ISSN NO: 2770-2936
August 2025

Experimental Assessment Of The Effect Of Rutan On The Level Of Endogenous Intoxication Syndrome In Prepubertal An

Ziyaviddin Zainutdinovich Hakimov[1], Fatima Adilbaevna Khalmuratova[2], Alisher Khudayberdievich Rakhmanov[3]

[1] Doctor of Medical Sciences, Professor, Department of Pharmacology, Tashkent State Medical University

[2] Assistant, Department of Pharmacology and Pharmaceutical Technology, Karakalpak Medical Institute

[3] Doctor of Medical Sciences, Professor, Researcher, Biomedical Research Center, Tashkent State Medical University, Uzbekistan

Abstract.
An acute hepatitis model was induced in one-month-old rabbits using carbon tetrachloride, followed by experimental therapy with Karsil and Rutan to evaluate their effectiveness in eliminating endogenous intoxication syndrome. It was found that acute toxic hepatitis in prepubertal rabbits is accompanied by a high degree of endotoxemia, which manifests as an increase in the sorption capacity of erythrocytes, the concentration of middle molecular weight molecules in the blood, and a reduction in the lifespan of paramecia. Experimental treatment of acute hepatitis with Karsil, and especially with Rutan, led to normalization of the parameters of endogenous intoxication syndrome to levels comparable to those of healthy animals. Rutan may be recommended as a therapeutic agent for the treatment of conditions accompanied by endotoxemia in pediatric practice, following appropriate clinical trials.

Keywords: prepubertal period, acute toxic hepatitis, endogenous intoxication syndrome, middle molecular weight molecules, paramecia lifespan, experimental therapy, Karsil, Rutan.

Conflict of Interest.
The authors declare no conflict of interest related to the publication of this article.

Introduction

The investigation and interpretation of the pathogenetic aspects of endogenous intoxication syndrome (EIS) as a universal symptom complex is an important direction in medical research, since it not only reflects the severity of disease but also serves as a criterion for predicting its outcome. Among the primary biological barriers for most xenobiotics entering the human body is the liver. EIS is associated not only with hepatobiliary disorders but also with many extrahepatic conditions (burn disease, peritonitis, chronic renal failure, infectious diseases, etc.) [1–5]. Despite a significant body of research, key elements in the pathogenesis of EIS require further study, particularly with the implementation of specific syndrome markers in pediatric practice, where liver pathologies of various etiologies have been increasing over the past two decades [6,7]. Restoration of hepatocellular detoxification capacity is considered an effective non-invasive strategy for managing EIS. In this context, hepatoprotectors with antioxidant properties—due to their polyphenolic compounds—are proposed for therapeutic use [8,9]. Previously, we demonstrated that Rutan enhances the biotransformation of xenobiotics during therapeutic application [10]. However, the efficacy of this compound in acute toxic hepatitis (ATH) in growing animals, particularly in relation to EIS parameters, has not yet been studied. Solving this issue could improve the effectiveness of pharmacotherapy for both hepatic and extrahepatic diseases associated with endotoxemia.

Objective

To investigate the effect of Rutan on the parameters of endogenous intoxication syndrome in acute toxic hepatitis in prepubertal animals.

Materials And Methods

ISSN NO: 2770-2936
August 2025

The study was conducted on one-month-old male and female Chinchilla breed rabbits, born and raised under vivarium conditions. The animals were kept in standard laboratory conditions with free access to food and water, a natural light-dark cycle, and room temperature maintained at 20–24°C. All procedures involving animals were carried out in accordance with the "Rules for Working with Experimental Animals" and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123, Strasbourg, March 18, 1986). The experimental protocol was approved by the Ethics Committee of the Tashkent Medical Academy under the Ministry of Health of the Republic of Uzbekistan (Protocol No. 9, dated May 26, 2025).

Acute toxic hepatitis (ATH) was induced in the rabbits by intragastric administration of carbon tetrachloride (CCl_4) at a dose of 0.25 mL/100 g once daily for four consecutive days [11]. Each experimental group included six animals. One day after the last dose of CCl_4, the animals were divided into four groups. The first and second groups received Rutan at doses of 25 mg/kg and 50 mg/kg, respectively; the third group received Karsil at a dose of 40 mg/kg. The fourth group served as a control (untreated) and was administered an equivalent volume of distilled water. All treatments were administered once daily for six days. The study aimed to evaluate the effects of these preparations on the severity of endogenous intoxication syndrome (EIS) in rabbits with ATH.

