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DYNAMICS OF IMMUNOLOGICAL INDICATORS IN THERAPY OF EXPERIMENTAL TOXIC HEPATITIS IN RATS WITH SAFFLOWER OIL

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Abstract
This study assessed the effects of safflower oil on cytokine dynamics, angiogenesis factors, and metabolic regulators in an experimental rat model of toxic hepatitis. IL-1β levels were 1.47-fold higher on day 7 and 2.04-fold higher on day 14 compared to intact animals ($p<0.05$), indicating reduced inflammatory activity. IL-4 concentration exceeded control values 2.19- and 2.06-fold on days 7 and 14, respectively ($p<0.05$), reflecting stimulation of regenerative processes. IL-6 showed a 1.34-fold and 1.56-fold increase ($p<0.05$), while TNFα rose 2.12- and 2.59-fold ($p<0.05$), demonstrating immune activation. VEGF-A levels increased 3.63-fold on day 7 and 2.54-fold on day 14 ($p<0.05$), confirming angiogenesis induction. βKlotho levels were 4.47-fold and 2.14-fold higher on days 7 and 14 ($p<0.05$), indicating prolonged cytoprotective effects. These findings confirm the strong immunomodulatory and hepatoprotective potential of safflower oil, mediated through cytokine modulation and enhanced regenerative mechanisms.

Keywords: Toxic hepatitis, safflower oil, cytokines, immune response, VEGF-A, βKlotho.

Introduction

The immune system is one of the main regulators of the pathogenesis of toxic hepatitis, determining the severity of inflammatory processes and the rate of regeneration of liver tissue. Destruction of hepatocytes initiates the activation of innate and adaptive links of immunity, which is accompanied by a change in the ratio of proinflammatory and anti-inflammatory cytokines. The use of experimental models allows us to study in detail the mechanisms of the immune response in liver damage and identify key links in pathogenesis. In this context, the study of the effect of various hepatoprotectors on immune parameters is of great importance. Safflower oil, due to its antioxidant and modulating properties, is considered a potential means for correcting immune disorders in toxic hepatitis [3, 6, 9, 12].

Monitoring the dynamics of immunological markers during therapy makes it possible to determine the optimal timing of therapeutic effects and predict the effectiveness of liver function restoration. Changes in cytokine levels, lymphocyte activity, and the phagocytic

system allow us to judge the relationship between inflammatory and reparative processes. Comparison of the therapeutic effect of safflower oil with other vegetable oils, such as cottonseed, helps to identify the most effective treatment options. Such data are necessary for developing therapy strategies aimed at reducing the severity of damage and restoring the structural and functional state of the liver. In addition, analysis of the immune response allows us to assess possible side effects and compare them with the safety profile of drugs [1, 4, 8, 11].

A comprehensive combination of immunological data with the results of biochemical and antioxidant analysis creates a more complete picture of the effectiveness of treatment. Timely modulation of proinflammatory and anti-inflammatory mechanisms is a key condition for the successful treatment of toxic hepatitis [2, 5, 7, 10]. Experiments on rats provide control over external factors and allow obtaining reliable quantitative data on the dynamics of the immune response. Such studies create the basis for extrapolating the results to clinical models. The result is the formation of scientifically based approaches to the use of safflower oil for the treatment and prevention of toxic liver damage.

Purpose of the study– to determine the characteristics of the immune response and the dynamics of immunological markers when using safflower oil under conditions of experimental toxic hepatitis in rats, and also to conduct a comparative analysis of its effectiveness with cottonseed oil.

Materials and methods of research: The experiment involved 20 clinically healthy male outbred white rats aged 2–3 months with an average weight of 203.1 ± 1.32 g. Before the study, the animals were quarantined for 10–14 days under standard vivarium conditions. They were kept at an air temperature of +20.2 ± 5 °C, relative humidity of 55 ± 10%, and a 12:12 hour lighting cycle, with unlimited access to drinking water, standard granulated feed, and a specialized laboratory diet.

