



## Components of colostrum regulate the activity of the immune system in animals with liver fibrosis

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Regulation of the functional activity of the cellular component of the body's immune system against the background of various liver pathologies, primarily fibrosis, represents a promising approach to developing treatment strategies for such altered functional states. Low molecular weight protein components (LMW) obtained from whole cow colostrum were analysed as natural regulators of immune system activity. The effect of LMW on the number of cytotoxic components in blood serum, the activity of oxygen-dependent and oxygen-independent phagocytosis, and the number of circulating immune complexes in the blood of animals with Cu-induced liver fibrosis was determined. Liver fibrosis was induced in Wistar rats by 6 consecutive injections of copper sulphate at a dose of 1 mg/100 g body weight, and after 3 days one of the groups of animals was administered LMW at a dose of 0.1 mg/100 g body weight per os daily for 6 days. It was found that animals with Cu-induced liver fibrosis had a twofold increase in cytotoxic components in the body compared to the control group. This was accompanied by a twofold increase in the spontaneous level of phagocytic activity of neutrophils (without additional stimulation with zymosan), while the functional reserve of neutrophils was depleted (there was no stimulation with zymosan); the ability of phagocytic cells to absorb foreign substances was increased by only 34%, while the number of circulating immune complexes was reduced by 33%. This immune status of animals with Cu-induced liver fibrosis can be defined as a risk factor for the development of an autoimmune component. Administration of LMW to animals with liver fibrosis for 6 days at a dose of 1 mg/100 g of body weight led to a decrease in phagocytic activity to the level of control indicators, restoration of the reserve of oxygen-dependent phagocytosis of neutrophils and normalisation of oxygen-independent phagocytosis indicators, while the number of circulating immune complexes corresponded to the control values. The restoration of cellular parameters under the influence of LMW may prevent the chronic development of liver fibrosis and its transition to cirrhosis, and further systemic studies will allow the development of treatment tactics using natural LMW.

**Keywords:** Cu-induced liver fibrosis; low molecular weight protein components of colostrums; LMW; cytotoxic components; DAMPs; immune system; phagocytosis.

### Introduction

It has been shown in numerous studies that colostrum has a wide range of effects on the newborn's body. However, the most important of them is the formation and regulation of the activity of the immune system (Struff & Sprotte, 2008; Ghosh & Iacucci, 2021; Khan et al., 2024). It was shown that colostrum components are able to regulate the activity of the redox system not only in newborns, but also in adults, to influence the functional activity of the bone marrow and to form the body's resistance to stress factors (Bozhkov et al., 2019). Despite the fact that this natural product has unique properties related to a wide range of biological activity, there are currently no pharmacological preparations based on colostrum components. It is explained by the fact that the composition of colostrum is extremely dynamic. Colostrum is formed by the mammary gland in a very short period (1–3 days) after the start of lactation (Puppel et al., 2019; Playford & Weiser, 2021). In addition, its composition depends on age, the cow's breed, housing conditions and season (El-Fattah et al., 2012; Wąsowska & Puppel, 2018). All these factors do not allow us to obtain medicines with a stable composition and, as a result, the same properties.

Our laboratory has developed a 'Selective-integrative technology' for obtaining individual basic fractions from colostrum, which makes it possible to obtain low-molecular-weight protein components of colostrum with a relatively stable composition (Ivanov et al., 2024). The study of the biological effects of low molecular weight protein components (LMW) of colostrum can make it possible to obtain functional food products or pharmacological agents of natural origin (Kusumaningrum et al., 2021; Poonia & Shiva, 2022). It should

be noted that substances of natural origin, unlike synthetic drugs, do not have such pronounced side effects (Barzegar et al., 2023). It is known that mortality caused by liver pathology ranks 5th in the structure of mortality in the world (Devarbhavi et al., 2023), and currently there is an increase in such diseases as hepatitis, fibrosis, cirrhosis and hepatocellular cancer (Castaneda et al., 2021; Liu & Chen, 2022).

The problem of treatment of this group of pathologies is aggravated by the fact that symptoms and pain appear in late stages of development of these pathologies, when the disease progresses to the chronic stage and is practically untreatable (Ginès et al., 2021; Abeysekera et al., 2022).

Hepatitis and liver fibrosis can be provoked by a wide range of external factors (Yao et al., 2020; Barouki et al., 2023; Pellicano et al., 2023), including food and various drinks (Zheng et al., 2022). As a rule, the development of liver pathologies is characterised by stages, and the final stage of such diseases is liver cirrhosis, which is an irreversible pathological condition (Le, 2023). Currently, there are no effective ways and methods of prevention and treatment of liver fibrosis and cirrhosis.

A large proportion of fibrosis is caused by the ingestion of synthetic drugs and other chemical compounds (Yan et al., 2018; Bao et al., 2021).

Experimental models are used to study the mechanisms of fibrosis development and ways to treat it. One of the most commonly used models of chemical toxicosis is the tetrachloromethane model (Unsal et al., 2020; Qiuling et al., 2023).

However, tetrachloromethane is extremely toxic and leads to the death of a large number of animals during the development of fibrosis. Therefore, an experimental model of Cu-induced liver fibrosis

was developed (Bozhkov et al., 2019). It was shown that CCL<sub>4</sub>- and Cu-induced fibrosis had similar changes at the level of the redox system (Ivanov et al., 2024), but in the case of Cu-induced fibrosis there was no lethality in the process of pathology development and there was more intensive formation of extracellular matrix.

