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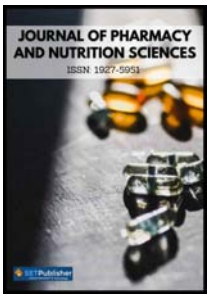

Mitigation of the Oxidative Damage in Liver Caused by Influenza Virus Infection in Mice by an Effective Combination of Oseltamivir and S-adenosyl-L-methionine (SAM)

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Abstract:

This study aimed to estimate the protective effect of a combination of S-Adenosyl-L-methionine (SAM) as a precursor of glutathione and oseltamivir as a specific inhibitor of virus replication on oxidative damages caused by influenza infection in the liver of infected mice. Albino mice were inoculated with $10\times LD_{50}$ of influenza virus A/Aichi/2/68(H3N2). Oseltamivir was applied for five days after infection, twice per day, in a dose of 2.5 mg/kg. SAM was applied for ten days in a 100 mg/kg dose, starting five days before inoculation. Markers of oxidative stress, mortality rate, mean survival time, index, and protection coefficient were followed. Influenza infection causes severe oxidative damage to the liver. All combinations of SAM and oseltamivir restored the levels of the biochemical markers to those in healthy animals and improved the virological parameters. A combination of SAM 100 mg/kg and oseltamivir 2.5 mg/kg, which is 1/4 of the optimal therapeutic mice dose, exhibits protection index and affects most effectively all the tested parameters. This study provides an easy-to-apply approach with a good therapeutic potential for co-medicating influenza infection with a specific antiviral agent and an antioxidant precursor.

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1. INTRODUCTION

The influenza viruses, belonging to the *Orthomyxoviridae* family, have segment single-stranded negative-strand RNA genome organization that allows them to easily undergo mutations and spread through different hosts – animals, birds, and humans [1]. In human population influenza is one of the most common pathogens, causing a severe public health problem. Seasonal influenza epidemics are estimated to cause more than 500,000 deaths per year worldwide [2].

Experts from the WHO have made a parallel between influenza epidemics in the last century and the COVID-19 pandemic in early 2020, as serious human threats were causing concern worldwide. The similarity between the two virus infections is that both induced hyperinflammatory conditions with a broad spectrum of symptoms – from none through severe illness to sometimes lethal exit. Besides the symptoms of fever, cough, runny nose, and body aches, both infections can induce severe pneumonia, trigger a cytokine storm in pulmonary tissues through hyperactivation of the immune system, and the uncontrolled release of cytokines to cause multiple organ disorders. The incubation period of influenza (up to 3 days) and the time between consecutive cases (serial intervals) are short. For comparison, for the COVID-19 virus, the serial interval is 5-6 days. The influenza virus can spread faster, even from COVID-19 [3-5].

Influenza virus infection starts with viral penetration in the ciliated bronchial epithelium. After that, it induces a cascade of immune processes - activation of phagocytosis, NK-cells, and T-cytotoxic lymphocytes, production of antibodies, and various proinflammatory and anti-inflammatory cytokines [4]. Extrapulmonary complications could concern all systems in the body – cardiovascular, renal, digestive, endocrine, nervous, etc. There is data that shows that this impact is related to changed cell redox balance because of the overproduction of reactive oxygen/nitrogen species (ROS/RNS), accompanied by a severe cytokine production in the airways the influenza infection, as well as dynamic changes in oxidative metabolism [4-6]. Produced during influenza infection, ROS/RNS have crucial effects on both – the host cells and the virus. Virus-induced oxidative stress plays a significant part in regulating the host immune system [6].

The mediators of inflammation can spread systemically and cause a multiorgan failure. Hepatic damage could result from the immune response or associated inflammatory infiltrates in the liver [7]. Liver disorders, which are due to the oxidative imbalance in influenza infections, are known not only in experimental animal models but also in children and adults as hepatic decompensating, quite often in patients with underlying chronic liver disease or a condition associated with the impact on the liver [8]. Currently, two types of drugs as specific inhibitors of influenza virus replication are used in clinical practice. The first one is the adamantane derivative - rimantadine, which is helpful in the early stage of infection. It blocks the influenza M2 protein that forms ion channels enveloping the virus particles [9]. Unfortunately, the exceptionally high range of genetic drifts and shifts made it resistant to those drugs; because of the high number of resistant to adamantane drugs serotypes, the WHO recommends inhibitors of a viral membrane enzyme neuraminidase as oseltamivir (Os) and zanamivir that destroy the sialic-acid-containing receptor on the surfaces of the infected cells [3]. However, drug resistance development is an important problem in flu medication. That makes its treatment and prophylaxis very challenging. The dramatic increase in drug resistance to specific anti-influenza drugs such as rimantadine, oseltamivir, marboxil, and baloxavir emphasizes the instance of novel anti-influenza virus therapeutic strategies. Oseltamivir is suitable for a wide range of patients of different ages because it has a predictable linear pharmacokinetic profile [10]. There are data showing that small molecular neuraminidase inhibitors are mighty instruments against influenza viruses [11]. The existing reports of a worldwide deficit of trustworthy antiviral drugs, in parallel with the development of drug-resistant influenza virus strains, as well as the technological period of the 6-month delay in antifly vaccine elaboration, emphasizes the need for the development of new therapeutic approaches that could be widely useful during future pandemics [12].