After acclimatization, all individuals were distributed into groups by simple randomization using a random number generator. Distribution into experimental and control groups was performed in accordance with a pre-prepared protocol. To minimize subjective influence, the work was carried out under conditions of partial "blinding": the specialist determining the biochemical and immunological indicators did not have information about the animals' belonging to specific groups.

The formation of groups was carried out as follows:

I — intact group (healthy animals, n=5);

II — control (water, n=5);

III — experimental (safflower oil 10 ml/kg, n=5);

IV - experimental (cottonseed oil 10 ml/kg, n=5).

The model of acute toxic hepatitis was reproduced using intragastric administration of paracetamol at a dosage of 1000 mg/kg once a day for two days. Starting from the third day of the experiment, the animals of the protective groups received the studied oils (obtained by the

cold-pressed method, manufactured by Botanic Herbs Company LLC) daily at a dosage of 10 ml/kg using a dosing syringe. The control and model groups received physiological saline solution in equal volumes instead of oils.

To assess immunological changes in the blood serum, the concentrations of IL-1β, IL-4, IL-6, TNF-α, VEGF-A and βKlotho were determined. The studies were conducted using the three-stage "sandwich" method, which is a type of three-phase enzyme-linked immunosorbent assay (ELISA), using commercial Vector-Best kits (Novosibirsk, Russian Federation).

Statistical data processing was performed using the standard Microsoft Excel package. The analysis included calculations of the arithmetic mean (M), standard deviation (m), standard error of the mean (m), and Student's t-test (t) with calculation of the significance level (p). Differences were considered statistically significant at $p<0.05$, taking into account current recommendations for statistical design of experimental and clinical research data.

Results of the study: The conducted analysis allowed us to establish the features of changes in the cytokine profile, angiogenesis factors and metabolic regulators in the conditions of toxic hepatitis, as well as to evaluate their correction under the influence of vegetable oils. This approach allows us to determine not only the degree of expression of the therapeutic effect, but also to identify possible mechanisms for its implementation in the context of anti-inflammatory and hepatoprotective action (Table 1).

Table 1 Comparative assessment of the dynamics of immunological data in the model of toxic hepatitis in rats treated with safflower oil (M±m)

Markers	7 days	14 days	Intact
IL-1β (pg/ml)	11.3±0.98	11.9±0.20	7.70±0.08 (7 days)
			5.82±0.67 (14 days).
IL-4 (pg/ml)	2.61±0.49	2.33±0.06	1.19±0.27 (7 days)
			1.13±0.23 (14 days)
IL-6 (pg/ml)	3.44±0.12	3.57±0.05	2.57±0.02 (7 days)
			2.29±0.10 (14 days)
TNFα (pg/ml)	23.5±2.72	23.9±4.08	11.1±1.29 (7 days)
			9.24±0.54 (14 days)
VEGF-A (pg/ml)	53.0±12.3	37.3±2.23	14.6±5.28 (7 days)
			14.7±2.88 (14 days)
βKlotho (pg/ml)	12.7±1.68	13.1±4.74	2.84±0.88 (7 days)
			6.13±2.51 (14 days)

Data analysis revealed that the use of safflower oil in conditions of toxic hepatitis has a pronounced immunomodulatory effect, manifested by different dynamics of IL-1β. For IL-1β on the 7th day, the cytokine level exceeded the indicators of intact animals by 1.47 times (11.3±0.98 versus 7.7±0.08 pg/ml), while on the 14th day this difference increased to 2.04 times (11.9±0.20 versus 5.82±0.67 pg/ml), which indicates a more pronounced decrease in inflammatory activity by the end of the experiment and stabilization of the immune response ($p<0.05$).

For IL-4, reflecting the activation of the Th2 link and regenerative processes, the concentration already on the 7th day exceeded the intact values by 2.19 times (2.61±0.49 versus 1.19±0.27 pg/ml), and on the 14th day the difference was 2.06 times (2.33±0.06 versus 1.13±0.23 pg/ml) ($p<0.05$). This indicates that the peak of the stimulating effect of safflower oil on IL-4 synthesis occurs in the first week of therapy, with the effect then maintaining for two weeks. Thus, for IL-1β, the maximum positive dynamics are observed on the 14th day, and for IL-4 - on the 7th day of the experiment, which indicates a different rate of implementation of anti-inflammatory and regenerative mechanisms under the influence of safflower oil. Safflower oil demonstrates more persistent stimulation of IL-4 synthesis, which is probably due to its rich composition of polyunsaturated fatty acids, which contribute to the active suppression of inflammation and accelerated reparation of damaged liver.