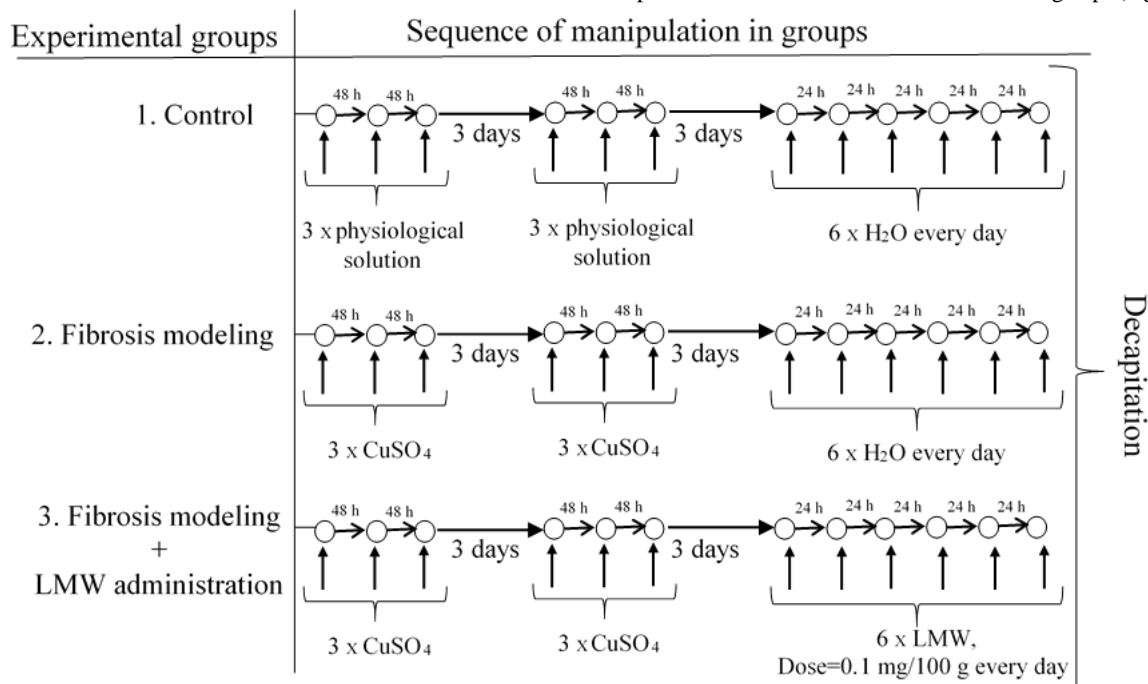
In the model of Cu-induced liver fibrosis, it was shown that LMW colostrum regulates the activity of the redox system and increases the survival rate of rats (Ivanov et al., 2021). The study of possible mechanisms of action of LMW components and the role of the immune system in these processes is an important step in the development of means to combat liver pathologies.

In this regard, we determined the participation of low molecular weight components of the colostrum as a regulator of the immune response in the Cu-induced liver fibrosis model.

## Materials and methods

The study was conducted with male Wistar rats weighing 120–130 g (3-month-old animals). During the whole experiment, the animals were kept under standard vivarium conditions and had free access to food and water. All manipulations were performed in accordance with the decision of the Bioethical Committee of the V. N. Karazin Kharkiv National University (Protocol of the Bioethical Commission of Kharkiv University No. 4 of September 18, 2024), which is guided by the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, March 18, 1986).

The control group received physiological solution instead of copper sulphate in the same volume as the copper sulphate solution received by the experimental animals in the second and third test groups (Fig. 1).



**Fig. 1.** The scheme shows the time course of copper sulphate administration to experimental animals for the induction of Cu-induced liver fibrosis (group 2), a control group (group 1) that received physiological solution instead of copper sulphate, and a group of animals that received low molecular weight components of colostrum at a dose of 0.1 mg/100 g body weight for 6 days (group 3)

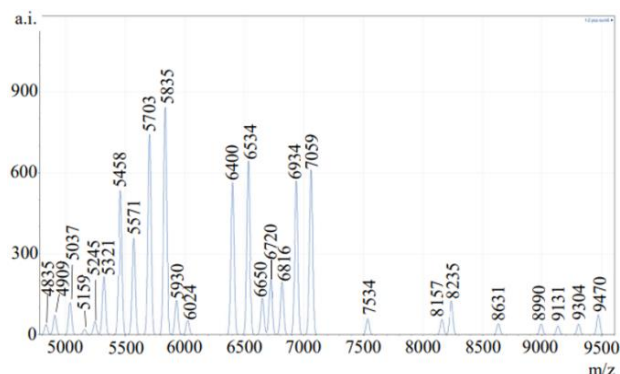
The second group was animals with Cu-induced liver fibrosis. They were injected three times with copper sulfate at a dose of 1 mg/100 g with an interval of 48 hours between injections, which was about 33% of the lethal dose for rats. After 3 days, the same cycle of administration was repeated (Fig. 1). At the end of two cycles of intoxication, the animals received water for 6 days. This procedure has previously been shown to be accompanied by the development of liver fibrosis (Ivanov et al., 2024).

Animals in the second group were a control group in comparison with group 3, which after intoxication received low molecular weight components of colostrum at a dose of 0.1 mg/100 g body weight for 6 days after intoxication (Fig. 1).

The low molecular weight components of the colostrum include peptides with mass to charge ratio from 4835 to 9470 Da (Fig. 2).

To study the composition of proteins in whole colostrum and LCF, we prepared samples so that 1 ml contained 2 mg of protein from each fraction. Mass spectrometric studies were carried out using the device Autoflex II LRF 20 “Bruker Daltonics” (Germany), equipped with a pulsed nitrogen laser ( $\lambda = 337$  nm, pulse duration – 3 ns). The samples of proteins, after mixing with the matrix, which was prepared according to the standard procedure: 12 mg of synaptic acid (Fluka) dissolved in 1 ml of the mixture water–isopropanol alcohol (1:2 V/V) with the addition of 0.1% trifluoroacetic acid, were applied to a steel target and dried at room temperature. The analysis was carried out under a linear mode of device operation with the detection of positive and negative ions. The results were analyzed using the

open software ProteoWizard (<http://proteowizard.sourceforge.net>) mMasc (<http://mmasc.org>).



**Fig. 2.** Mass spectra of low molecular weight proteins from colostrum including peptides with m/z from 4835 to 9470 (mass to charge ratio): a typical spectrum of low molecular weight components of colostrum is shown

Oxygen-dependent phagocytosis was evaluated by the intensity of spontaneous or induced activation of NADPH-N-oxidase reactions of neutrophils. The test involved the reduction of soluble nitroblue tetrazolium nitrate dye (NTN) absorbed by the phagocyte into insoluble difformazan under the action of the formed superoxide anion. Light

microscopy (Olympus BX53, Japan) was used in this work (Khudan et al., 2021). The number of blue-violet diformazan granules in the neutrophil corresponded to the localization of NADPH-H-oxidase, and their size corresponded to the total activity of this enzyme and the formation of ROS (reactive oxygen species). The number of cells containing diformazan granules was determined. The number of diformazan granules was used to estimate the activity of spontaneous, i.e. not induced or baseline phagocytosis (SL, up to 10%) and induced (ZIL, up to 70%) by zymosan in the *in vitro* system of oxygen-dependent phagocytosis. The phagocytosis stimulation index (SI) was calculated using to the formula:  $SI = ZIL/SL$ .