In our previous studies, we found that pharmacological management of oxidative imbalance in the lung through combining oseltamivir (1/4 and 1/8 of the optimal therapeutic dose) and S-Adenosyl-L-methionine - SAM (100 mg/kg, *i.p*., once daily) resulted in a reduction in airway inflammation, a reduction in lung viral titer, and an improvement in the level of lung protection against oxidative destruction. The co-medication with a specific influenza virus inhibitor plus a precursor of antioxidants such as glutathione prevents the development of therapeutic resistance. This approach achieves a

protective effect with lower doses of antigripal, which unambiguously means less toxic effect and less oxidative damage on the membrane of the cells [5]. SAM plays many key functions in the liver as a precursor for glutathione synthesis. SAM is significant in opposing the toxicity of over-generated free radicals produced by various pathogen infections [13]. Data show that all kinds of chronic liver injury result in decreased hepatic GSH biosynthesis [14]. Glutathione, a well-described intracellular antioxidant, is a potential redox regulator molecule responsible for cellular protection from damage by free radicals, peroxides, and toxins [15]. In this context, glutathione is very indicative and a potential target for investigation regarding influenza damages.

Modern influenza treatment is the simultaneous application of specific viral replication inhibitors and biological response modifiers or antioxidants [6,16]. Combined therapy is a way to increase treatment efficacy, prevent drug resistance development, and reduce the time length of treatment [7,8]. This work aims to research the mitigation of oxidative damage in the liver caused by influenza by combining the specific antiviral drug oseltamivir in a dose of 1/4 of optimal therapeutic as applied together with SAM as a precursor of glutathione.

2. MATERIALS AND METHODS

2.1. Materials

Oseltamivir phosphate (Os) was purchased from Hoffmann-La Roche (Switzerland). For the *in vivo* experiments, it was dissolved *extempore* in PBS (phosphate-buffered saline). S-adenosyl-L-methionine (SAM), purchased from Haya Labs pharmacy Ltd, was diluted in PBS for *in vivo* application. Influenza virus A/Aichi/2/68 (H3N2) was gained from the D. I. Ivanovsky Institute of Virology, Moscow (Russia), and modulated to mice.

2.2. Animals and Treatment

The experiment used ICR male mice from Slivnitsa Animal Pharm (Bulgarian Academy of Sciences (BAS), Bulgaria). They were transported to the Animal House facility of the Stephan Angeloff Institute of Microbiology, BAS, and put in cages with free access to food and water. The experiments were performed by Bulgaria's Directorate of Health Prevention and Humane Behavior toward Animals. Bulgarian Food Safety Agency (BFSA) published Certificate number 125 and standpoint 45/2015 for five years to use animals in experiments for the Stephan Angeloff's Institute. The Ethical Committee of The Stephan Angeloff Institute approved the experimental design and protocols of the work with a decision from 4.10.2020.

For the aim of our work, the mice underwent slight ether anesthesia and were infected intranasally with $10\times LD_{50}$ influenza virus strain A/Aichi/2/68 (H3N2). The following experimental groups (n = 5) were defined: Group 1. Healthy control group; Group 2. Influenza-infected mice (IVI); Group 3. IVI + Os 2.5 mg/kg; Group 4. IVI + SAM 100 mg/kg; Group 5. IVI + SAM 100 mg/kg + Os 2.5 mg/kg.

Oseltamivir was given twice daily, *per os*, for five days, from the day of viral inoculation; SAM was applied in the peritoneum once a day, starting 5 days before the viral inoculation.

The virological markers were followed for 14 days. The markers of oxidative stress were tested on the 5th day after viral inoculation when a peak of the viral infection was observed [5, 6]. The histologic study was also conducted on the 5th day after viral inoculation.

2.3. Determination of Oxidative Stress Markers

Each liver (n = 5 per group) was perfused with PBS, homogenized, suspended to 10 % mass/volume, and centrifuged at 2000 rpm for 10 minutes. The amount of protein was calculated by the method of Lowry [17]. The obtained supernatant was used for the determination of malondialdehyde (MDA) as a late product of lipid peroxidation (LPO) by the TBA test, adapted by Mileva *et al*. [16]. The level of glutathione (GSH) was measured by Tietze [18]. Part of the supernatant was re-centrifuged at 12000 rpm for 20 min to obtain post-mitochondrial supernatant where the activities of superoxide dismutase (SOD) - according to Beauchamp and Fridovich [19] glutathione peroxidase (GPx) - according to Gunzler *et al*. [20]; glutathione reductase (GSSG-red) - by the method of Pinto & Bartley [21]; and catalase (CAT) - by the method of Aebi [22] were measured. The results of our experiment are expressed as a percentage of values obtained from the samples of healthy mice, which are assumed 100%.

