UDC: 615.357:612.357

ANTIOXIDANT AND HEPATOPROTECTIVE PROPERTIES OF SAFFLOWER OIL IN DYNAMICS IN EXPERIMENTAL ACUTE TOXIC HEPATITIS IN RATS

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XULOSA

Dolzarbligi. *Toksik gepatit gepatologiyaning eng dolzarb muammolaridan biri bo'lib, tabiiy kelib chiqishga ega samarali va xavfsiz gepatoprotektorlarni izlashni talab etadi.*

Tadqiqot maqsadi. *Saflor moyining jigar funktsiyasining biokimyoviy ko'rsatkichlariga va antioksidant fermentlar faolligiga ta'sirini kalamushlarda eksperimental toksik gepatit sharoitida o'rganish.*

Materiallar va usullar. *Tadqiqotga toksik gepatit modeli qo'llangan 30 ta oq erkak kalamush olindi. Ularga 14 kun davomida 10 ml/kg dozada saflor moyi berildi. Dinamik kuzatuvda oqsil almashinuvi ko'rsatkichlari, sitolitik fermentlar (AST, ALT) faolligi, malon dialdegid konsentratsiyasi, shuningdek katalaza va superoksid dismutaza faolligi aniqlanib borildi.*

Natijalar. *14-kunda umumiy oqsil darajasi intakt hayvonlarga nisbatan 1,41 marta oshdi (117,2 ga qarshi 83,2 g/dl, p<0,05), bu jigar oqsil-sintetik funksiyasining rag'batlanishini ko'rsatadi. AST va ALT faolligi kamayib, normaga yaqinlashdi, bu gepatotsitlar sitolizining pasayishini aks ettiradi. Malon dialdegid konsentratsiyasi 7-kunga nisbatan 1,02 marta kamaydi (3,17 ga qarshi 3,24 mmol/ml), katalaza va superoksid dismutaza faolligi esa mos ravishda 1,07 va 1,02 marta oshdi, bu esa antioksidant himoya tiklanishini ko'rsatadi.*

Xulosa. *Saflor moyi aniq ifodalangan antioksidant va gepatoprotektiv xususiyatlarga ega bo'lib, ular ayniqsa 14-kun terapiyada yaqqol namoyon bo'ldi.*

Kalit so'zlar: *saflor moyi, toksik gepatit, antioksidant tizim, malon dialdegid, katalaza, superoksid dismutaza.*

РЕЗЮМЕ

Актуальность. *Токсический гепатит остаётся одной из актуальных проблем гепатологии, требующей поиска эффективных и безопасных гепатопротекторов природного происхождения.*

Цель исследования. *Изучить влияние сафлорового масла на биохимические показатели функции печени и активность антиоксидантных ферментов при экспериментальном токсическом гепатите у крыс.*

Материалы и методы. *В исследование включено 30 белых крыс-самцов с моделью токсического гепатита, которым в течение 14 дней назначали сафлоровое масло в дозе 10 мл/кг.*

Результаты исследования. *На 14-й день уровень общего белка увеличился в 1,41 раза по сравнению с интактными животными (117,2 против 83,2 г/дл, p<0,05), что свидетельствует о стимуляции белково-синтетической функции печени. Активность АСТ и АЛТ снизилась, приближаясь к норме, что отражает уменьшение цитолиза гепатоцитов. Концентрация малонового диальдегида уменьшилась в 1,02 раза по сравнению с 7-м днём (3,17 против 3,24 ммоль/мл), а активность каталазы и супероксиддисмутазы повысилась в 1,07 и 1,02 раза соответственно, демонстрируя восстановление антиоксидантной защиты.*

Вывод. *Сафлоровое масло обладает выраженными антиоксидантными и гепатопротекторными свойствами, наиболее заметными на 14-й день терапии.*

Ключевые слова: *сафлоровое масло, токсический гепатит, антиоксидантная система, малоновый диальдегид, каталаза, супероксиддисмутаза.*

Toxic hepatitis remains one of the pressing problems of modern hepatology and is accompanied by damage to liver cells under the influence of chemical compounds, drugs and biologically active substances. In its pathogenesis, the key role is played by oxidative stress processes, decreased activity of the antioxidant system and activation of inflammatory cascades. Despite the existence of various pharmacological agents, the search for safe and effective hepatoprotectors of natural origin is of particular importance, which makes this area of research a priority [3,6,8,11].

Safflower oil, which contains a significant amount of polyunsaturated fatty acids and biologically active substances, is considered a promising source of natural antioxidants and regulators of inflammatory processes. Previously published data indicate its ability to reduce the intensity of lipid peroxidation and normalize metabolic reactions in liver damage of various etiologies.