Oxygen-independent phagocytosis was determined by the degree of antigen uptake and adhesion; *Saccharomyces cerevisiae* yeast was used as an antigen by phagocytizing cells. The efficiency of phagocytosis was evaluated by the digestive capacity of phagocytic cells – the phagocytosis completion index (PhCI) (Yanko et al., 2022). We determined the phagocytic index (PhI) – the number of neutrophils involved in phagocytosis (as a percentage of the total number of neutrophils) and phagocytic number (PhN) – the average number of *S. cerevisiae* cells engulfed by one neutrophil (in conventional units). Endocytosis of satellite samples was evaluated after 120 min. The index of phagocytosis completion was calculated in conventional units by the ratio of PhN after 30 min to PhN after 120 min. Light microscopy (Olympus BX53, Japan) was used in this technique.

Determination of cytotoxicity of serum components was carried out *in vitro* by the degree of degradation of autolymphocyte membrane cells after exposure to auto serum in the presence of exogenous complement. The suspension of lymphocytes was isolated in a density gradient of ficoll-verographin (density 1.077) and incubated with auto serum at 37 °C for 30 minutes. In the stained preparations, the ratio of live intact cells to dead cells with destroyed membranes was counted by light microscopy (Olympus BX53, Japan) (Hayashida et al., 2011).

Integral cytotoxic components of blood serum were determined in the test with *D. viridis* cell culture by the degree of change in cell motility, the presence of cell aggregates, and the release of exometabolites by the cells. The integral cytotoxicity coefficient (Cc) was calculated and expressed as previously described (Klimova et al., 2016).

The concentration of CIC was measured spectrophotometrically (Shimadzu UV-2600, Japan) as a result of the interaction between antigens, antibodies and complement system proteins after precipitation in polyethylene glycol 6000 at room temperature. Optical density was measured at a wavelength of 450 nm against borate buffer (Riha et al., 1979) and expressed in relative units.

Statistical analysis was performed using the program Statistica 10 (Statsoft, USA). We used a method of visualizing the distribution of data in each group – boxplot with inclusive medians. Significant differences between groups were determined using ANOVA (Kruskal–Wallis H test) with post hoc comparisons of Mann–Whitney U test with Bonferroni correction. Differences between the control and experimental groups were considered significant at  $P \leq 0.05$ .

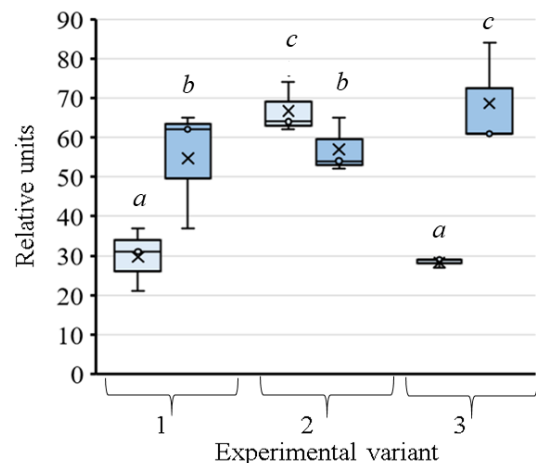
## Results

It was found that in animals with Cu-induced liver fibrosis, at the early stage of fibrosis development – F1, the spontaneous level of neutrophil phagocytic activity (without zymosan stimulation) was increased 2-fold compared to the control group of animals (Fig. 3).

If animals with Cu-induced fibrosis received LMW components at a dose of 0.1 mg/100 g body weight for 6 days, the spontaneous level of phagocytic activity did not differ from the control variant and it was 2.4 times lower compared to the phagocytic activity of neutrophils in animals with liver fibrosis (Fig. 3). Consequently, a 6-fold dose of LMW in experimental animals with Cu-induced liver fibrosis in the early stages of its development was accompanied by a normalization of the activity of oxygen-dependent phagocytosis to control levels.

In the next series of experiments, we determined the functional reserve of neutrophils in animals from the three groups studied. It was found that when neutrophils were obtained from animals of the con-

trol group, their phagocytic activity increased by 83% after zymosan activation compared to the initial level (Fig. 3).



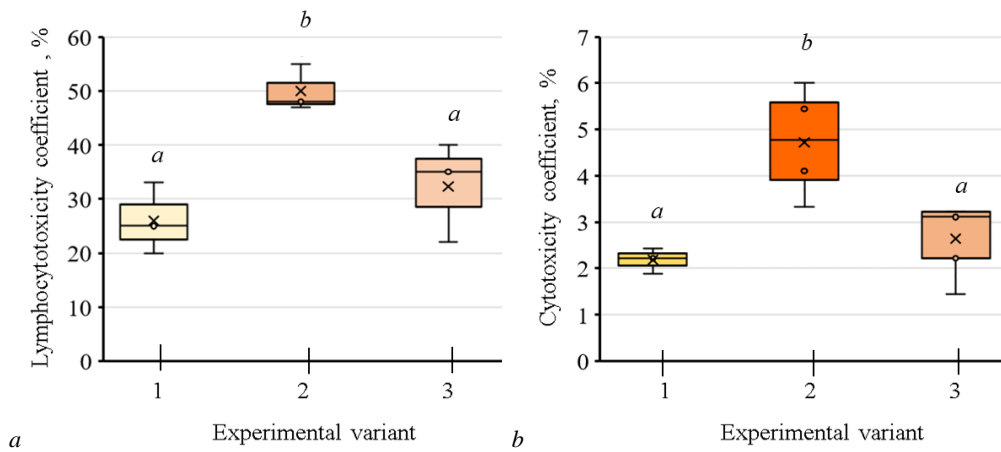
**Fig. 3.** Phagocytic activity of blood neutrophils in the NST-test at spontaneous level (light blue) and at the zymosan-induced level (dark blue) in the control (intact) group of rats (1), in animals with Cu-induced liver fibrosis (2) and in a group of animals with Cu-induced liver fibrosis, which received low molecular weight components of colostrum daily for 6 days at a dose of 0.1 mg/100g (3); different letters in the same coordinate system indicate samples that differ significantly from each other according to the results of the Tukey test ( $P < 0.05$ ); midline – median, upper and lower rectangle borders – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers;  $n = 6$

If phagocytic cells were obtained from animals with Cu-induced liver fibrosis, zymosan had no stimulating effect on them, i.e. the functional reserve of the cells was exhausted in animals with Cu-induced fibrosis (Fig. 3). If phagocytic cells were obtained from animals with liver fibrosis, which received a course of 6-day administration per os LMW components at a dose of 0.1 mg/100g of body weight, their phagocytic activity was increased by zymosan 2.4 times compared to their initial values, which is more than in the control variant (Fig. 3).