2.4. Histological Study

The histological analysis was prepared according to the standard protocol of the Department of Anatomy and Histology of Medical University, Sofia. Microscopic and

macroscopic assessments and histological analyses of ulcers were performed. Livers were perfused and fixed in neutral formalin 4%. After 7 days, dehydration in alcohol was performed, following lightening in cedar oil. After that, the samples were embedded in paraffin, and a series of cuts 20 μm in thickness were prepared.

2.5. Cumulative Mortality

This parameter was determined by the 14th day as the number of dead animals in each group.

2.6. Determination of Virological Markers

We tracked the following virological parameters of infected animals (n=10):

Mortality rate in the groups (%);

Average survival time (AST), calculated by the formula:

ST = [f (d - 1)] / n;

Where *f* is the number of dead mice recorded on day *d* (the survivors on day 14 were included in the calculation), and n is a number of mice in a group [23].

Coefficient of protection (CP) in the groups – mortality rate in the infected with influenza virus group divided by the mortality rate in the drug-treated group.

CP = % mortality in influenza-infected group/%mortality in the drug-treated group.

The equation evaluated index of protection (IP):

IP= (CP - 1/CP) * 100 [24]

Pulmonary viral titer of infected animals (n=5) on the day 5th after inoculation.

The assay was performed in 96-well flat-bottomed plates on a cell monolayer of MDCK (Madine-Darby canine kidney) cells from ATCC (Manassas, VA, USA) and grown in DMEM (Gibco BRL, Paisley, Scotland, UK) with added 10% fetal bovine serum (Gibco BRL, Paisley, Scotland, UK), 3.7 mg/ml Na_2HCO_3, 10 mM HEPES buffer (AppliChem GmbH, Darmstadt, Germany), 100 mg/ml streptomycin, 100 IU/ml penicillin and 50 mg/ml gentamicin, in a 5% CO_2 incubator (Thermo Scientific 311, Thermo Fisher Scientific, USA). Ten-fold dilutions were made from each virus sample and transferred to the plates. The result was read at 48 hours and calculated as log10 $CCID_{50}$ /0.1 ml.

2.7. Statistical Analysis

The experiments were performed in triplicate, and data are presented as mean values ± SEM. The statistical analyses were conducted using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA). Data distribution was assessed before deciding on the statistical test to use. For statistical analysis of the effect of different concentrations of the tested substances on oxidative stress parameters, the Kruskal-Wallis test followed by Dunn multiple comparison post-hoc was conducted. Significant differences were accepted up to $P \leqq 0.05$.

3. RESULTS

Table **1** presented the mortality rate, average survival time (AST), coefficient of protection (CP), index of protection (IP), depending on pulmonary influenza virus titer in the experimental groups. Mice treated with the combination of SAM 100 mg/kg +Os 2.5 mg/kg showed a lower viral titer as compared to those supplemented with Os or SAM only. When oseltamivir 2.5 mg/kg was combined with SAM 100 mg/kg, the mortality rate was 10%. In this group, the AST was 12.5 days.

The combination of oseltamivir 2.5 mg/kg and SAM 100 mg/kg demonstrated very good antiviral protection in the experiments *in vivo*. In this one group coefficient of protection was 7.0, and the index of protection - 85.71.

The effect of SAM and Os, alone and in combinations, on the cumulative mortality of mice, is shown in Figure **1**. SAM only could not significantly affect this parameter. Oseltamivir in a dose of 2.5 mg/kg showed 50% mortality. SAM supplementation in infected mice in a dose of 100 mg/kg showed 70 % morality, but combination therapy of Os in a dose of 2.5 mg/kg with SAM 100 mg/kg showed 30 % mortality.

The influenza virus infection increased MDA values as a marker of lipid peroxidation by about 75% relative to controls (Figure **2A**). The 2.5 mg/kg oseltamivir dose reduced MDA by about 25% ($P \leqq 0.01$) compared to infected animals, but the value is higher than that in healthy mice. In animals supplemented with SAM 100 mg/kg + Os 2.5 mg/kg, MDA levels were close to those of the control group ($P \leqq 0.05$).

In animals infected with the influenza virus but not treated with any of the administered therapeutic regimens, we observed a depletion of the total glutathione level (Figure **2B**) by about 40% compared

Table 1: Mortality Rate, Average Survival Time (AST) for 15 Days after Inoculation, Coefficient of Protection (CP) and Index of Protection (IP) in the Experimental Groups

Experimental group	Mortality rate (%)	AST (days)	CP	IP (%)	Pulmonary viral titer lg CCID50/0.1 ml SEM;
Noninfected mice	0	14	-	-	-
Influenza infected animals	70	8.8±0.67	-	-	8±0.45
IVI + Os 2,5	30	11.9±0.56	2.3	56.5±8.9*	4.7±0.12*
IVI + SAM 100	80	7.6±0.65	0.88	0	8±0.65 *n.s.*
IVI + SAM 100 + Os 2,5	10	12.5±0.79	7	85.71±12.9*	3.7*

Note: Data are expressed as mean value ± SE. Influenza virus (H3N2) inoculation dose: 10 ×LD_{50} intranasal. Albino mice ICR 12-14 g. Os (per os): five days treatment course since virus inoculation. IP% = [(CP-1)/CP] x 100, where CP=lethality in infected (%) /lethality in treated group (%); Pulmonary infection titers recorded on day 5th after virus inoculation. Values are expressed as means lg $CCID_{50}$/0.1 ml SEM; * Presented a significant statistical level of P<0.01 vs. IVI group (n=10).