However, information on the optimal dosages and effectiveness of safflower oil in toxic hepatitis is limited, which necessitates further targeted research [1,4,7,12].

Studying the hepatoprotective and immunomodulatory potential of safflower oil in experimental toxic hepatitis will allow us to better understand the mechanisms of its effect on liver cells and determine the possibilities of its use in complex therapy. This is of particular importance given the growing integration of herbal remedies into clinical practice and the growing interest in agents that combine antioxidant and anti-inflammatory properties [2,5,9,10].

In continuation of the analysis aimed at identifying the features of the influence of vegetable oils on the functional state of the liver, it seems appropriate to consider the dynamics of biochemical and antioxidant indicators during the treatment of experimental toxic hepatitis in rats.

THE AIM OF THE STUDY

To study the effect of safflower oil on biochemical parameters of liver function and the activity of antioxidant enzymes in a model of acute toxic hepatitis in laboratory rats.

MATERIALS AND METHODS OF RESEARCH

The study included 30 healthy male white mongrel laboratory rats aged 2–3 months with a body weight of 203.1 ± 1.32 g. Before the experiment, the animals were quarantined for 10–14 days under standard vivarium conditions. The air temperature was maintained at +20.2 ± 5 °C with a relative humidity of 55 ± 10% and a light regime of 12:12. All animals had free access to water, standard feed and a specialized laboratory diet.

At the end of the adaptation period, the rats were divided into groups by simple randomization using a random number generator. Distribution into control and experimental groups was carried out on the basis of a pre-prepared protocol. To exclude systematic errors, the study was carried out under conditions of partial “blinding”: the specialist who analyzed the biochemical and antioxidant indicators did not know the animals’ belonging to the groups.

The animals were divided into four groups:

I – intact (healthy animals, n=10);

II – control group (OTG without treatment), (n=5);

III – experimental group (safflower oil 10 ml/kg, n=10);

The model of acute toxic hepatitis was created by intragastric administration of paracetamol solution at a dose of 1000 mg/kg once a day for two days. Starting from the third day, animals from the groups receiving protective treatment were daily administered the test oil (cold-pressed method, Botanic Herbs Company LLC) at a dose of 10 ml/kg using a dosing syringe. The control and model groups received an equivalent volume of physiological solution. The duration of safflower oil administration was 14 days.

The functional state of the liver was assessed on the 9th and 16th days of the experiment. The concentration of glucose and the activity of key enzymes were determined in the blood serum: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP). The determinations were performed using commercial highly sensitive test systems (Cypress Diagnostics BV, Belgium).

To characterize the antioxidant status, the content of malondialdehyde (MDA), as well as the activity of catalase and superoxide dismutase (SOD) in the blood serum were measured. The studies were carried out by the enzyme immunoassay method using reagent kits (Wuhan Fine Biotech Co., Ltd., China).

Morphological evaluation was performed on liver samples fixed in 10% neutral formalin for 24 hours. After fixation, the tissues were dehydrated in increasing concentrations of alcohol, treated with xylene, and embedded in paraffin. Sections stained with hematoxylin and eosin were prepared from paraffin blocks and examined under a Cilika BT-P microscope (MedPrime Technologies Pvt. Ltd., India) using a digital imaging system.

The study was approved by the Ethics Committee of the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan (protocol No. 2025-0001) and was carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 2010.

Statistical data processing was performed using the standard Microsoft Excel package and Student’s t-test. The arithmetic mean (M), standard deviation (m), standard error of the mean (m) and Student’s t-test (t) with determination of the significance level (p) were calculated. Differences between groups were considered statistically significant at $p<0.05$, in accordance with generally accepted recommendations for processing clinical and experimental data.

RESEARCH RESULTS

Analysis of dynamic changes will allow us to identify the hepatoprotective and antioxidant properties of the oil under study, as well as to clarify the mechanisms of their action under conditions of acute toxic liver damage.

Analysis of the dynamics of biochemical parameters showed that after 7 days of safflower oil application, the level of total protein exceeded the value in intact animals by 1.05 times (88.4 versus 84.6 g/dl), and by the 14th day the increase reached 1.41 times relative to intact animals (117.2 versus 83.2 g/dl), which indicates an increase in the synthetic function of the liver by the end of the experiment. The glucose concentration by the 7th day was 1.01 times higher than intact values (5.15 versus 5.12 mmol/l), and by the 14th day - 1.08 times (5.98 versus 5.55 mmol/l), which may reflect the normalization of carbohydrate metabolism with longer exposure.

The ALT values on the 7th day were 1.04 times higher than the level of intact rats (38.5 versus 36.9 U/l), and on the 14th day - 1.27 times relative to the intact

ones (38.9 versus 30.6 U/l). AST on the 7th day was 0.91 times lower than the values of intact ones (31.9 versus 35.0 U/l), whereas by the 14th day a reliable decrease to 0.90 times from the intact ones was observed (36.8 versus 41.1 U/l), which indicates stabilization of cytolytic processes.

