The effect of oral administration of tauroside Sx1 on the accumulation of influenza virus and histological changes in the lungs of mice

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Abstract. The constant threat of a new viral pandemic gives special urgency to the search for new effective means of preventing and treating influenza infection. The article examines the effect of oral administration of saponin tauroside Sx1, obtained from the leaves of Crimean ivy, on the development of infection caused by influenza virus A/WSN/1/33(H1N1), and histological changes in the lungs of infected mice. It was revealed that oral administration of saponin tauroside Sx1 at a dose of 200 mcg/mouse day or 11.8 mg/kg/day for three days after infection led to an almost twofold statistically significant increase in the average life expectancy of infected animals from 6.50±0.67 to 11.10±2.19 days. The protective activity of tauroside Sx1 was established when administered orally in the early stages of influenza infection in mice. The protective effect of saponin is manifested in a significant increase in the average life expectancy and normalization of the structure of lung tissue in infected animals. The results obtained indicate the prospects for further study of saponin tauroside Sx1 as a potential component of anti-influenza drugs.

1 Introduction

Cyclical influenza pandemics are the result of the appearance of viruses with a new variant of the genotype that can be transmitted from animals to humans, to which most of the population has no immunity. Such viruses spread rapidly around the world, often circulating outside the normal flu season, and pose a danger to people of all ages [1]. Despite the fact that previously acquired immunity due to previous infection with antigenically related virus strains can provide partial protection, as happened in seniors during the influenza A(H1N1) pandemic in 2009-2011, according to the World Health Organization (WHO), more than 646 thousand patients die annually from seasonal influenza in the world [2]. For influenza therapy and prevention, adamantane series drugs (remantadine, amantadine), neuraminidase

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inhibitors (zanamivir, oseltamivir, peramivir), as well as the relatively new baloxavir marboxil are currently used [3].

The disadvantages of these drugs are rather high toxicity, narrow spectrum of action, and rapid formation of virus resistance, which occurs most quickly in people with a weakened immune system [4]. Currently, a full-fledged drug against influenza A/H1N1 viruses that effectively interrupts the viral reproduction cycle, not developed. In this regard, to increase the effectiveness of influenza prevention and treatment, it is advisable to use indirect drugs with an immunomodulatory effect.

For a long time, ivy-based preparations have been widely used as expectorants and mucolytic agents. The therapeutic effect of such drugs depends on the combination of many plant components. For example, it was found that ivy α–hederin has an antispasmodic effect on the bronchi as a result of its binding to β-adrenoreceptors, which causes relaxation of smooth muscles. Ivy saponins are also able to enhance the secretion of bronchial glands due to the alkaloid emetine, which irritates the receptors of the gastric mucosa and induces a vagus reflex that spreads to the lungs and enhances the secretion of mucus from the alveoli [5]. Nevertheless, histological studies of lung tissue against the background of the use of immunomodulatory properties of ivy have not been practically studied.

The purpose of this research was to study the effect of oral administration of saponin tauroside Sx1, obtained from the leaves of Crimean ivy, on the pathogenesis of infection caused by influenza virus A/WSN/1/33(H1N1), and histological changes in the lungs of infected mice.

2 Materials and Methods

The objects of the study were 68 male mice aged 4-6 weeks of the BALB/c line weighing 16-18 g. The animals were divided into the following groups: control group (K, 8 animals) who were orally injected with isotonic sodium chloride solution (IR); saponin control group (KS, 8 animals) who received oral saponin; group infected with influenza virus A/WSN/1/33(H1N1) (V, 26 animals), and a group of infected animals that received saponin (VS, 26 animals). Animals were infected intranasally with influenza A/WSN/1/33(H1N1) virus under ether anesthesia by injecting 50 ml of allantois fluid containing 10 LD_{50} of the virus. The initial version of the influenza virus (IV) was obtained from the Institute of Virology n.a. D. I. Ivanovsky RAMS (Moscow, Russia). Tauroside Sx1 was used as a correction agent, which is a triterpene glycoside with the formula 3-O-a-L rhamnopyranosyl (1-"2)-a-L-arabinopyranoside of hederagenin isolated from the Crimean ivy Hedera taurica (Hibberd) Carrière [6]. Animals from the KS and VS groups were watered twice a day for 3 days after infection with a saponin solution with a concentration of 5 mg/ml. The dose of saponin received by these animals was 200 mcg/mouse/day or 11.8 mg/kg/day. This therapeutic scheme of saponin administration has previously shown its effectiveness [7, 8] in animals with experimental influenza infection and with influenza vaccination. The percentage of mortality and the average life expectancy of animals were calculated based on 21 days of observation, the percentage of weight loss of mice.