As a result, LMW restored the reserve of oxygen-dependent neutrophil phagocytosis to control levels.

Such an effect of LMW on the phagocytic activity of neutrophils may be due to several reasons. A significant increase in copper content, which takes place when it is administered to the organism at a dose of 1 mg/100 g body weight (Bozhkov et al., 2021), may be accompanied by partial necrosis of hepatocytes and, as a consequence, lead to the formation of a variety of cell degradation products. Such products are called damage-associated molecular fragments (DAMPs) (Scheibner et al., 2006) and may be immunotropic. Since the degradation products of cells and macromolecules are extremely diverse and their direct determination is difficult, their presence can be assessed by the manifestation of cytotoxicity in model systems, in particular the Terasaki test.

Lymphocytotoxicity, as determined by the Terasaki method, was found to be 26% in intact animals (Fig. 4a). In animals with Cu-induced liver fibrosis, it was increased 2-fold (Fig. 4a). When animals with liver fibrosis received LMW components for 6 days, their cytotoxicity index did not differ from that of control rats (Fig. 4a). Consequently, the action of copper ions is associated with an increase in degradation products of cellular components that have a toxic effect on the cells of the body's immune system. In this case it is important to determine the “directionality” or specificity of the action of these cytotoxic components, i.e. whether they inhibit the functional activity of cells of the immune system only, or other types of cells i.e. whether they have cell specificity. Previously, a method was developed to evaluate the cytotoxic effects of biological substances on a culture of the microalgae *D. viridis* (Klimova et al., 2016) and it was shown that it can be used to evaluate the presence of cytotoxic components in the serum of patients with various pathologies (Klimova et al., 2022).



**Fig. 4.** Coefficient of lymphocytotoxicity determined by the Terasaki test (a) and coefficient of cytotoxicity determined by *D. viridis* cell culture test (b), for the control group of animals (1), for animals with Cu-induced liver fibrosis (2) and for the group of animals with Cu-induced liver fibrosis that received low molecular weight components of colostrum daily for 6 days at a dose of 0.1 mg/100 g of weight (3): midline – median, upper and lower rectangle borders – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; n = 6

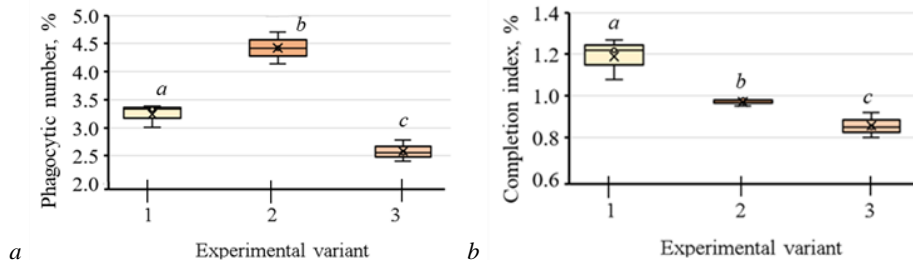
It was found that the coefficient of cytotoxicity of serum components from intact rats was 2.2 conventional units, whereas in rats with liver fibrosis it was 4.7 (Fig. 4b). The results obtained in the *D. viridis* test were in agreement with the lymphocytotoxicity test, which convincingly indicates the lack of specificity of the inhibitory effect of the cytotoxic components formed in animals with liver fibrosis.

The coefficient of cytotoxicity of serum components in animals with liver fibrosis that received LMW components of cow's colostrum did not differ from that in control animals (Fig. 4b).

Consequently, components of colostrum, which were given to animals with Cu-induced liver fibrosis for 6 days daily, could provide elimination of toxicity by forming complexes with them or accelerating their elimination from the body and/or reducing their formation.

In the next stage of the work we determined some indices of the activity of oxygen-independent phagocytosis, in particular, the ability of phagocytic cells to absorb foreign substances (phagocytic number – PhN) and the ability to digest or destroy the absorbed components (phagocytosis completion index – PhI).

It was found that PhN in rats with liver fibrosis was insignificantly increased (34%), compared to the control level (Fig. 5a). Whereas this index was reduced by 42% after administration of LMW components of cow's colostrum compared to the animals with liver fibrosis and was not significantly different from the control level (Fig. 5a), and the phagocytosis completion index was slightly decreased compared to the control and was not different from that of the rats with fibrosis (Fig. 5b).

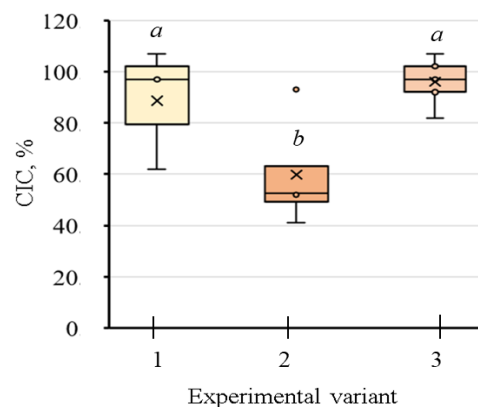


**Fig. 5.** Ability of phagocytic blood cells to absorb foreign substances – phagocytic number (a) and ability of phagocytic cells to digest absorbed foreign substances – completion index (b) respectively for the control group of animals (1), for animals with Cu-induced liver fibrosis (2) and for the group of animals with Cu-induced liver fibrosis that received low molecular weight components of colostrum daily for 6 days at a dose of 0.1 mg/100 g of weight (3); n = 6

Consequently, the administration of low molecular weight components of colostrum daily for 6 days at a dose of 0.1 mg/100 g of body weight to animals with Cu-induced liver fibrosis was accompanied by a normalization of the altered parameters of the immune system, which were similar to those of the control animals.