The degree of endogenous intoxication was assessed using several parameters:

Erythrocyte Sorption Capacity (ESC) was measured according to the method described in [12]. The principle of this method is based on the increased ability of erythrocytes to absorb vital dyes such as methylene blue under the influence of toxins. The optical density of the dye solution was measured before and after erythrocyte sedimentation using a spectrophotometer at a wavelength of 630 nm. The percentage of absorbed dye was calculated using the formula:

$$A(\%)=C\times100100-BA\ (\%) = \frac{C \times 100}{100 - B}A(\%)=100−BC×100$$

where A – percentage of dye absorbed, B – optical density of the initial dye solution (in extinction units), and C – optical density of the dye solution after incubation with erythrocytes (in extinction units).

Determination of Medium Molecular Weight Molecules (MMWM) was performed as described in [13]. Coarse proteins were precipitated by adding 10% trichloroacetic acid, and the eluate was analyzed spectrophotometrically at a wavelength of 254 nm. The MMWM level was expressed in arbitrary units corresponding to the extinction values obtained.

Serum Toxicity was assessed based on the **Paramecia Lifespan (PL)** test [14]. In this method, 0.01 mL of blood serum was mixed with 0.01 mL of a suspension containing 8 to 10 paramecia in an erythrocyte melangeur. The time taken for half and then all of the paramecia to die was recorded in seconds and used as an indicator of serum toxicity.

The obtained data were statistically processed using the **Statistica for Windows** software package. Standard methods of variational statistics were applied, and significance was evaluated using the Student's *t*-test. The results are presented as mean ± standard error (M±m), and differences were considered statistically significant at a confidence level of 95% ($p < 0.05$).

Research Results And Discussion

The excessive accumulation of intermediate and end products of impaired metabolism due to destructive processes is a primary cause of the development of intoxication syndrome. This is further exacerbated by the dysfunction of physiological systems responsible for the inactivation and elimination of both natural metabolites and toxic xenobiotics. Typically, the failure of adaptive-compensatory mechanisms during any pathological process—especially when detoxifying and excretory organs are compromised—also leads to the development of endogenous intoxication syndrome (EIS). In modern clinical practice, the most common indicators for evaluating EIS include erythrocyte sorption capacity (ESC), medium molecular weight molecules (MMWM), and paramecia lifespan (PL).

The involvement of erythrocytes in maintaining chemical homeostasis provides a rational basis for using screening methods to assess erythrocyte membrane properties under endotoxicosis conditions. The implementation of the ESC method in practical medicine has enabled better prediction of disease progression

ISSN NO: 2770-2936
August 2025

and outcomes, as well as the selection of rational pharmacotherapy schemes. In our study, using this test on growing rabbits with acute toxic hepatitis (ATH), it was found that carbon tetrachloride significantly increased ESC by 59.5% ($p < 0.05$). However, in animals treated with Rutan and Karsil, ESC was significantly reduced by 20.0%–30.9%, with the most pronounced effect observed with Rutan at a dose of 25 mg/kg (see Table 1). Notably, the ESC values in these animals were not statistically different from those of healthy rabbits.

Thus, carbon tetrachloride-induced ATH in one-month-old rabbits leads to a marked increase in ESC, which is effectively reduced to near-normal levels by treatment with Rutan or the hepatoprotector Karsil. Importantly, in terms of pharmacological activity, Rutan—especially at a dose of 25 mg/kg—clearly outperforms Karsil.

It is well known that in addition to their primary function of gas transport, red blood cells are capable of participating in the regulation of acid-base balance, water-electrolyte homeostasis, micro-rheological blood properties, immune responses, and the binding and transport of infectious agents and pharmaceutical compounds. Accumulating evidence suggests that erythrocytes are involved in pathological processes not only in hematologic disorders but also undergo significant structural and functional changes in diseases of various origins [15].