To determine the dynamics of the accumulation of influenza virus in the lung tissue of animals, some mice from the experimental groups were removed from the experiment under ether anesthesia 1, 2, 3, 4, 5 and 6 days after infection. The lungs of these mice were homogenized in a tenfold volume of a sterile phosphate-salt buffer containing 400 micrograms/ml of gentamicin, and a series of tenfold dilutions were prepared from homogenates on the same buffer. The tissue homogenate was incubated in a thermostat for 1 hour at 370C, then the liquid part was separated from the tissue sediment by centrifugation at 3000 rpm for 15 minutes and 10-fold dilutions of the supernatant were prepared, which were used to infect 10-day-old chicken embryos to detect the virus titer. The embryos were

cultured in a thermostat at 37^0C 48 h, after which they were cooled and opened for the selection of allantois fluid (AF). The level of reproduction of the virus was assessed according to the hemagglutination reaction (RHA) of erythrocytes.

To study the effect of tauroside Sx1 on the development of pathological changes in lung tissue, mice were removed from the experiment on day 4 after infection, and organs were extracted for further study. Lung areas were fixed in 10% neutral buffered formalin followed by standard histological treatment. Paraffin sections were stained with hematoxylin and eosin for descriptive analysis of morphological transformations followed by morphometric evaluation using the Aperio Image Scope program (Leica Biosystems, USA). The percentage ratio of the areas of damaged (with foci of acute swelling and distelectasis) and unchanged lung areas was determined on the slices using the Aperio Image Scope software. Measurements were made at 200 magnification in 20 fields of view on an image area of 1 mm^2.

All histological studies were carried out on the basis of the Central Research Laboratory of the Institute Medical Academy n.a. S. I. Georgievsky FSAEI HE "KFU named after V. I. Vernadsky". The study was approved by the minutes of the meeting of the Ethics Committee No. 3 dated 21.03.2023 of the FSAEI HE "Crimean Federal University named after V.I. Vernadsky". The animals were kept in standard vivarium conditions in accordance with the norms and rules for the treatment of laboratory animals according to the "Rules for carrying out work using experimental animals".

Statistical processing of the obtained quantitative results was performed using Microsoft Excel and specialized software "Statistica 10" (StatSoft Inc., USA). The statistical analysis included the construction of variation series of quantitative data, the determination of the normality of their distribution using the Kolmogorov-Smirnov criterion and the degree of homogeneity of the variances of the compared samples, as well as the calculation of descriptive statistical parameters, including the mean (M) and the error of the mean (m). Due to the relatively small sample sizes, the reliability of the differences in the compared values was determined using the nonparametric Mann-Whitney U-criterion. The results were presented as M±m. The differences between the compared quantitative indicators were considered significant at the significance level α=5% ($p < 0.05$).

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3 Results and Discussion

A(H1N1) viruses are increasingly being used to study the effects on the host body and to search for new effective antiviral drugs for the treatment and prevention of influenza infection. The potential protective effect of the studied substances is assessed by the final survival rate, average life expectancy, the onset of death and weight loss of animals, as well as according to pathohistological research [9].

In this work, the dynamics of the accumulation of influenza virus in the lungs of infected animals during the first 6 days of the course of a lethal influenza infection was studied. On the 3rd and 4th days of the experimental influenza infection, the titer of the virus in the lungs of mice was close to peak values. Due to the fact that with the therapeutic administration of saponin, oral therapeutic administration of tauroside Sx1 to animals was discontinued on the 3rd day of the experiment, the 4th day of the experiment was chosen to evaluate the effectiveness of this substance on the accumulation of virus and the formation of histopathological changes in the lungs. Another important criterion is the ability of the studied substance to influence the course of the infectious process, manifested in changes in the survival rate and life expectancy of infected animals. As shown in Table 1, oral administration of saponin to mice at a dose of 200 mcg/mouse/day significantly increases the average life expectancy of influenza-infected mice (experimental group VS) by 5 days,

compared with the control group (V). The remaining differences in the studied parameters between these groups were statistically unreliable.

Table 1. The effect of tauroside Sx1 on the survival of mice with 3-day oral therapeutic administration of saponin against the background of influenza infection.