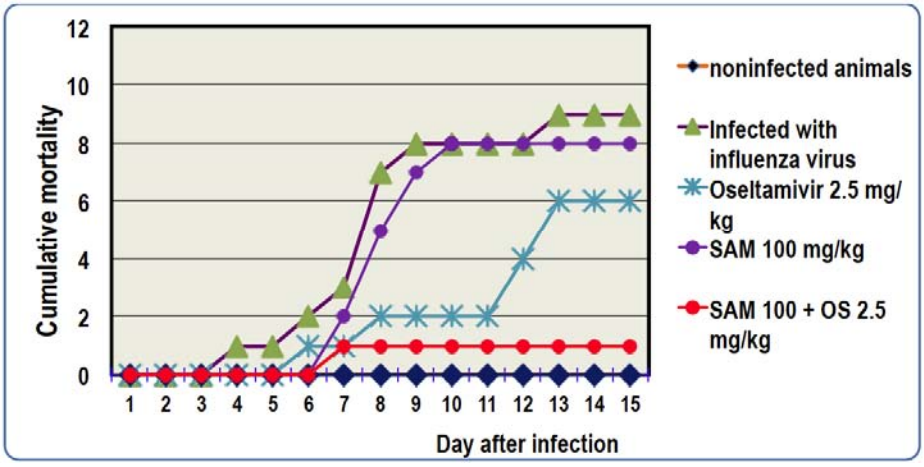

Figure 1: Effect of SAM (100 mg/kg b. w.), oseltamivir (2.5 mg/kg b. w.), and their combination on the number of deaths of infected mice monitored until the 15th day after virus challenge (n=10).

with the uninfected control group (P ≦ 0.05). Oseltamivir loading at a dose of 2.5 mg/kg, which is 1/4 of the therapeutic dose for mice, resulted in a slight increase in GSH levels above those of infected and untreated animals (IVI group). SAM at a 100 mg/kg dose did not alter endogenous glutathione. However, co-medication administered to infected animals showed an increase in GSH levels close to the control value. The results were considered significant at P ≦ 0.05.

Figure **3** illustrates the data with the changes in the enzymatic activity of the antioxidant enzymes SOD and CAT under the applied experimental design. The influenza virus increased SOD activity in the liver by approximately 40% (P ≦ 0.05). Oseltamivir and SAM administered alone reduced SOD activity compared with influenza-infected mice. In animals treated with the combination regimen of SAM 100 mg/kg + Os 2.5 mg/kg b. w., values very similar to those of the uninfected control group were obtained (Figure **3A**).

CAT activity increased by about 15% in influenza-infected mice compared with uninfected animals (Figure **3B**). The level was higher in the group treated with Os 2.5 mg/kg - by almost 30% compared to healthy controls. The group treated with the combination of SAM 100 mg/kg + Os 1.25 mg/kg showed CAT activity similar to that of the uninfected control group.

Figure **4** presents the GSSG-red and GPx activity in the liver of the experimental animals. Influenza virus

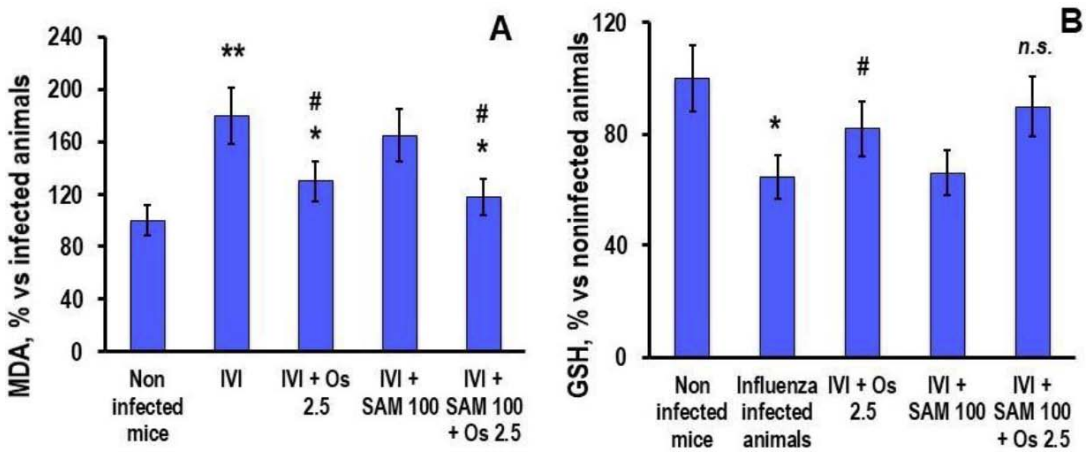

Figure 2: Changes in MDA (**A**) and GSH (**B**) levels in the liver of the experimental groups.