A rather important and informative indicator of the activity of the immune system on the action of both foreign and autoimmune components is the number of circulating immune complexes (CIC) contained in blood serum. It is known that CICs are complexes formed from antigens and their associated corresponding antibody with the participation of complement components C3, C4 and C1q. Such complexes are removed from the body by phagocytosis and/or through the reticulo-endothelial system. The balance between the rate of formation of CICs and the rate of their elimination from the body can lead to changes in their levels in the body and in the case of accumulation they can accumulate in the perivascular space and cortical layer of the kidneys inducing complement activation and inflammatory processes.

It was found that in animals with Cu-induced liver fibrosis the amount of CICs in the serum was slightly decreased (33%) compared to their amount in the group of control group (Fig. 6).



**Fig. 6.** The amount of circulating immune complexes in blood serum of the control group of animals (1), in animals with Cu-induced liver fibrosis (2) and animals with Cu-induced liver fibrosis, which received low molecular weight protein components of colostrum at a dose of 0.1 mg/100 g body weight for 6 days (3); n = 6

When LMW components of cow's colostrum were administered per os for 6 days to animals with liver fibrosis, the amount of CICs did not differ from that of control rats (Fig. 6). The obtained results suggest that the rate of CIC excretion from the organism is slightly increased in animals with Cu-induced liver fibrosis.

## Discussion

Despite the availability of a large number of hepatotropic drugs, the treatment of liver pathologies is ineffective, especially in the late stages of the pathological process (Makri et al., 2021; Paternostro et al., 2022). The search and development of new substances capable of preventing and treating liver fibrosis remains an urgent task (Powell et al., 2021).

When developing new drugs, it is important to consider both the effectiveness of the treatment and the absence of side effects. Natural substances may be particularly promising in this regard. Bovine colostrum is known to contain traces of a wide range of natural substances capable of regulating metabolism and thus of restoring and maintaining homeostasis in pathological conditions (Yalçıntaş et al., 2024). However, the extremely high variability of its composition and the resulting problems of standardisation do not allow the use of colostrum as a medicinal substance.

We have developed a two-step 'selective-integrative' technology that has allowed us to partially standardise and produce the so-called low molecular weight components of colostrum (Ivanov et al., 2024). The study of the biological activity of low molecular weight components of colostrum for more than 10 years on the experimental model of liver fibrosis showed that LMW are a promising substance in the prevention and treatment of liver pathologies. The present work is one of the stages of research into the possible mechanism of action of the polycomponent protein substance obtained from milk.

It is known that one of the primary reactions of the organism in the development of various pathologies is a shift in the balance of the redox system in favour of pro-oxidants, i.e. a change in the balance between pro- and antioxidants and, as a consequence, the manifestation of oxidative stress (Tretter et al., 2021). However, the use of antioxidants does not ensure the restoration of the necessary balance in the redox system. The problem of regulating the redox balance has proved to be much more complicated, because in biological systems there is a huge repertoire of metabolic reactions involving products of free radical reactions (Sadi, 2023). In addition, the products of free radical reactions are not only signaling molecules of many metabolic reactions, but the characteristics of redox regulation differ in different compartments of the cell, depending on metabolic needs, cell cycle phases, circadian rhythms and disease states. To address this fundamental problem of metabolic regulation, it is necessary to use specific 'smart' regulators of oxidative balance. 'Smart' in the sense that they can act as both pro-oxidants and antioxidants, and their direction of action may depend on both their concentration and the microenvironment in which they are currently located in a particular biological compartment. It has been shown that low molecular weight components of colostrum have both antioxidant and pro-oxidant properties (Ivanov et al., 2021). Administration of low molecular weight components of colostrum to animals with hepatic fibrosis resulted in restoration of balance in the redox system, partial normalisation of liver functions and reduction in mortality of such animals, i.e. there was a systemic effect of LMWs on the organism (Ivanov et al., 2024).

In addition, low molecular weight components of colostrum had an effect on the functional properties of the bone marrow in animals with Cu-induced liver fibrosis, as evidenced by changes in the number and characteristics of immunocompetent cells (Bozhkov et al., 2020). These results led to the conclusion that the effect of LMWs is polyfunctional. Further studies on the mechanisms of low molecular weight components of colostrum action are of great interest in terms of the fine regulation of redox balance, in the sense that different components of colostrum may have different properties in different cells and cellular components. Such differences can be explained by the fact that their concentration may be different in different compartments, as they can selectively penetrate different cell types and their

compartments, and their activity depends both on their concentration and on the characteristics of the compartment itself. Furthermore, due to their polyfunctionality, low molecular weight components of colostrum can exert specific regulation not only at the level of the redox system, but also at other levels of metabolic regulation.

It is known that oxidative stress is associated with the appearance of degradation products of macromolecules and even whole cells (DAMPs) in cells and the organism as a whole, and when the degradation products are caused by infectious agents, they are usually defined as PAMPs (pathogen-associated molecular fragment of molecules) (Dallio et al., 2021; Li et al., 2021). The formation of biodegradation products can be caused by various factors, and the composition of DAMPs is extremely diverse. They can induce the manifestation of different responses: synthesis of various stress proteins; inflammatory process; change in the activity of the immune system and general metabolism. Depending on the balance between these types of changes at the physiological level, this can manifest itself as the restoration of the previous level of homeostasis, the formation of a quasi-homeostatic level, a new level of homeostasis (chronic condition) or the destruction of the metabolic system (death of the organism).

It is known that copper is an element with variable valence, which induces the formation of free radicals, forms complexes with proteins and other macromolecules and, as a result, can lead to changes in the functional activity of these macromolecules (Kovaleva et al., 2012; Valko et al., 2016; Coskun et al., 2021).

The present work shows that against the background of Cu-induced liver fibrosis development, cytotoxic components (DAMPs) are formed in the organism, which affect the structural and functional characteristics of different cell types (leukocytes and *D. viridis* cells), i.e. these cytotoxic components are not species-specific and probably tissue-specific. In the presence of DAMPs, there was a twofold increase in oxygen-dependent phagocytosis and a slight increase in oxygen-independent phagocytosis (by 34% compared to the control). At the same time, the amount of CICs was 33% lower compared to the control. These results suggest that in Cu-induced liver fibrosis, significantly more degradation products of macromolecules are formed, which were 'utilised' in neutrophils by oxygen-dependent phagocytosis or oxidative burst (Buonocore et al., 2016).