Animal groups	Survival rate of mice by 21 days of experiment (%)	Titer of the virus in the lungs on day 4	Average life expectancy (days after infection)	Beginning of death (day after infection)
Control (V)	0	2.8±0.7	6.5±0.7	4
Experiment (VS)	30	2.1±0.4	11.1±2.1*	5

* the difference between control and experiment is significant, $p<0.05$

The obtained data on the protective effect of tauroside Sx1 were comparable with the data on the effect of antiviral drugs oseltamivir and riamilovir, at least with respect to increasing the average life expectancy of mice infected with influenza A (H1N1): from 9.5 (infection control) to 13.4 days (dose of oseltamivir 5 mg/kg/day) and up to 10.2-10.7 days (the dose of riamilovir is 12.5 – 25 mg/kg/day), respectively [10].

Microscopic examination of the lung tissue of influenza-infected animals of group V was characterized by the presence of foci of pneumonia against the background of pronounced hemodynamic disorders and loss of airiness of the lung tissue (Fig.1).

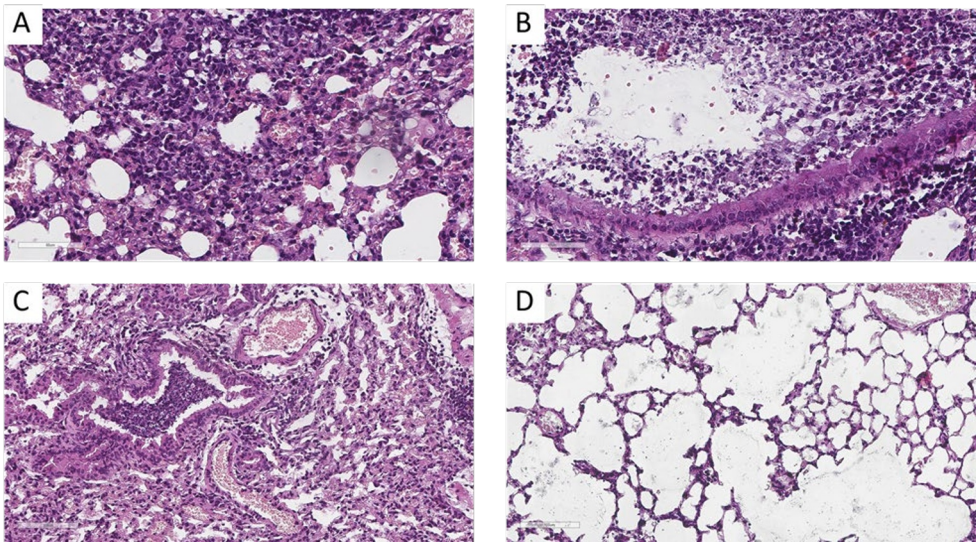

Fig. 1. Sections of the lungs of mice V on the 4th day of influenza infection. Stained with hematoxylin and eosin.

A – destruction and phenomena of acute swelling of the respiratory parts of the lung with foci of intraalveolar serous hemorrhagic inflammation. Thickening of the interalveolar septa due to mixed lymphohistiocytic infiltration with an admixture of neutrophils, × 1000; B – destructive changes on the part of the bronchial mucosa with accumulation of a significant

amount of exudate in the lumen, ×400; C – area of distelectase with the formation of perivascular inflammatory infiltrate against the background of hemodynamic disorders; destruction of the bronchial epithelium with accumulation of purulent-necrotic exudate in the lumen, ×400; D – extensive areas of acute pulmonary tissue swelling with rupture of the interalveolar septa, ×400.

The affected areas of the lung were characterized by accumulations of intraalveolar exudate with an admixture of shaped blood elements and exfoliated alveolocytes. The cellular component of the inflammatory reaction was represented by a mixed lymphohistocytic infiltrate with an admixture of neutrophils. In some areas, the alveoli were lined with hyaline membranes, which was regarded as a morphological sign of diffuse alveolar damage and acute respiratory distress syndrome (Fig. 1A). Changes on the part of both cartilaginous bronchi and terminal bronchioles were also characterized by an acute inflammatory reaction with the accumulation of mixed lymphohistiocytic infiltrate with an admixture of neutrophils in their lumen. The respiratory epithelium of terminal bronchioles, as a rule, was flattened and characterized by degenerative changes, the phenomena of hyperplasia of goblet cells were noted (Fig. 1B). Changes in the interalveolar septa were characterized by unexpressed lymphohistocytic infiltration against the background of full blood vessels of the microcirculatory bed with the phenomena of stasis and microthrombosis. In general, the walls of most vessels were characterized by edema; in the areas of exfoliation of the endothelium, the basement membrane looked fibrous. The general picture of histopathological changes, as a whole, was characterized by the presence of foci of hemorrhages, abscessing and alternation of foci of dis- and atelectasis (Fig. 1C) with acute focal swelling of the lung tissue (Fig. 1D).