Table 1

Comparative Study of the Effects of Rutan and Karsil on the Endogenous Intoxication Syndrome in Prepubertal Rabbits with Acute Toxic Hepatitis

Groups	Dose, mg/kg	SSE, in %	MMWM, conventional units	Permanent residence, in seconds
Intak	-	44,76±2,89	0,271±0,033	229,83±7,19
OTG	-	71,41±5,68*	0,595±0,058*	144,33±6,28*,#
OTG+Rutan	25	49,33±4,05#	0,305±0,025#	196,81±10,11*,#
OTG+Rutan	50	51,71±6,27#	0,341±0,031#	188,75±10,03*,#
OTG+Karsil	40	57,15±3,88#	0,366±0,039#	179,67±13,02*,#

Note: * – P <0.05 compared to the intact group,
– P <0.05 compared to the control group.

There is evidence supporting the existence of typical erythrocyte membrane abnormalities in various pathological processes and conditions. Oxidative stress in toxic hepatitis is known to cause significant disruptions in the lipid composition of erythrocyte membranes, increased viscosity of the lipid bilayer, disturbances in intermolecular protein–lipid and lipid–lipid interactions, as well as disorganization of the surface architecture of erythrocytes. The severity of molecular damage to erythrocyte membranes is most pronounced in severe pathological conditions accompanied by complications [16].

It is important to note that to date, the adsorptive and transport function of erythrocytes has been shown to play a critical role in the following processes:

It facilitates rapid and selective entry of endogenous and exogenous xenobiotics into the exchange layer of blood capillaries, where mechanical replacement of substances from the parietal exchange layer by molecules adsorbed on erythrocytes occurs;

Erythrocytes participate in the removal of metabolites, denatured proteins, and other chemically active substances from the blood, enabling their rapid delivery to the liver — the primary detoxification organ;

The adsorptive–transport function helps stabilize the concentration of plasma substances through a dynamic “adsorption–desorption” mechanism.

First, the selective delivery of many substances to tissues, including exogenous compounds, is largely determined by their varying ability to adsorb on the surface of erythrocytes. Based on this property, substances are conventionally classified as weakly, moderately, or strongly adsorbed. The percentage of strongly adsorbed substances increases with proximity to the erythrocyte membrane.

Second, erythrocytes aid in the removal of metabolites, denatured proteins, atherogenic lipids, and other chemically active compounds. As proteins age and undergo denaturation, their adsorptive capacity increases. Atherogenic lipids are significantly more adsorptive than non-atherogenic ones. Proteins and lipids with high

ISSN NO: 2770-2936
August 2025

adsorption affinity can partially displace glucose from the erythrocyte surface. Conversely, glucose can displace native proteins and non-atherogenic lipids from the erythrocyte-bound pool. These features promote the rapid delivery of "elimination-targeted" substances to the liver.

Third, this function provides an additional anti-edematous mechanism. A portion of adsorbed glucose is consistently delivered to tissues. The vacated binding sites on erythrocytes are mainly occupied by proteins, which leads to a reduction in protein concentration in venous plasma and in the parietal layer of venous capillaries. This shifts the transcapillary concentration gradient and promotes the return of proteins from the interstitial space to the blood through the highly permeable walls of the microcirculatory bed. This mechanism is enhanced by the increased volume and adsorption surface area of erythrocytes saturated with carbon dioxide. This effect is absent in the lungs, where the risk of acute edema is greater. This mechanism is consistent with known data showing a higher incidence of edema in diabetes mellitus, hypoproteinemia, anemia, and other pathologies.

Finally, the adsorptive–transport function of erythrocytes stabilizes the concentration of substances in plasma by means of reversible adsorption–desorption. The plasma pool and erythrocyte-bound pool of substances are closely linked. Not only in control groups but also in diseased individuals and animal experiments under various conditions, a reliable positive correlation is usually observed between plasma levels of specific substances and their concentration on erythrocyte surfaces. Dilution or concentration of the blood affects the ratio between these pools minimally.

From this perspective, it becomes clear that hepatoprotectors, including Rutan, likely suppress the intensity of free radical lipid peroxidation in erythrocyte membranes, thereby eliminating destructive changes in red blood cells and enabling the restoration of their sorptive capacity. This hypothesis is supported by data indicating that, under the influence of pharmacological agents, erythrocyte sorption capacity is restored to levels observed in healthy animals. This effect is largely attributable to enhanced biotransformation of xenobiotics in hepatocytes under the influence of the studied agents in juvenile animals with acute toxic hepatitis (ATH) [10].