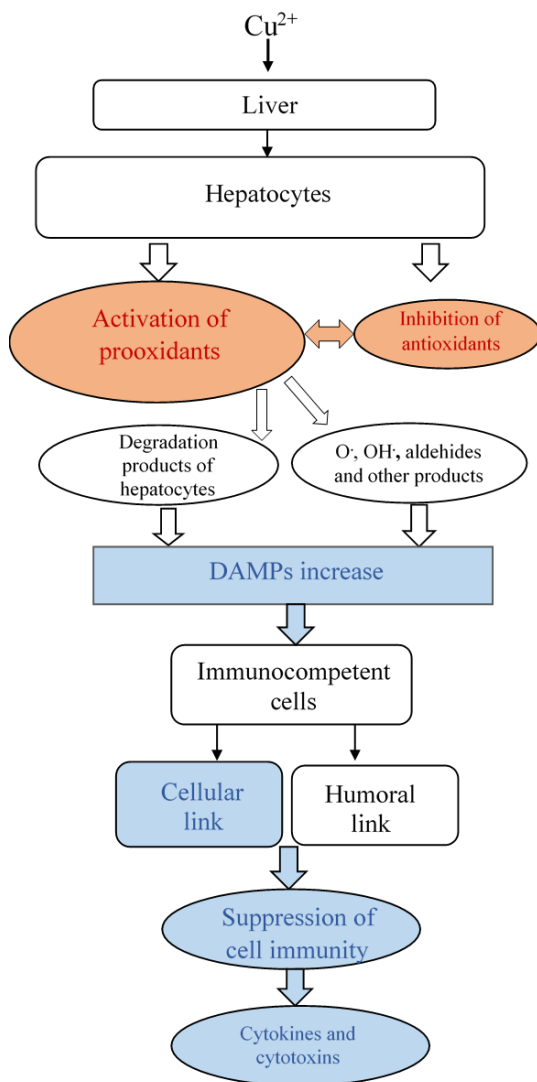
It should be noted that the oxidative burst induced in neutrophils is a response of the organism at the level of the first line of immune defense aimed at destruction (inactivation) of altered components by activation of oxidative enzymes (NADPH H-oxidase) in neutrophils (Jiang et al., 2022). It is important to note that against the background of Cu-induced liver fibrosis development, the activation of oxygen-dependent phagocytosis reached its limit, in the sense that additional stimulation of oxygen-dependent phagocytosis by zymosan did not lead to an increase in this index in the *in vitro* system compared to the control variant.

An additional confirmation of the activation of the cellular link of the immune system in animals with Cu-induced liver fibrosis may be an insignificant decrease in the amount of circulating immune complexes in the bloodstream of such animals. At the same time, fragments of destroyed cells, which are normally 'utilised' by oxygen-dependent phagocytosis, are much less in Cu-induced liver fibrosis. This is also supported by histological data on the morphological characteristics of the liver in Cu-induced liver fibrosis (Bozhkov et al., 2024). A certain reduction of CICs in the bloodstream of Cu-induced liver fibrosis animals compared to the control suggests that in this fibrosis model, the ratio between the contribution of cellular and humoral immunity in the 'utilisation' of degradation products changes in favour of the cellular part of the immune system. This may be indirectly supported by data on changes in bone marrow activity in Cu-induced liver fibrosis (Bozhkov et al., 2019).

The results of the present work and the early data obtained on the characterisation of the metabolic and immunological changes in Cu-induced liver fibrosis allow us to propose the following mechanism of the development liver fibrosis (Fig. 7).

When, in the early stages of Cu-induced liver fibrosis development, experimental animals were injected daily for 6 days with LMW, which contains various low molecular weight protein compo-

unds (Fig. 2), at a dose of 1 mg/100 g of body weight, there was a normalisation of the balance of the redox system, a decrease in the number of DAMPs and a restoration of phagocytosis activity to the level of control animals.



**Fig. 7.** Schematic showing the effect of high doses of copper sulphate (30% of the lethal dose) on shifting the equilibrium in the liver towards pro-oxidants (shown by increasing area), formation of a variety of degradation products (DAMPs) which activate phagocytic activity, accompanied by the development of inflammatory reactions, activation of hepatic stellate cells and development of the initial stages of liver fibrosis

It can be assumed that low molecular weight components of colostrum with different rates can bind to different compartments of hepatocytes (mitochondria, cytosol, etc.) and restore redox balance and as a consequence reduce the amount of formed DAMPs, which in turn leads to normalisation of phagocytosis activity.

Further studies of the systemic action of the components of colostrum on the organism will contribute not only to the understanding of the mechanisms of the choice of adaptation strategy of the organism to the action of toxic compounds, but also to the development of ways to eliminate such pathological conditions.

## Conclusions

The development of Cu-induced liver fibrosis was accompanied by an increase in the number of cytotoxic components in the blood serum of the experimental animals. These cytotoxic components were not species- or tissue-specific. Against the background of a twofold

increase in the cytotoxicity of serum components, there was an activation of phagocytosis. The administration of low-molecular-weight components of cow's colostrum to experimental animals with Cu-induced liver fibrosis was accompanied by a restoration to control values of the number of DAMPs and of activity indicators of the cellular link of the immune system. Low molecular weight components of colostrum can provide modulation of the cellular links of the immune system in animals with liver fibrosis and other types of toxicosis.

On the basis of the data obtained earlier and according to the results of the present work, it is suggested that the biological effect of the low molecular weight components of the colostrum is realised by restoring the balance of the redox system of the liver.

The authors declare no conflict of interests.