Against the background of saponin therapy in the VS group, the pathogenic effect of the virus on the lung tissue of mice was significantly less pronounced. In general, the lungs retained an airy spongy structure (Fig. 2A); the respiratory sections of the lung were characterized by less pronounced destructive changes while maintaining, as in the previous group, signs of intraalveolar focal serous hemorrhagic inflammation (Fig. 2B). At the same time, there was also a tendency to focal formation of hyaline membranes with the phenomena of desquamation of the alveolar epithelium and lymphohistiocytic inflammatory infiltration with an admixture of fibrin. The vessels of the microcirculatory bed were characterized by the phenomena of fullness and stasis of shaped elements. Small areas of distelectasis and acute bloating were detected in the respiratory sections of the lungs.

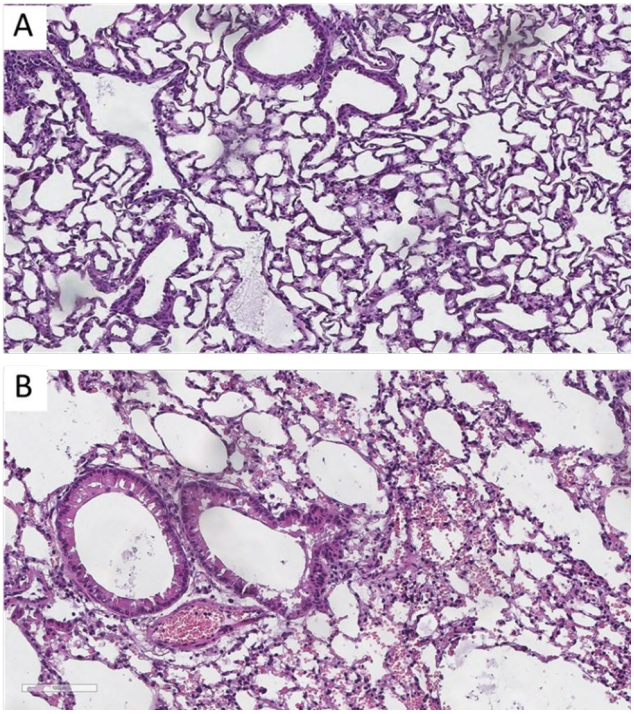

Fig. 2. Lungs of mice of the VS group on day 4 of influenza infection. Stained with hematoxylin and eosin.

A – focal inflammatory changes are detected among practically unchanged lung tissue, small areas of distelectase alternate with the phenomena of acute swelling of the alveoli; inflammatory exudate is present in a small amount in the lumen of terminal bronchioles, ×200; B – the epithelium of terminal bronchioles is flushed in some places, but in most cases retains its integrity (pointers), with the phenomena of hyperplasia goblet cells. The lumen of the blood vessels is dilated, there are foci of hemorrhages (arrow). Interalveolar septa with minor manifestations of inflammatory lymphohistiocytic infiltration, ×400.

At the same time, all of the above signs were much less pronounced than in group V in infected mice without saponin therapy. A characteristic difference was also the absence or significantly lower severity of inflammatory cellular exudate in the lumen of the cartilaginous bronchi. Histopathological changes on the part of most bronchioles were characterized by the presence of single cells of lymphohistiocytic inflammatory infiltrate and minor desquamation of the epithelium with the formation of a small amount of eosinophilic masses in the lumen.

On the 4th day after infection in infected animals treated with saponin (group VS), the proportion of sites with acute swelling of the respiratory lung was statistically significantly 30% less than in group V without therapy ($p<0.05$) (Fig. 3A). At the same time, the proportion of distelectases in both infected groups V and VS did not differ significantly (5.5% and 6.1%, respectively) (Fig. 3B)