**P ≦ 0.01 vs healthy controls; *P ≦ 0.05 vs healthy controls.

P ≦ 0.05 vs. influenza-infected mice; *n. s.* - nonsignificant vs. healthy controls.

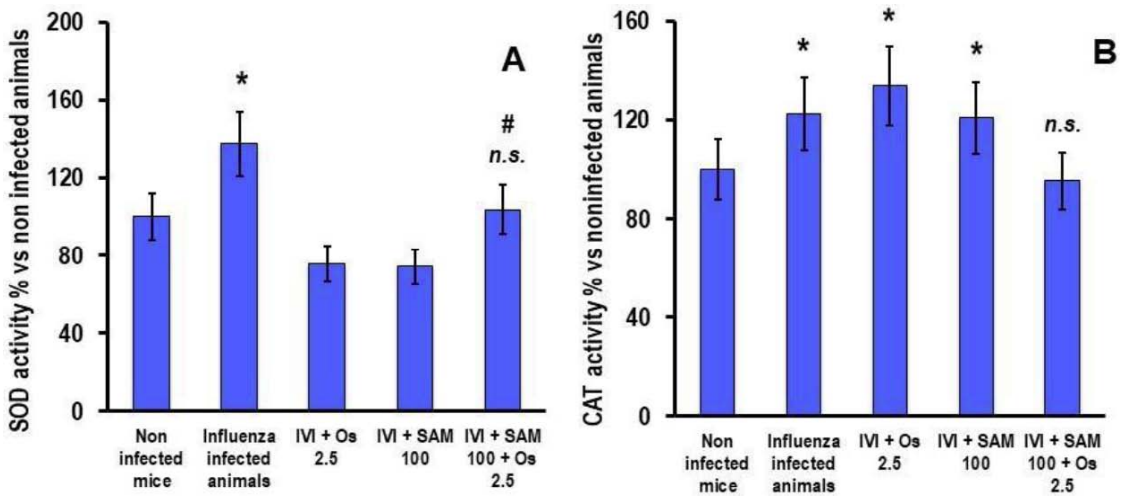

Figure 3: Activity of SOD (**A**) and CAT (**B**) in the liver of the experimental groups

* P ≦ 0.05 vs healthy control; # P ≦ 0.05 vs influenza infected mice; *n.s.* - nonsignificant vs. healthy controls.

increased GSSG-red activity by about 40% versus the noninfected group (Figure **4A**). After Os supplementation, a slight decrease in the activity of GSSG-red versus infected animals was found. The GPx activity in the liver of experimental mice was increased by about 50% in the influenza-infected group. In SAM and Os-supplemented animals GPx activity decreased even more than in healthy animals. In a group treated with both drugs, GPx enzyme activity almost reached the control values at P ≦ 0.05 (Figure **4B**).

A normal cytoarchitectonic picture of the liver of healthy animals was observed at medium microscopic magnification. As seen in Figure **5**, the parenchyma of liver cells was organized in lamellae formed by mononuclear and binuclear hepatocytes covered with supporting reticuloendothelial cells. This is where the blood from the hepatic artery mixes with the blood flow from the *vena portae* to the *vena centralis* of the hepatic lobe.

In Figure **6**, pathologic changes in the liver of infected animals may be observed. The cytoarchitectonic structure damage of the liver lobe was present. There was an aberration in the organization and arrangement of the lamellar structure. Compared to healthy liver parenchyma (Figure **5**), the cell organization presented massifs of cells formed as radial structures in the structure of hepatocyte plates around the central veins. It was observed clearly expressed the formation of hexagonal structures with the deformation of the portal triads.

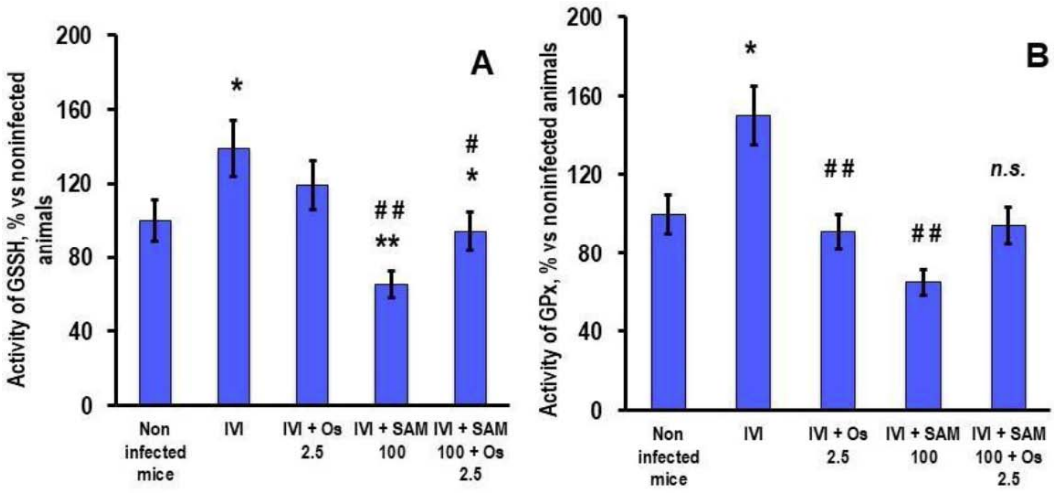

Figure 4: Activity of hepatic GSSG-red (**A**) and GPx (**B**) in the liver of the experimental groups.