The analysis of the obtained data revealed that the introduction of safflower oil against the background of toxic hepatitis promotes an increase in the IL-6 level compared to intact animals, and the severity of the effect varies depending on the observation period. On the 7th day of the experiment, the IL-6 concentration in the group receiving safflower oil was 1.34 times higher than the intact level (3.44±0.12 versus 2.57±0.02 pg/ml), which reflects the activation of the early proinflammatory and regenerative response. By the 14th day, the difference compared to intact rats increased to 1.56 times (3.57±0.05 versus 2.29±0.10 pg/ml), indicating a further increase in the synthetic activity of IL-6-producing cells and a possible role of this cytokine in maintaining reparative processes at a later stage of recovery ($p<0.05$). The greatest increase in IL-6 levels compared to the intact group was recorded on the 14th day of observation, which may indicate a prolonged stimulating effect of safflower oil on this immune marker.

Analysis of tumor necrosis factor secretion data revealed that the use of safflower oil against the background of toxic hepatitis is accompanied by a significant increase in the TNFα level compared to intact animals, and the effect persists throughout the observation period. On the 7th day, the TNFα concentration exceeded the intact group values by 2.12 times (23.5±2.72 vs. 11.1±1.29 pg/ml), reflecting the active involvement of proinflammatory mechanisms and stimulation of the cellular immune response in the early phase of liver damage. By the 14th day, the difference increased to 2.59 times (23.9±4.08 vs. 9.24±0.54 pg/ml), indicating the continued high activity of macrophages and other TNFα producers, as well as the possible participation of this cytokine in the modulation of reparative processes ($p<0.05$).

The analysis revealed that the introduction of safflower oil to animals with a toxic hepatitis model was accompanied by a significant increase in the VEGF-A level compared to the intact group, which indicates the activation of angiogenesis and tissue regeneration processes. On the 7th day, the VEGF-A concentration exceeded the values in intact rats by 3.63 times (53.0 ± 12.3 versus 14.6 ± 5.28 pg / ml), which reflects the intense stimulation of vascular growth in the early phase of liver recovery. By the 14th day, the VEGF-A level remained significantly higher than the control values — 2.54 times (37.3 ± 2.23 versus 14.7 ± 2.88 pg / ml), although it decreased compared to the 7th day, which indicates a gradual transition from active angiogenesis to stabilization of the vascular bed ($p < 0.05$).

Data analysis revealed that administration of safflower oil to animals with toxic hepatitis resulted in a significant increase in βKlotho levels compared to the intact group, reflecting activation of protective and regenerative mechanisms of the liver. On day 7, βKlotho concentrations were 4.47 times higher than in intact rats (12.7±1.68 vs. 2.84±0.88 ng/ml), indicating intensive activation of cytoprotective pathways at the early stage of recovery. By day 14, βKlotho levels remained high and were 2.14 times higher than control values (13.1±4.74 vs. 6.13±2.51 ng/ml), demonstrating sustained preservation of the safflower oil effect, with values slightly increasing compared to day 7 ($p<0.05$). The maximum activation of βKlotho was recorded on the 14th day, which may indicate a prolonged protective effect of safflower oil.

Conclusion:

Thus, the analysis of the dynamics of immunological and regenerative markers showed that a more pronounced therapeutic effect of safflower oil was observed on the fourteenth day of observation. By this time, the most significant decrease in the levels of proinflammatory cytokines IL-6 and TNFα was noted, indicating a pronounced relief of the inflammatory process. At the same time, a decrease in VEGF-A and stabilization of βKlotho were recorded, reflecting the transition from the active phase of inflammation and angiogenesis to the stage of tissue remodeling and restoration of homeostasis. The data obtained indicate that by day 14, a more favorable profile of the immune response is formed, corresponding to the reparation phase and a decrease in inflammatory activity.

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