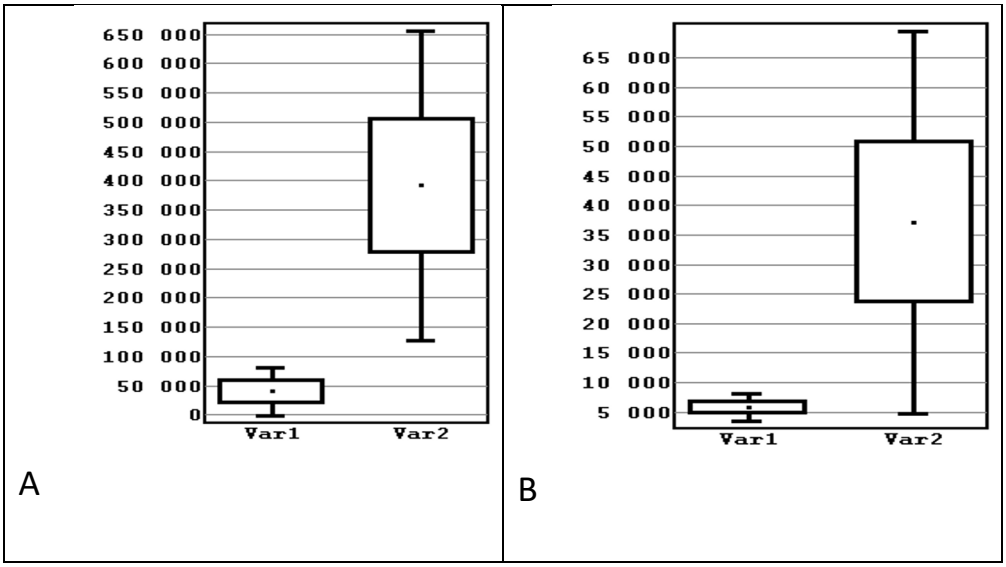

Fig. 3. The average area (mm^2) of swellings (A) and distelectases (B) in the lungs of mice on day 4 of experimental influenza infection in mice treated with tauroside Sx1 (Var 1 – VS), and without its administration (Var 2 – V).

Thus, the severity of lung damage in influenza-infected mice treated with saponin was significantly less than in infected animals of the control group. This was manifested in a significant 30% decrease in the proportion of areas with acute pulmonary tissue swelling in mice treated with saponin. The obtained data of histopathological examination of the lungs of mice infected with IV and treated with saponin can be realized by increasing the number of free β2-receptors on the surface of bronchial cells and the duration of their excitation phase, which leads to a broncholytic effect, as well as the likely presence of a secretolytic effect due to the gastropulmonary reflex mechanism of action of saponins, as shown by other authors when using extracts ivy leaves [11].

4 Conclusions

The search and study of the mechanisms of action of new anti-influenza drugs are of great practical importance. The protective activity of tauroside Sx1 has been established with its oral administration at the early stages of the development of influenza infection in mice. This protective effect manifests itself in a significant increase in the average life expectancy and a lower severity of histopathological changes in the lung tissue in infected animals. Thus, the results obtained indicate the prospects for further study of saponin tauroside Sx1 as a potential component of anti-influenza drugs.

References

1. A.D. Iuliano, K.M. Roguski, H.H. Chang, D.J. Muscatello, R. Palekar, S. Tempia et al., *Lancet*. **391(10127)**, 1285–300 (2018).
2. Weekly national bulletin on influenza and ARVI for the 36th week of 2023. (04.09.23 – 10.09.23). Available from: https://www.influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/ (accessed 20.09.2023).
3. V.V, Zarubaev, A.V. Garshinina, A.V, Slita, S.V. Belyaevskaya, I.N. Lavrentieva,

Antibiotics and Chemotherapy. **65(1–2)**, 15–20 (2020).

4. A. Kossyvakis, A.A. Mentis, K. Tryfinopoulou, V. Pogka, A. Kalliaropoulos, E. Antalis et al., Eur Clin Microbiol Infect Dis. **36(2)**, 361–71 (2017).
5. T.A. Brezhneva, N.D. Samsonova, A.A. Solodukhina, M.V. Popova, A.I. Slivkin, VSU Bulletin, Series: Chemistry. Biology. Pharmacy. **1**, 127–141 (2019).
6. M.Yu. Maligina, I.B. Andronovskaya, Yu. L. Krivorutchenko, V.I. Grishkovets, Scientific notes of the V.I. Vernadsky Crimean Federal University. Biology. Chemistry. **70(3)**,125–133 (2018).
7. L.M. Somova, E.A. Kotsyurbiy, E.I. Drobot, I.N. Lyapun, M.Yu. Shchelkanov, Clinical and experimental morphology. **10(1)**,11–20 (2021).
8. V.V. Zarubaev, A.L. Kovalenko, T.V. Sologub, M.G. Romantsov, A.Yu. Petrov, Preventive and clinical medicine.**1(34)**,122–130 (2010).
9. E.A. Glubokova, I.A. Leneva, N.P. Kartashova, I.N. Falynskova, R.M.Tikhov, N.Yu. Kuznetsov, Acta nature. **2(49)**,116–125 (2021).
10. S.V. Chepur, M.A. Tyunin, V.A. Myasnikov, I.I. Alekseeva, O.V. Vladimirova, N.S. Ilinskiy et al., Clinical and experimental morphology.**10(4)**, 25–34 (2021).
11. J. Schulte-Michels, C. Keksel, H. Häberlein, S. Franken, Inflammopharmacology. **27**, 339–47 (2019).