The causes of endogenous intoxication syndrome (EIS) are conventionally divided into two groups. The first includes destructive processes leading to the accumulation of excessive amounts of intermediate and end products of aberrant metabolism, which exert toxic effects on vital systems. The second group comprises functional impairments of physiological systems responsible for the binding, inactivation, and elimination of both natural metabolites and toxic products. Primary damage to these systems or failure of their adaptive-compensatory mechanisms during any pathological process can also lead to the development of EIS [17]. This syndrome is regarded as polyetiological and multipathogenetic, characterized by the accumulation of endogenous toxic substances in tissues and biological fluids — an excess of products of normal or aberrant metabolism or cellular responses.

Three major biochemical mechanisms of endotoxicosis development are identified:

Activation of tissue proteolysis;
Activation of free radical oxidation processes;
Effects of bacterial toxins.

The activation of proteolysis — hydrolytic degradation of proteins by tissue proteases (cathepsins) — is one of the most common molecular mechanisms of tissue damage under pathological conditions. Middle-mass molecules (MMM, 500–5000 Da) are primarily peptide-based substances formed from proteolytic breakdown of proteins, contributing to intoxication. The toxic effect of MMM is due to the combined influence of all constituent compounds through potentiation and synergistic effects.

Biochemical analyses revealed that the concentration of MMM in the serum of animals in the control group was 2.2 times higher than in healthy animals. This finding confirms both the development of EIS and increased proteolysis, resulting in the accumulation of abnormal protein metabolism products. The likely mechanism involves intensified lipid peroxidation of lysosomal membranes, leading to the release of hydrolytic enzymes and the buildup of protein degradation products due to proteolytic activity. This hypothesis is supported by results from animals treated with Rutan and Karsil, which possess antioxidant properties [18,19]. Rutan treatment reduced MMM levels by nearly half compared to untreated animals and showed no statistically significant difference from healthy controls. Increasing the Rutan dose twofold did not enhance this effect.

ISSN NO: 2770-2936
August 2025

The well-known hepatoprotector Karsil also reduced MMM levels, although to a lesser extent (by 38.5%) than Rutan.

The development of EIS is generally accompanied by the accumulation of toxic metabolic products in plasma, negatively affecting cell longevity. This is the basis for using *Paramecium* vitality (PJV) as an indicator of EIS severity. Studies showed that when serum from healthy animals was added to the culture medium, the PJV was 229.83 ± 7.19 seconds, whereas serum from untreated animals reduced it by 37.0%, indicating the presence of substances in the serum that are lethal to *Paramecium*. These results confirm the validity of conclusions based on CCC and MMM data, indicating that ATH in juvenile animals is associated with severe EIS.

In contrast, the serum of animals treated with Rutan and Karsil increased PJV compared to untreated animals by 34.6%, 30.8%, and 24.5% respectively for Rutan at doses of 25 mg/kg and 50 mg/kg, and for Karsil at 40 mg/kg. These findings suggest that these pharmacological agents mitigate the biochemical processes responsible for cellular and organelle membrane destruction, ultimately reducing the formation of toxic metabolic byproducts. It is also possible that enhanced xenobiotic biotransformation contributes to this effect [10].

Conclusion:

1. Acute toxic hepatitis during the growth period is accompanied by the development of severe endogenous intoxication syndrome, as evidenced by increased erythrocyte sorption capacity and middle-mass molecules, as well as reduced *Paramecium* lifespan.
2. Experimental treatment with Karsil and especially Rutan led to a clear reduction in endotoxemia parameters to levels observed in healthy animals.
3. Rutan may be recommended as a therapeutic agent for treating pathologies associated with endogenous intoxication syndrome in pediatric practice, following appropriate clinical trials.

