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INVESTIGATION OF THE HEPATOPROTECTIVE EFFECT OF ANACARDIUM IN CARBON TETRACHLORIDE- AND ETHANOL–INDUCED HEPATITIS MODELS

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Abstract

Toxic liver injury is among the most common etiological factors of hepatobiliary pathology and is strongly associated with the increasing impact of hepatotoxic xenobiotics from household, industrial, and agricultural chemicals. These exogenous agents not only possess specific mechanisms of action but also trigger universal endogenous damage in hepatocytes through oxidative stress. In this context, polyphenolic compounds with antioxidant properties are considered promising hepatoprotective agents. The present study investigates the hepatoprotective potential of anacardic polyphenol in experimental models of carbon tetrachloride- and ethanol-induced toxic liver injury in Wistar rats. Different doses of the compound were administered orally and intraperitoneally, and its influence on biochemical markers such as total protein, ALT activity, lipid peroxidation products (diene conjugates and MDA), as well as functional indicators (hexobarbital-induced sleep duration) were evaluated. The findings demonstrated that anacardic polyphenol significantly reduced biochemical alterations, restored hepatocellular integrity, and exhibited dose-dependent hepatoprotective activity, with the most effective results observed at 100 mg/kg orally and 5 mg/kg intraperitoneally. These results suggest that anacardic polyphenol may serve as a promising hepatoprotective agent, potentially comparable to established drugs such as Karsil.

Keywords: *toxic liver injury, hepatoprotection, anacardic polyphenol, oxidative stress, ALT, MDA, carbon tetrachloride, ethanol*

Introduction

Toxic liver injury is one of the most common etiological factors of hepatobiliary pathology. It is associated with the increasing exposure to hepatotoxic xenobiotics, including household, industrial, and agricultural

chemicals. These exogenous factors not only have their own specific mechanisms of action, but also trigger a universal and powerful pathway of endogenous hepatocyte damage in the form of oxidative stress (Li, S. et al., 2015). Its products – highly reactive and abundant oxygen metabolites – are responsible for the development of metabolic disorders, membranopathies, functional impairments, mutations, accelerated apoptosis, and other forms of cellular pathology.

At the same time, the multifactorial nature of toxic liver injury requires therapeutic agents with multilevel protective mechanisms, a property currently characteristic of only certain hepatoprotectors (e.g., Legalon, Sylibor, Hepaton). A distinctive feature of modern hepatoprotectors is that they are derived from plant raw materials and contain polyphenolic compounds with antioxidant activity – flavonoids, flavolignans, cinnamic acids, and other bioactive components (Saha, P., et al., 2019).

At present, the share of effective domestic hepatoprotectors on the global pharmaceutical market is relatively small, and they constitute only a minor proportion compared to similar foreign drugs. Therefore, there is an urgent need to identify new agents capable of enhancing liver resistance to toxic injury. Preference should be given to plant-based preparations, which are generally characterized by low toxicity combined with sufficient efficacy and a broad spectrum of therapeutic action (Ma, S., et al., 2025).

Material and Methods

The studies were conducted on 240 mature white Wistar rats of both sexes, weighing 170–280 g, in accordance with international recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Acute liver injury induced by carbon tetrachloride (CCl_4) was modeled by oral administration of a 50% CCl_4 solution using a metal atraumatic probe. Acute alcoholic liver injury was induced by intraperitoneal administration of a 33% ethanol solution. The studied polyphenolic compound, anacardic acid, was administered to model animals both orally and intraperitoneally (Chiu, Y. J., et al., 2017).

The tested anacardic polyphenol was administered orally at doses of 100, 300, and 500 mg/kg twice daily for 12 days via gastric intubation (tube feeding), and intraperitoneally at doses of 5, 15, and 25 mg/kg twice daily for 12 days. It should be noted that the tested polyphenol was administered 5 days prior to the introduction of the hepatotoxin solution (carbon tetrachloride or ethanol), and then concomitantly for 7 days, i.e., 1 hour before CCl_4 or ethanol administration (Zhang, X., et al., 2022).

Determination of the acute toxicity of the studied drugs was carried out in accordance with methodological guidelines for studying the general toxic effects of pharmacological agents. The functional activity of the liver was evaluated by the duration of sleep in animals, which reflects the state of the microsomal system responsible for the metabolism of xenobiotics, particularly sodium ethaminal. The experiment was conducted according to the method of V. V. Gatsura. After 14 days of administration of the studied preparation to animals with carbon tetrachloride- and ethanol-induced models, sodium ethaminal was injected intraperitoneally at a dose of 40 mg/kg. The sleep duration (in the lateral position) was recorded in minutes. Statistical results were processed and expressed as the arithmetic mean (M) and its standard error (m).