## References

- Abeyskera, K. W. M., Macpherson, I., Harris, R., Mansour, D., McPherson, S., Rowe, I., Rosenberg, W., Dillon, J. F., & Yeoman, A. (2022). Identification of liver disease: Why and how. *Frontline Gastroenterology*, 13, 367–373.
- Bao, Y., Wang, L., Pan, H., Zhang, T., Chen, Y., & Xu, S. (2021). Animal and organoid models of liver fibrosis. *Frontiers in Physiology*, 12, 666138.
- Barouki, R., Samson, M., Blanc, E. B., Colombo, M., Zucman-Rossi, J., Lazaridis, K. N., Miller, G. W., & Coumoul, X. (2023). The exposome and liver disease – How environmental factors affect liver health. *Journal of Hepatology*, 79(2), 492–505.
- Barzegar, F., Nabizadeh, S., Kamankesh, M., Ghasemi, J. B., & Mohammadi, A. (2023). Recent advances in natural product-based nanoemulsions as promising substitutes for hazardous synthetic food additives: A new revolution in food processing. *Food and Bioprocess Technology*, 17, 1087–1108.
- Bozhkov, A. I., Novikova, A. V., Klimova, E. M., Ionov, I. A., Akzhyhitov, R. A., Kurhuzova, N. I., Bilovetska, S. G., Moskalov, V. B., & Haiovy, S. S. (2024). Vitamin A reduces the mortality of animals with induced liver fibrosis by providing a multi-level body defense system. *Journal of Clinical and Experimental Hepatology*, 13(1), 48–63.
- Bozhkov, A. I., Ohienko, S. L., Bondar, A. Y., Ivanov, E. G., & Kurguzova, N. I. (2020). Low-molecular weight components of cow colostrum regulate bone marrow functions by modelling the redox-system of the organism. *Regulatory Mechanisms in Biosystems*, 11(2), 272–277.
- Bozhkov, A. I., Ohienko, S. L., Bondar, A. Y., Klimova, E. M., & Ivanov, E. G. (2019). Induced liver fibrosis is accompanied in young and old animals by age-dependent changes in bone marrow cells. *Advances in Gerontology*, 32(1–2), 45–54.
- Bozhkov, A. I., Sidorov, V. I., Alboqai, O. K., Akzhyhitov, R. A., Kurguzova, N. I., Malyshev, A. B., Albegai, M. A. Y., & Gromovoi T. Y. (2021). The role of metallothioneins in the formation of hierarchical mechanisms of resistance to toxic compounds in young and old animals on the example of copper sulfate. *Translational Medicine of Aging*, 5, 62–74.
- Buonocore, D., Seneci, A., Carrabeta, M. E., Pialorsi, F., Zurlo, M., Bottone, M. G., Veneroni, P., Verri, M., & Dossena, M. (2016). *In vitro* oxidative burst assay to evaluate the efficacy of Immune-G-matrix™, an innovative yeast-β-glucans, zinc and copper-based formulation. *Nutrafoods*, 15, 21–26.
- Castaneda, D., Gonzalez, A. J., Alomari, M., Tandon, K., & Zervos, X. B. (2021). From hepatitis A to E: A critical review of viral hepatitis. *World Journal of Gastroenterology*, 27(16), 1691–1715.
- Coskun, M., Kayis, T., Yilmaz, M., Dursun, O., & Emre, I. (2021). Copper and zinc impact on stress biomarkers and growth parameters in a model organism, *Galleria mellonella* larvae. *Biomaterials*, 34(6), 1263–1273.
- Dallio, M., Sanginetto, M., Romeo, M., Villani, R., Romano, A. D., Loguercio, C., Serviddio, G., & Federico, A. (2021). Immunity as cornerstone of non-alcoholic fatty liver disease: The contribution of oxidative stress in the disease progression. *International Journal of Molecular Sciences*, 22(1), 436.
- Devarbhavi, H., Asrani, S. K., Arab, J. P., Nartey, Y. A., Pose, E., & Kamath, P. S. (2023). Global burden of liver disease: 2023 update. *Journal of Hepatology*, 79(2), 516–537.
- El-Fattah, A. M. A., Rabo, F. H. R. A., El-Dieb, S. M., & El-Kashef, H. A. (2012). Changes in composition of colostrum of Egyptian buffaloes and Holstein cows. *BMC Veterinary Research*, 8, 19.
- Ghosh, S., & Iacucci, M. (2021). Diverse immune effects of bovine colostrum and benefits in human health and disease. *Nutrients*, 13(11), 3798.
- Ginès, P., Krag, A., Abraldes, J. G., Solà, E., Fabrellas, N., & Kamath, P. S. (2021). Liver cirrhosis. *The Lancet*, 398(10308), 1359–1376.
- Hayashida, M., Matsuura, T., Saeki, I., Yanagi, Y., Yoshimaru, K., Nishimoto, Y., Takahashi, Y., Fujita, K., Takada, N., Taguchi, S., Uesugi, T., Hirose, R., Nakamura, M., Nakao, M., & Taguchi, T. (2021). Association of lym-