* P ≦ 0.05 vs healthy controls, ** P ≦ 0.01 vs healthy controls; # P ≦ 0.05 vs influenza infected mice; ## P ≦ 0.01 vs influenza infected mice; *n.s.* - nonsignificant vs. healthy controls.

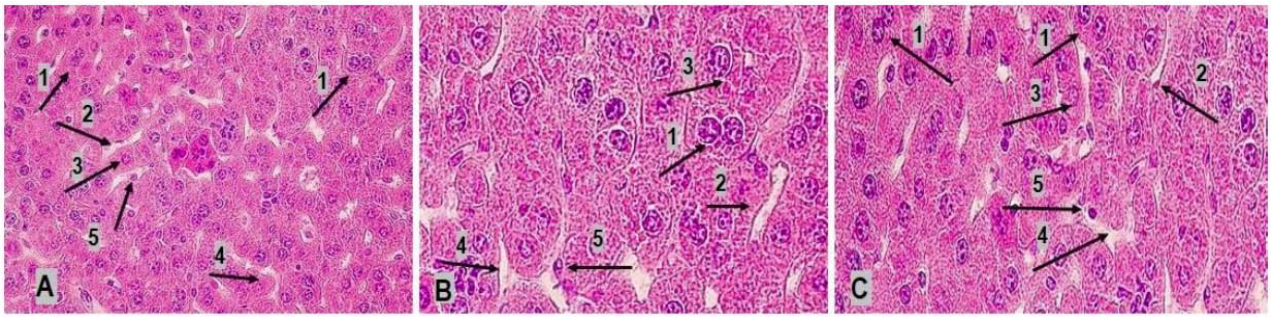

Figure 5: Histological observations of the liver – normal histologic structure of non-infected animals. 1 - binuclear hepatocytes; 2 - reticuloendothelial cells; 3 - hepatocyte lamellae; 4 – sinusoids; 5 - Kupffer cells. Staining HEx40.

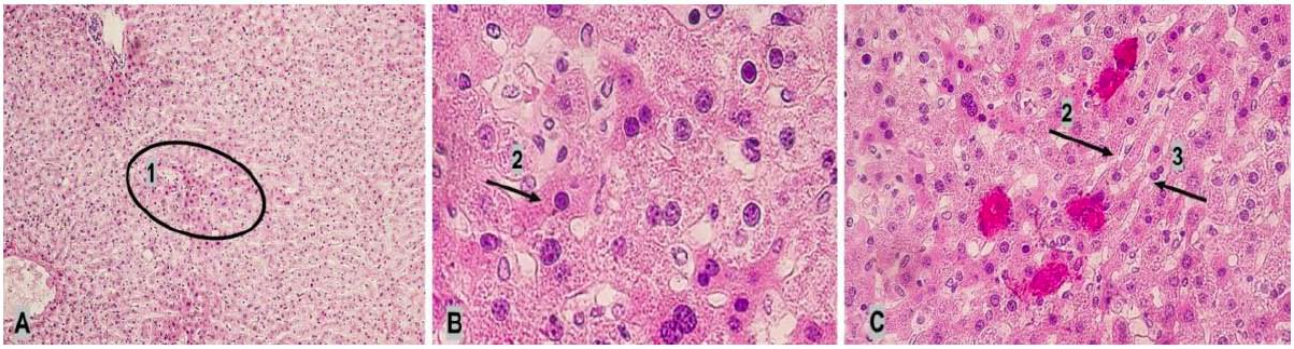

Figure 6: Histological changes in the liver in influenza-infected mice.

1 - triade, 2 – lamellae, 3 - reticuloendothelial cells. A - HE x 20; B – HE x 40, C – HE x 40.

After supplementation with Os 2.5 mg/kg of IVI-infected mice, recovery of liver parenchyma was observed (Figure **7A**). Between the recovered hepatocytes, there was hepatocyte entrapment in the liver parenchyma with mild intracellular and extracellular edema (Figure **7A-1**). Even at low magnification, they were lighter in color, both on the body and their nucleus (Figure **7A - 2**).