Sodium ethaminal and the studied anacardium polyphenol were administered orally at different doses (100, 300, and 500 mg/kg) and intraperitoneally at different doses (5, 15, 125 mg/kg) under conditions of acute toxic liver injury induced by carbon tetrachloride and ethanol. On the 14th day of substance administration, sodium ethaminal was injected intraperitoneally at a dose of 40 mg/kg. Animals that received the same volume of purified water were used as the control group. According to the obtained results, compared to the control group, administration of anacardin led to a significant and reliable reduction in sleep duration, especially at a dose of 100 mg/kg, which accounted for 55.7%.

The reduction of ethaminal sleep duration during administration of the studied preparations most likely indicates the presence of a hepatoprotective effect, which contributes to maintaining the activity of the liver microsomal system. This system, through

cytochrome P450 participation, provides biotransformation reactions catalyzed by endoplasmic reticulum enzymes. Our research was devoted to determining the optimal therapeutic dose of the studied preparation for normalizing altered biochemical parameters in hepatocytes under conditions of carbon tetrachloride- and ethanol-induced liver damage. To achieve this goal, the effect of the preparation was evaluated based on its ability to normalize all biochemical parameters, including the reduced total protein level observed during cytolysis and the increased activity of the ALT enzyme.

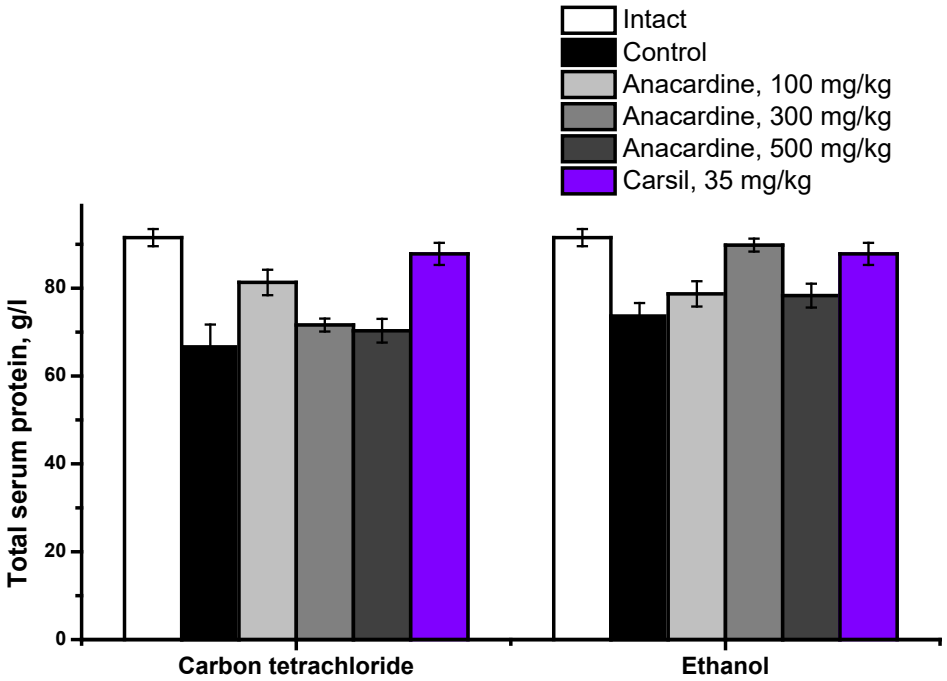
Results

In our experiments, the composition of diene conjugates was initially determined. The content of diene conjugates was expressed in μmol/L. Carbon tetrachloride intoxication disrupted all liver functions: protein-synthetic activity (the total protein level decreased by 27.2%), and severe hepatocellular damage was observed, manifested by an increase in the serum concentration of diene conjugates and TBARS products (MDA), by 41.2% and 175.1%, respectively, compared with the group of intact animals.

When anacardine was administered orally at a dose of 100 mg/kg, the levels of serum lipid peroxidation (LPO) primary products (diene conjugates) and LPO end-products (TBARS-active MDA), as well as total protein, remained significantly elevated compared to intact controls-109.9%, 145.2%, and 88.5% of the control values, respectively.