References

1. Bogatov V.V., Burova N.M. Integral scheme for assessing endotoxicosis syndrome in patients with maxillofacial phlegmons. Stomatology. 2013; 2:33-35.
2. Akalaev R.N., Stopnitsky A.A., Ariphodzhaeva G.Z., Saidova M.K. Toxic liver injury in acute poisoning and endogenous intoxication (Literature review). Bulletin of Emergency Surgery. 2020; 13(6):95-102.
3. Pshenichnaya E.V., Astafyeva E.V. Significance of middle-mass molecules level in blood plasma as an indicator of endogenous intoxication for predicting glomerulonephritis in children with hemorrhagic vasculitis. Mother and Child in Kuzbass. 2024; 1(96):66-72.
4. Zolotukhina V.A., Bezruk E.L. Characteristics of endotoxemia in different treatments of experimental burns in rats. International Journal of Veterinary Medicine. 2022;(4):440-444. (In Russian) https://doi.org/10.52419/issn2072-2419.2022.4.440; https://doi.org/10.17816/psaic1570
5. Khakimov Z.Z., Dzhanaev G.Yu., Mustanov T.B., Mamadjanova M.A. Role of hepatocyte monooxygenase system in liver dysfunction development after thermal burns. Kazakh Journal of Medicine and Pharmacy. 2024; 6:33-41.
6. Goriacheva L.G., Ventslovayte N.D., Greshnyakova V.A. Viral hepatitis in children: current state and prospects. Children's Infections (Detskie Infektsii). 2021; 20(4):35-41. doi.org/10.22627/2072-8107-2021-20-4-35-41.
7. Chen F., Huang Y., Huang Z., Fang F., Zhou H., Shu S. Epidemiological characteristics of liver disease in hospitalized children: a 10-year single-center retrospective study. Frontiers in Pediatrics. 2024; 12:1344714. doi:10.3389/fped.2024.1344714.
8. Musaeva E.M., Guseynova G.A., Polukhova Sh.M. et al. Possibilities of pharmacological correction of hepatobiliary system pathologies. Bulletin of the Russian Military Medical Academy. 2018; 2(62):221-225.
9. Dronova A.V., Dyakova N.A. Yacon (Smallanthus sonchifolius): applications and prospects (review). Chemistry of Plant Raw Materials. 2023; 1:35-60.

ISSN NO: 2770-2936
August 2025

10. Khakimov Z., Rakhmanov A., Khalmuratova F. Effectiveness of Rutan in correcting xenobiotic biotransformation disorders in acute toxic hepatitis in prepubertal rats. International Bulletin of Medical Sciences and Clinical Research. 2025; 5(5):63–69. Zenodo. https://doi.org/10.5281/zenodo.15488966.
11. Rakhmanov A.Kh., Khakimov K.Z. Influence of hepatoprotectors on the intensity of lipid peroxidation processes in acute hepatitis during prepubertal period. Pharmaceutical Bulletin of Uzbekistan. 2013; 1:46-50.
12. Togayboev A.A., Kurkuzkin A.V., Rikin I.V., Karibzhanova R.M. Method for diagnosing endogenous intoxication. Laboratory Business. 1988; 9:22-24.
13. Gabrielyan N.N., Levitsky E.R., Shcherbanova O.I. Middle molecules hypothesis in clinical nephrology practice. Therapeutic Archive. 1983; 6:76-78.
14. Pafomov G.A., Burdiche F.A., Shirinova M.I. Rapid method for determining toxic properties of blood and lymph using Paramecium in exo- and endotoxicology. Soviet Medicine. 1980; 1:42-44.
15. Novitsky V.V., Urazova O.I. Pathophysiology. Textbook in 2 volumes. Vol. 2. 5th ed., revised and supplemented. GEOTAR-Media Publishing; 2025. 592 p.
16. Novitsky V.V., Ryazantseva N.V., Stepovaya E.A. et al. Molecular erythrocyte membrane disorders in pathologies of various origins are typical organism reactions: problem outline. Bulletin of Siberian Medicine. 2006; 5(2):62-69. https://doi.org/10.20538/1682-0363-2006-2-62-69.
17. Kuznetsov P.L., Borzunov V.M. Endogenous intoxication syndrome in the pathogenesis of viral hepatitis. Experimental and Clinical Gastroenterology. 2013; 4:44-50.
18. Aripov T.F., Ghaibov U.G., Ghaibova S.N. et al. Antioxidant and antiradical activity in vitro of the sum of polyphenols (active substance of antiviral drug Rutan) from leaves of sumac (Rhus coriaria L.). Chemistry of Plant Raw Materials. 2024; 4:138–147. DOI: 10.14258/jcprm.20240413433.
19. Azonov D.A., Kholov A.K., Ganiev Kh.A., Azonov I.D. Antitoxic and antioxidant properties of Zeravshan juniper essential oil in toxic hepatitis. Science and World. 2020; 2(8):59-63.