In infected mice supplemented with SAM 100 mg/kg, a hepatic lobule with impaired cytoarchitectonics may be established in Figure **7B - 1**. A seal with fibroblast growth was observed around the vessels (Figure **7B - 2**). In the liver lobule, completely recovered hepatocytes could be observed (Figure **7B - 3**). After the treatment of IVI mice with SAM 150 mg/kg, the

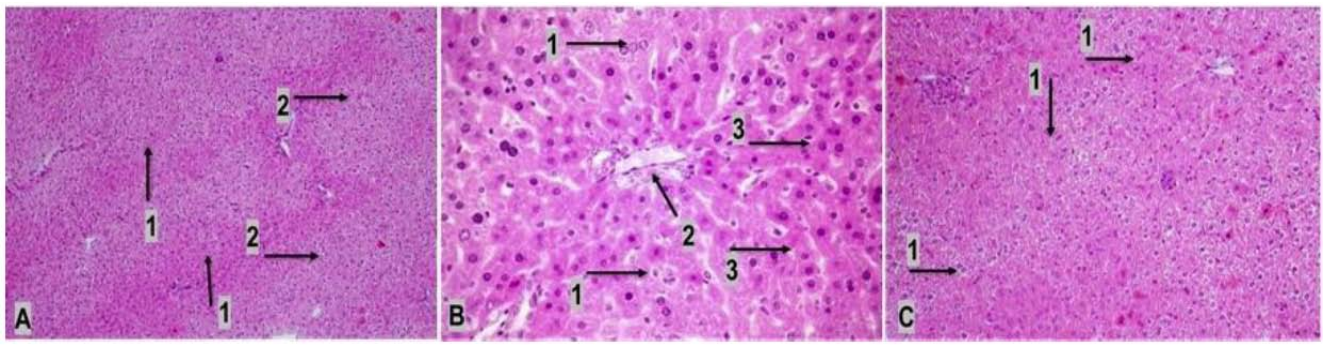

Figure 7: Histological observations of the liver in influenza-infected mice treated with:

A - Oseltamivir 2.5 mg/kg, HE X 10. 1 - recovery, starting from the periphery of the hepatic lobe; 2 - zone with present damage.

B - SAM 100 mg/kg, HE x 40. 1 - zone with the destruction of the hepatocyte;2 - vena centralis; 3 - normal hepatocyte.

C - SAM 100 mg/kg + Os 2.5 mg/kg, HE x 20. 1 – regeneration of hepatocyte

picture shows a peripheral area of the liver covered with the liver capsule (Figure **7C - 1**). The capsule was peeled off due to edema (Figure **7C - 2**). Rows of preserved hepatocytes could be found in part of the tissue (Figure **7C - 3**). However, in some hepatocytes, they was expressed destruction of the cytolemma and nucleus (Figure **7C - 4**).

Almost complete recovery of liver tissue around the central venous vessels may be ascertained in IVI mice treated with SAM 100 mg/kg + Os 2,5 mg/kg. Hepatocytes with a lighter color and larger nuclei could be seen around the liver lobes, a sign of intracellular edema (Figure **7C - 1**).

4. DISCUSSION

The influenza virus invasion in the body provokes lung inflammation and a cascade of extrapulmonary processes, affecting almost all organs, incl. the liver [25-27]. Using animal models, it has been demonstrated that hepatocellular injury is not related to virus replication directly [28]. However, the concomitant production of cytokines induces oxidative stress responsible for this pathology. Moreover, several studies have reported the association of this influenza virus-induced hyperinflammatory syndrome with the disease severity of influenza infections [7,28,29]. Our results suggest that oxidative modification of hepatic tissue - i.e., proteins, lipids, and DNA results in liver structural and functional abnormalities at the cytoarchitectonic. Under previously reported data [5,30], the results showed a disturbance of oxidative status in livers from infected with influenza virus mice, expressed by elevated levels of lipid peroxidation (MDA marker) (Figure **2A**). The activation of antioxidant enzymes in the infected animals was insufficient to protect the liver from the development of oxidative stress. In addition, decreasing levels of GSH, the main non-enzymatic cellular antioxidant, were found (Figure **2B**). Some data demonstrated that low molecular antioxidants such as α-tocopherol and GSH in the liver decreased sharply in the early stage of infection, before the beginning of disease symptoms, and remained low until the end of the experiment [31]. In addition to its antioxidant role, protecting cellular structures from the adverse effect of ROS, GSH participates in the immune response. Its decrease could lead to local immunosuppression [32], resulting in the growth of diverse pathogens, such as bacteria and fungi. Consequently, severe structural and functional changes in the liver may occur.

The reticuloendothelial network makes up the structure of the liver lobe and includes different cell types. Resident macrophages in the liver - Kupffer cells are in the sinusoidal space. The single cells between the hepatocytes and star-shaped endothelial cells (lipocytes) are fat-storing cells involved in the metabolism of lipophilic xenobiotics such as vitamin A, vitamin E, etc. [33]. The disruption of the lamellar arrangement of the hepatocytes is an important diagnostic marker for some forms of inflammatory processes of the parenchyma during viral infections [34]. The liver's cellular compartments actively maintain their redox state. This is necessary to perform their specialized tasks [35]. In the liver of infected mice (Figure **6**), reticuloendothelial cells are irregularly configured and reduced in size, probably due to the disturbance of their specific functions, such as phagocytosis, cytokine secretion, and support of intercellular communication with surrounding hepatocytes.