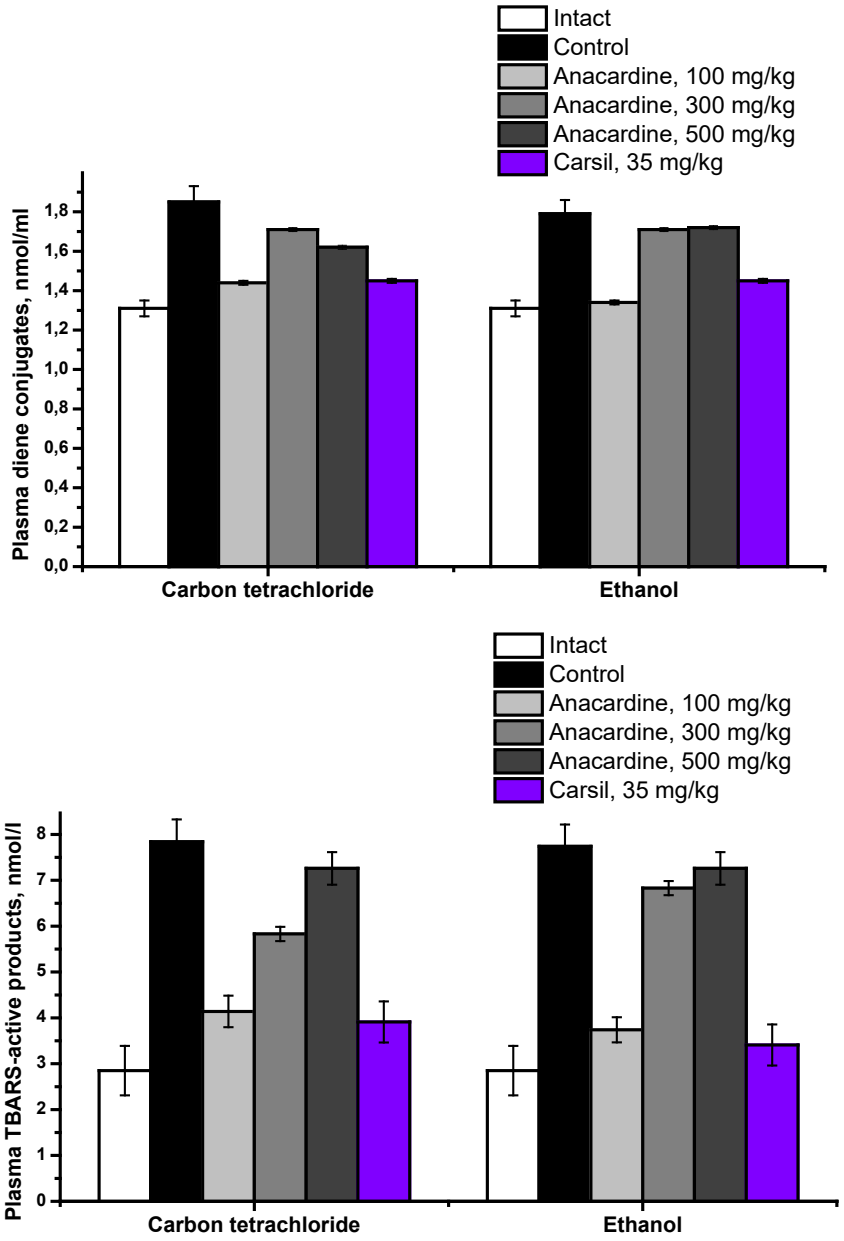
At a dose of 300 mg/kg, anacardine administration, compared to the 100 mg/kg dose, resulted in a significant decrease in serum diene conjugates, TBARS-active products, and total protein levels – by 10.2%, while diene conjugates and TBARS-active products increased by 20.6% relative to controls (Figure 1).

Figure 1. *Changes in the serum total protein level under oral administration of different doses of anacardine in liver toxicity induced by carbon tetrachloride and ethanol*



According to the results obtained in ethanol-induced liver pathology, when the dose was increased to 300 mg/kg, serum total protein concentration decreased by 22% compared with the control, while the levels of diene conjugates and TBARS-active products significantly decreased to 102.29% and 131.22%, respectively. However, these values were still lower than those observed with the 100 mg/kg dose. Subsequent escalation of the anacardine dose to 500 mg/kg did not exert any additional beneficial effect on these parameters and did not lead to significant changes (Figure 2).

Figure 2. *Changes in the levels of diene conjugates and TBARS-reactive substances in blood serum under oral administration of different doses of anacardine in liver toxicity induced by carbon tetrachloride and ethanol*



At the next stage of the study, the doses of intraperitoneal administration of anacardine were determined in order to confirm its efficacy at 5 mg/kg. The effects of different doses (5, 15, and 25 mg/kg) on the dynamics of changes in biochemical markers in models of toxic liver injury induced by carbon tetrachloride and ethanol were investigated. According to the results, administration of the studied polyphenol anacardine by two different routes in the selected pathologies did not show significant differences and demonstrated almost identical outcomes.

