergenic properties of biologically active food supplements

Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” were tested for allergenic properties on guinea pigs. For the experiment, 3 individuals were selected in each group (total 3 groups). The initial weight of the animals was 340-370 g.

Initially, 0.02 ml of a suspension dissolved in saline 1:500 (20 μg) of each biological food supplement was injected subcutaneously under the skin of the outer ear of the animal using a tuberculin syringe. Animals of the control group were injected only with saline. After 12 days, the skin surface of the right side of the experimental animals, devoid of hair, was incised with a scarifier 1-1.5 cm long, twice the test dose (40 μg) was instilled than the initial sensitizing test dose (40 μg) and evaluated using a specially adapted scale (template).

The experiments showed that when BAA was introduced into the skin and instilled into the incision, the results were the same as in control animals, redness and other changes in the skin were not observed.

Conclusions: Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” are not allergenic.

PATHOLOGICAL STUDIES

At the end of toxicological experiments on animals to test biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin”, the animals were dissected, focusing on changes in the internal organs - the heart, lungs, liver, kidneys, stomach, spleen, intestines.

The results of the study showed that the colour, size, weight and location of the internal organs of the experimental animals did not differ from those of the control animals.

Conclusions: Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” do not cause pathomorphological changes in the internal organs when administered to animals once.

GENERAL CONCLUSIONS ON TOXICOLOGICAL STUDIES

1. The average lethal dose of biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” in the stomach of white rats is more than 5000 mg/kg in a single injection. According to this indicator, it complies with state standards (GOST 12.1.007-76) and belongs to substances of the 4th class of low hazard.

2. When biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” are applied to the skin, the total score of local changes (redness - 0 and swelling - 0) is 0. According to a special classification, this substance does not cause local effects (inflammation) in the skin;

3. Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” are not absorbed through the skin when applied to the skin, i.e. do not have negative resorptive properties;

4. Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” refer to substances that do not damage the eyes (mucous membrane and cornea) according to the degree of local action;

5. Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” belong to the class of substances, the cumulative property of which is functional;

6. Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” are not allergenic;

7. Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” do not cause pathomorphological changes in the internal organs when administered to animals once.

FINAL CONCLUSION

Biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin” according to the average lethal dose ($DL_{50\ per\ os} > 5000$ mg/kg), according to GOST GOST 12.1.007, belong to substances of the 4th class of low hazard, they are not having a harmful effect on the skin, eyes, have a functional cumulative property and do not cause allergies in animals.

Therefore, from a toxicological point of view, it is possible to produce and use biologically active food supplements “Steviamar”, “Skvaleamin Neo”, and “Skvalemarin”.

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