- phocyte crossmatch and the outcome of intestinal transplantation in swine. *Pediatric Surgery International*, 27(3), 279–281.
- Ivanov, E. G., Lebid-Biletska, K. M., Bozhkov, A., & Nikitchenko, Y. V. (2024). Copper sulfate and carbon tetrachloride induces a uniform response at the level of the redox system and the nature of this response depends on age. *Regulatory Mechanisms in Biosystems*, 15(3), 496–503.
- Ivanov, I., Goltvjansky, A., Bozhkov, A., & Gromovoy, T. (2024). Selective-integrative technology for the separation of colostrum into components and the possibilities of obtaining protein substances from different sources. *Innovative Biosystems and Bioengineering*, 8(3), 60–67.
- Ivanov, I., Kozheshkurt, V., Bozhkov, A., Goltvjansky, A., Katrich, V., Sidorov, V., & Gromovoy, T. (2021). Low-molecular components of colostrum as a regulator of the organism redox-system and biological antidote. *Eureka: Life Sciences*, 2, 56–64.
- Jiang, L., Liu, W., Xu, J., Gao, X., Zhao, H., Li, S. H., Huang, W., Yang, Z., & Wei, Z. (2022). CuO-NPs-triggered heterophil extracellular traps exacerbate liver injury in chicks by promoting oxidative stress and inflammatory responses. *Archives of Toxicology*, 96(11), 2913–2926.
- Khan, T. S., Akram, N., Faisal, Z., Saeed F, Rasheed, A., Ahmed, F., & Afzaal, M. (2024). Bovine colostrum: Therapeutic potential and clinical evidence. *International Dairy Journal*, 157, 105996.
- Khudan, R., Bandas, I., Mykolenko, A., Svanishvili, N., & Krynytska, I. (2021). The influence of chronic hyperhomocysteinemia on phagocytic and metabolic activity of peripheral blood neutrophils in case of lipopolysaccharide-induced periodontitis. *Georgian Medical News*, (321), 119–125.
- Klimova, E. M., Bozhkov, A. I., Boyko, V. V., Drozdova, L. A., Lavinskaya, E. V., & Skok, M. V. (2016). Endogenic cytotoxic compounds and formation of the clinic forms of myasthenia. *Translational Biomedicine*, 7(3), 84.
- Klimova, E. M., Bozhkov, A. I., Lavinska, O. V., Drozdova, L. A., & Kurhuzova, N. I. (2022). Low molecular weight cytotoxic components (DAMPs) form the post-COVID-19 syndrome. *Immunobiology*, 228(1), 152316.
- Kovaleva, M. K., Menzyanova, N. G., Jain, A., Yadav, A., Flora, S. J. S., & Bozhkov, A. I. (2012). Effect of hormesis in *Dunaliella viridis* Teodor. (Chlorophyta) under the influence of copper sulfate. *International Journal on Algae*, 14(2), 44–61.
- Kusumaningrum, C. E., Widyasari, E. M., Sriyani, M. E., & Wongso, H. (2021). Pharmacological activities and potential use of bovine colostrum for peptide-based radiopharmaceuticals: A review. *Pharmacia*, 68(2), 471–477.
- Le, M. J. (2023). A review of liver fibrosis and cirrhosis regression. *Journal of Pathology and Translational Medicine*, 57(4), 189–195.
- Li, P., & Chang, M. (2021). Roles of PRR-mediated signaling pathways in the regulation of oxidative stress and inflammatory diseases. *International Journal of Molecular Sciences*, 22(14), 7688.
- Liu, Y. B., & Chen, M. K. (2022). Epidemiology of liver cirrhosis and associated complications: Current knowledge and future directions. *World Journal of Gastroenterology*, 28(41), 5910–5930.
- Makri, E., Goulas, A., & Polyzos, S. A. (2021). Epidemiology, pathogenesis, diagnosis and emerging treatment of nonalcoholic fatty liver disease. *Archives of Medical Research*, 52(1), 25–37.
- Paternostro, R., & Trauner, M. (2022). Current treatment of non-alcoholic fatty liver disease. *Journal of Internal Medicine*, 292(2), 190–204.
- Pellicano, R., Ferro, A., Cicerchia, F., Mattivi, S., Fagoonee, S., & Durazzo, M. (2023). Autoimmune hepatitis and fibrosis. *Journal of Clinical Medicine*, 12(5), 1979.
- Playford, R., & Weiser, M. (2021). Bovine colostrum: Its constituents and uses. *Nutrients*, 13(1), 265.
- Poonia, A., & Shiva (2022). Bioactive compounds, nutritional profile and health benefits of colostrum: A review. *Food Production, Processing and Nutrition*, 4, 26.
- Powell, E. E., Wong, V. W. S., & Rinella, M. (2021). Non-alcoholic fatty liver disease. *The Lancet*, 397(10290), 2212–2224.
- Puppel, K., Gołębiewski, M., Grodkowski, G., Słószarz, J., Kunowska-Słószarz, M., Solarczyk, P., Łukasiewicz, M., Balcerak, M., & Przysucha, T. (2019). Composition and factors affecting quality of bovine colostrum: A review. *Animals*, 9(12), 1070.
- Qiuling, L., Qilin, Y., Cheng, Y., Mingping, Z., Kangning, W., & Enhua, X. (2023). The application of a novel platform of multiparametric magnetic resonance imaging in a bioenvironmental toxic carbon tetrachloride-induced mouse model of liver fibrosis. *Environmental Research*, 238(1), 117130.
- Riha, I., Haskova, V., Kaslik, J., Maierova, M., & Stransky, J. (1979). The use of polyethylene glycol for immune complex detection in human sera. *Molecular Immunology*, 16(7), 489–493.
- Sadiq, Z. (2023). Free radicals and oxidative stress: Signaling mechanisms, redox basis for human diseases, and cell cycle regulation. *Current Molecular Medicine*, 23(1), 13–35.
- Scheibner, K. A., Lutz, M. A., Boodoo, S., Fenton, M. J., Powell, J. D., & Horton, M. R. (2006). Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. *Journal of Immunology*, 177(2), 1272–1281.
- Struff, W. G., & Sprotte, G. (2008). Bovine colostrum as a biologic in clinical medicine: A review – Part II. *International Journal of Clinical Pharmacology and Therapeutics*, 46(5), 211–225.
- Tretter, V., Hochreiter, B., Zach, M. L., Krenn, K., & Klein, K. U. (2021). Understanding cellular redox homeostasis: A challenge for precision medicine. *International Journal of Molecular Sciences*, 23(1), 106.
- Unsal, V., Cicek, M., & Sabancilar, İ. (2020). Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Reviews on Environmental Health*, 36(2), 279–295.
- Valko, M., Jomova, K., Rhodes, C. J., Kuča, K., & Musílek, K. (2016). Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Archives of Toxicology*, 90, 1–37.
- Wąsowska, E., & Puppel, K. (2018). Changes in the content of immunostimulating components of colostrum obtained from dairy cows at different levels of production. *Journal of the Science of Food and Agriculture*, 98(13), 5062–5068.
- Yalçıntaş, Y. M., Duman, H., Rocha, J. M., Bartkiene, E., Karav, S., & Ozogul, F. (2024). Role of bovine colostrum against various diseases. *Food Bioscience*, 61, 104818.
- Yan, M., Huo, Y., Yin, S., & Hu, H. (2018). Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. *Redox Biology*, 17, 274–283.
- Yanko, N. V., Kaskova, L. F., Kulai, O. O., Khmil, O. V., & Novikova, S. C. (2022). Neutrophil activities in adolescents with type I diabetes mellitus depending on periodontal state. *Wiadomości Lekarskie*, 75(11), 2826–2830.
- Yao, Q.-Y., Feng, Y.-D., Han, P., Yang, F., & Song, G.-Q. (2020). Hepatic microenvironment underlies fibrosis in chronic hepatitis B patients. *World Journal of Gastroenterology*, 26(27), 3917–3928.
- Zheng, J., Zhao, L., Dong, J., Chen, H., Li, D., Zhang, X., Hassan, M. M., Steck, S. E., Li, X., Xiang, Y.-B., & Wang, H. (2022). The role of dietary factors in nonalcoholic fatty liver disease to hepatocellular carcinoma progression: A systematic review. *Clinical Nutrition*, 41(10), 2295–2307.