The observed influenza-associated damage of hepatocyte membranes (Figure **7**) is probably due to the induced lipid peroxidation, which affects their permeability and integrity. Therefore, as the defense against oxidative processes in the organism involves the operation of the low molecular weight endogenous radical-scavengers as glutathione [5-7], our observations in the course of infection showed an insufficiency of glutathione (Figure **2B**). So, applying substances that can maintain endogenous GSH homeostasis is an appropriate and promising prospect for good prevention. Many studies show the positive effect of treatment with precursors of glutathione synthesis (such as N-acetylcysteine (NAC) or 2-oxothiazolidine-4-carboxylic acid) in liver pathologies of oxidative etiology (36,37]. In this study, we tested the capability of oseltamivir in combination with SAM as a GSH precursor to prevent liver damage induced by influenza virus A/Aichi/1968/(H3N2) infection in mice.

The design of a practical therapeutics approach against influenza virus infections is one of the most socially important goals of modern medicine due to the epidemic character of the disease, the high variability of the virus, and the development of serious complications, occasionally resulting in patient death. The problem became even more serious, especially after 2019, when the global pandemic COVID-19 broke out. Another serious problem that should not be underestimated is increased drug resistance to specific anti-influenza drugs. An important factor in influenza therapy is optimizing the therapeutic dose to avoid the development of resistance. A good therapeutic strategy should be able to reduce OS, measured by the level of LPO, in organs of virus-infected animals [38,39] and especially in combination with immune modulators and antioxidants [40]. Our results show that administered individually, in doses of SAM 100 mg kg and Os 2.5 mg/kg, do not show a sufficient protective effect on virological, biochemical, and histopathological markers of viral damage. Combining Oseltamivir with higher doses of SAM did not show a good enough protective effect on liver damage (data not shown). Oseltamivir 2.5 mg/kg and SAM 100 mg/kg showed the best protection on the parameters tested. It is important to note that Oseltamivir is at 1/4 of the optimal therapeutic dose.

Various hypotheses have been proposed for SAM's protective action on oxidative damage in the liver in infected animals. These include increased GSH levels by restoring its biosynthesis and modulating antioxidant enzyme expression [41]. A change in DNA methylation, normalization of CYP-dependent monooxygenase activities, decreased tumor necrosis factor-alpha expression, improved fluidity of the cell membrane, and inhibition of the production of collagen I by preventing transforming growth factor-beta induction of the COL1A2 promoter have also been suggested as mechanisms promoting the protective effect (14,42,43]. Furthermore, there are data that SAM performs direct antioxidant activity *in vitro* in chemical and biological lipid systems by the manner of (i) iron chelation and (ii) inhibition of Fe^{2+}autoxidation. SAM can act as a preventive antioxidant that lowers the initiation rate of oxidation reactions by chelating Fe^{2+} and retarding its ability to catalyze free radical formation [44]. Hepatocytic lipids, DNA, and proteins are among the cellular structures primarily targeted by overgenerated ROS produced during viral infection. Our data showed that the process leads to functional and structural disorders in the liver. So, the steering of oxidative stress must be studied by several mechanisms, including the investigation of biochemical markers.

Furthermore, monitoring of oxidative markers of the liver could be used for diagnosing the degree of liver damage, which is especially important in cases of co-medication of concomitant diseases (such as asthma, diabetes, neurodegenerative disorders, etc.) and allows the real assessment of the response to pharmacological therapies [45]. The results suggest that SAM could be important in the general therapy for influenza infection when given together with chemotherapeutic drugs such as neuraminidase inhibitors. SAM may be a trustworthy part of multitarget influenza infection therapy. To sum up, oxidative balance is critical in maintaining the normal functioning of a host. In contrast, oxidative stress caused by a virus affects the intracellular redox balance, which leads to significant changes in the defense system. This provides a therapeutic option for the prevention and control of virus infection. Antiviral therapeutic schemes should be characterized by their redox-modulating and antioxidant activity because they can affect therapeutic efficiency.

5. CONCLUSION

Experimental studies for regulating oxidative strategies in liver dysfunction caused by viral infections, through which cells control the balance between oxidative and antioxidant molecules, i.e., redox homeostasis of the infected body, have had serious success in recent years. The liver is a vital organ that plays an essential

role in the biochemical laboratory of living organisms, where most exogenous xenobiotics are metabolized. Liver damage during influenza infection is not typically registered in people, but it is often a consequence of severe influenza. Combining specific antiviral medicine (Oseltamivir) with the precursor of the main antioxidant glutathione - SAM, together with good virological protection parameters, leads to a rise in the level of antioxidant defense. This effect, plus lowering the doses of applied medicines, will certainly help the liver's better function. The proposed therapeutic strategy requires validation using other animal models of influenza infection and subsequent human clinical trials.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests, either financial or non-financial

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