Table 1

Comparative assessment of the dynamics of biochemical data of liver function in the model of toxic hepatitis in rats treated with safflower oil (M±m)

Safflower oil	7 days (n=5)	14 days (n=5)	Intact
Total protein, g/dl	88.4±6.31	117.2±2.76	84.6±1.24 (7 days)
			83.2±6.22 (14 days)
Glucose, mmol/l	5.15±0.22	5.98±0.18	5.12±0.21 (7 days)
			5.55±0.34 (14 days)
ALT, U/l	38.5±2.02	38.9±1.81	36.9±2.23 (7 days)
			30.6±2.62 (14 days)
AST, U/l	31.9±2.11	36.8±1.01	35.0±2.51 (7 days)
			41.1±3.61 (14 days)
Alkaline phosphatase, U/l	71.2±6.01	65.6±6.62	69.1±5.62 (7 days)
			55.3±5.64 (14 days)
GGT, U/l	0.57±0.02	0.72±0.03	0.65±0.09 (7 days)
			0.60±0.08 (14 days)

Alkaline phosphatase on the 7th day was practically no different from the intact ones (71.2 versus 69.1 U/l; 1.03 times), however, by the 14th day its level was 1.19 times higher than the intact values (65.6 versus 55.3 U/l; $p<0.05$), which can be considered as a sign of restoration of the membrane structures of hepatocytes. GGT indicators on the 7th day were 0.88 times lower than the intact ones (0.57 versus 0.65), and on the 14th day they exceeded them by 1.20 times (0.72 versus 0.60;, which may indicate activation of the liver enzyme systems with long-term intake of oil.

In general, the obtained data allow us to conclude that the main signs of a pronounced hepatoprotective and metabolic effect of safflower oil appear on the 14th day of treatment, which is accompanied by a significant improvement in protein and carbohydrate metabolism, stabilization of cytolytic enzymes and normalization of membrane-bound enzyme systems.

Table 2

Comparative assessment of the dynamics of antioxidant markers in a model of toxic hepatitis in rats treated with safflower oil (M±m)

Safflower oil	7 days	14 days	Intact
MDA mmol/ml	3.24±0.04	3.17±0.03	2.91±0.02 (7 days)
			2.91±0.02 (14 days)
Catalase μCat/mg protein	28.7±0.83	30.7±0.88	34.1±0.61 (7 days)
			34.1±0.61 (14 days)
Superoxide dismutase (units/mg protein)	1.23±0.01	1.26±0.01	1.32±0.01 (7 days)
			1.32±0.01 (14 days)

Based on the results of assessing antioxidant indices in rats with a toxic hepatitis model treated with safflower oil, it was found that after 7 days of treatment, the level of malondialdehyde (MDA) decreased from 3.24 ± 0.04 mmol/ml to 3.17 ± 0.03 mmol/ml by the 14th day, which is a decrease of 1.02 times, however, the indices remained higher than the level of intact animals (2.91 ± 0.02 mmol/ml) – 1.11 times on the 7th day and 1.09 times on the 14th day, which indicates a partial but incomplete restoration of lipid peroxidation.

Catalase activity during the treatment period demonstrated an increase from 28.7 ± 0.83 to 30.7 ± 0.88 μCat/mg protein, i.e. 1.07 times, however, both values were lower than those of the intact group (34.1 ± 0.61 μCat/mg protein) – 1.19 times on the 7th day and 1.11 times on the 14th day. This indicates a gradual restoration of the enzymatic link of antioxidant protection, but a full normalization effect was not achieved within 14 days.

The superoxide dismutase (SOD) values changed minimally – from 1.23 ± 0.01 to 1.26 ± 0.01 units/mg protein (1.02 times), while remaining below the control values (1.32 ± 0.01 units/mg protein) – 1.07 times on the 7th day and 1.05 times on the 14th day. This reflects a moderate increase in the antioxidant enzyme under the influence of safflower oil, but a slow recovery to the physiological norm.

Thus, the greatest positive changes in antioxidant markers were observed by the 14th day of treatment, but full compliance with the indicators of intact animals was not achieved, which indicates the need for a longer course of therapy for complete correction of oxidative stress.

CONCLUSIONS

The study found that toxic liver damage was accompanied by increased levels of ALT, AST, alkaline phosphatase, GGT, glucose and MDA, as well as a decrease in

total protein, catalase and superoxide dismutase activity. The use of vegetable oil led to a decrease in cytolytic and cholestatic syndromes, especially pronounced on the 14th day of therapy.

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