The presented data indicate that carbon tetrachloride intoxication impaired all liver functions: protein-synthetic function (total protein decreased by 26.2%), and deep hepatocellular damage was evidenced by an increase in serum diene conjugates and TBA-reactive products (MDA) by 41.2% and 175.1%, respectively, compared to the intact group.

In carbon tetrachloride-induced liver injury, oral administration of anacardine at a dose of 5 mg/kg resulted in serum levels of primary LPO products (diene conjugates), terminal LPO products (TBA-reactive MDA),

and total protein remaining significantly higher than in the intact group-109.9%, 145.2%, and 88.5%, respectively. At a dose of 15 mg/kg, compared to 5 mg/kg, significant decreases were observed relative to the control group: serum diene conjugates, TBA-reactive products, and total protein levels decreased by 10.2%, while diene conjugates and TBA-reactive products increased by 20.6%.

Our subsequent experiments focused on investigating certain biochemical markers, considered indicative in ethanol-induced liver pathology, under the influence of the studied polyphenol anacardine. When the dose was increased to 15 mg/kg, the total serum protein level decreased significantly by 22% compared to the control, while diene conjugates and TBA-reactive products decreased by 102.29% and 131.22%, respectively. However, these parameters remained lower than those observed at a dose of 5 mg/kg. Further increasing the dose of anacardine to 25 mg/kg did not produce positive effects on the above parameters and did not lead to significant changes (Akhmadova, M., et al., 2025).

Discussion

The overall indicators obtained, including integral survival indices, detailed biochemical analysis of serum and liver, and data calculated according to the hepatoprotection coefficient, make it possible to propose a dose of 5 mg/kg as an effective therapeutic dose for intraperitoneal administration of anacardine (Madrigal-Santillán, E., et al., 2014).

In models of carbon tetrachloride- and ethanol-induced hepatitis, comparison of the hepatoprotective effect of anacardine with Karsil showed that, relative to the control, serum diene conjugates decreased by 49%, TBA-reactive products were corrected by 36.4% in serum and 41.7% in liver. Furthermore, the increase in ALT activity, as a marker of cytolysis, and the decrease in total protein content were normalized under the influence of the applied preparation.

References

Akhmadova, M., Yunusova, M. and Mukhamedova, M. (2025). Investigation of the hepatoprotective effect of anacardium in an ethanol-induced hepatitis model. *International multidisciplinary journal for research & development*. – 1, 5 (Jul. 2025). – P. 319–322.

Chiu, Y. J., Chiu, C. S., Lin, C. H., Chen, C. J., & Wu, Y. C. (2017). Hepatoprotective effect of *Polygonum orientale* extract against CCl_4-induced liver injury. *Evidence-Based Complementary and Alternative Medicine*, 2017. – P. 1–10. URL: https://doi.org/10.1155/2017/9340123

Li, S., Tan, H. Y., Wang, N., Zhang, Z. J., Lao, L., Wong, C. W., & Feng, Y. (2015). The role of oxidative stress and antioxidants in liver diseases. *International Journal of Molecular Sciences*, – 16(11). – P. 26087–26124. URL: https://doi.org/10.3390/ijms161125942

Ma, S., Liu, Y., Zhang, R., & Chen, H. (2025). Hepatoprotective effects of *Citri reticulatae Pericarpium* and *Chaenomeles speciosa* extracts in alcohol-related liver injury. *Molecules*, – 30(2). – 356 p. URL: https://doi.org/10.3390/molecules30020356

Madrigal-Santillán, E., Madrigal-Bujaidar, E., Álvarez-González, I., Sumaya-Martínez, M. T., Gutiérrez-Salinas, J., Bautista, M., Morales-González, Á., García-Luna y González-Rubio, M., Aguilar-Faisal, J. L., & Morales-González, J. A. (2014). Review of natural products with hepatoprotective effects. *World Journal of Gastroenterology*, – 20(40). – P. 14787–14804. URL: https://doi.org/10.3748/wjg.v20.i40.14787

Saha, P., Das, S., & Biswas, A. (2019). Role of natural phenolics in hepatoprotection. *Frontiers in Pharmacology*, – 10. – 509 p. URL: https://doi.org/10.3389/fphar.2019.00509

Zhang, X., Wang, Y., Li, J., & Chen, L. (2022). Hepatoprotective activity of *Ganoderma tsugae* ethanol extract against CCl_4-induced acute liver injury. *International Journal of Molecular Sciences*, – 23(19). – 11628 p. URL: https://doi.org/10.3390/ijms231911628

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