MICROANATOMICAL CHANGES IN THE WALLS OF THE RIGHT CHAMBERS OF THE HEART IN 12-MONTH-OLD RATS POISONED WITH ALCOHOL AND THE EFFECT OF OLIVE OIL ON THESE CHANGES

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Abstract. *According to the World Health Organization report, in 2012, excessive alcohol consumption led to 3.3 million deaths. This figure accounts for 5.9% of all global causes of death (WHO, 2014). Numerous studies indicate that chronic alcohol consumption can cause cardiovascular diseases, including conditions such as hypertension, arrhythmia, cardiomyopathy, and heart failure. The effect of ethanol on the heart muscle occurs through direct and indirect mechanisms, including electrolyte imbalance, oxidative stress, and metabolic changes. Furthermore, heavy alcohol consumption can alter the functional characteristics of the heart, negatively impacting cardiac rhythm and contractility. Sudden excessive intake of alcohol can lead to arrhythmias, including atrial fibrillation and ventricular arrhythmias. At the same time, the long-term effects of alcohol can lead to the development of cardiomyopathy and cause dystrophy of the heart muscles.*

Keywords: *heart, rat, alcohol, olive oil.*

Introduction.

According to the World Health Organization, 3 million people die annually due to harmful alcohol use, accounting for 5.3% of all global deaths (WHO, 2020). Statistical data from 2017 indicates that approximately half of the world's adult population consumes alcohol, suggesting that 2.6 billion people are at risk of alcohol-related health issues [3]. Research suggests that low-level alcohol consumption may influence fatty acid levels in the blood, potentially affecting the development of atherosclerosis [9]. Studies also show that apoptosis (the death of cardiomyocytes) plays a significant role in the induction of alcoholic cardiomyopathy [1]. Various researchers have established models of chronic alcoholism in laboratory settings to observe the development of dependence over time [3]. Both acute and chronic alcohol exposure can decrease the conductivity and absolute refractory period of atypical cardiac cells, increasing the risk of atrial fibrillation [9]. Furthermore, ethanol increases the levels of saturated fatty acids, cholesterol, and apolipoprotein A in the blood [3]. While some findings suggest that small amounts of alcohol might offer a cardioprotective effect, the overall impact of ethanol on the cardiovascular system is considered negative. Excessive consumption is linked to arterial hypertension, alcoholic cardiomyopathy, and stroke [9].

Currently, numerous experiments are being conducted on medicinal plants containing antioxidants for disease treatment. This is because antioxidants possess a broad spectrum of biological activity, binding free radicals in the body and preventing lipid peroxidation. Pathological conditions associated with the formation of free radicals frequently lead to cardiovascular system diseases [4].

Thus, opinions regarding the effect of alcohol on the heart remain diverse and contradictory. Therefore, studying the impact of ethanol on the heart and developing new methods for treating damaged cells is a matter of significant relevance.

Material and methods of research.

In the course of this scientific study, experiments were conducted on 12-month-old rats. At the beginning of the experiment, all sexually mature rats were quarantined for one week; after confirming the absence of somatic or infectious diseases, they were transferred to a standard vivarium regimen.

Throughout the experiment, the behavior and physiological state of the animals in both the control and experimental groups were monitored.

The rats were divided into three groups (n = 43): Group I - control (n = 14); Group II (n = 15) - rats that received 40% ethyl alcohol at a dose of 7.0 g/kg body weight for 30 days; Group III (n = 14) - alcohol-poisoned rats that received 2 ml/kg of olive oil for 30 days.

To model and simulate ethanol poisoning, 40% ethyl alcohol was administered to the rats at a dose of 7 g/kg (Sidorova P.I., 2002) via an intragastric metal stomach tube for 30 days. Rats in the control group were administered 0.5 ml of distilled water via an intragastric metal stomach tube for the same 30-day period.

Detoxification of ethanol-poisoned rats was performed by administering olive oil at a dose of 2 ml/kg (Adetunji O. A., et al. 2022) via an intragastric metal stomach tube.The main stages of histological sample preparation included:

1. Sampling and sectioning. 2. Fixation. 3. Paraffin infiltration and embedding. 4. Microtomy. 5. Deparaffinization. 6. Staining. 7. Sample sorting. 8. Microscopy.The obtained samples were stained using Hematoxylin-Eosin and Van Gieson methods, and the wall thickness of the chambers was measured using an ocular micrometer.

Statistical analysis method

Statistical analysis was conducted using common statistical methods and software. The collected data were refined, and after systematizing the primary materials, an electronic spreadsheet was created using Microsoft Office Excel 2010 to achieve meaningful results. All obtained data underwent statistical analysis using IBM SPSS Statistics v.23 software (developed by IBM Corporation, USA)

Results.

Starting from the 12th month of postnatal ontogenesis in rats, it was observed that the thickness of the epicardial layer of the right atrial wall ranged from 23.99 to 29.10 μm (mean 27.14 ± 0.42 μm). The thickness of the outer plate of the epicardial layer ranged from 6.99 to 10.01 μm (mean 8.87 ± 0.25 μm), while the thickness of its inner plate ranged from 15.90 to 21.1 μm (mean 18.33 ± 0.43 μm).

During this period of rat development, the thickness of the right atrial myocardial layer was found to range from 589.0 to 752.0 μm, with a mean of 664.93 ± 13.37 μm (Figure 1).

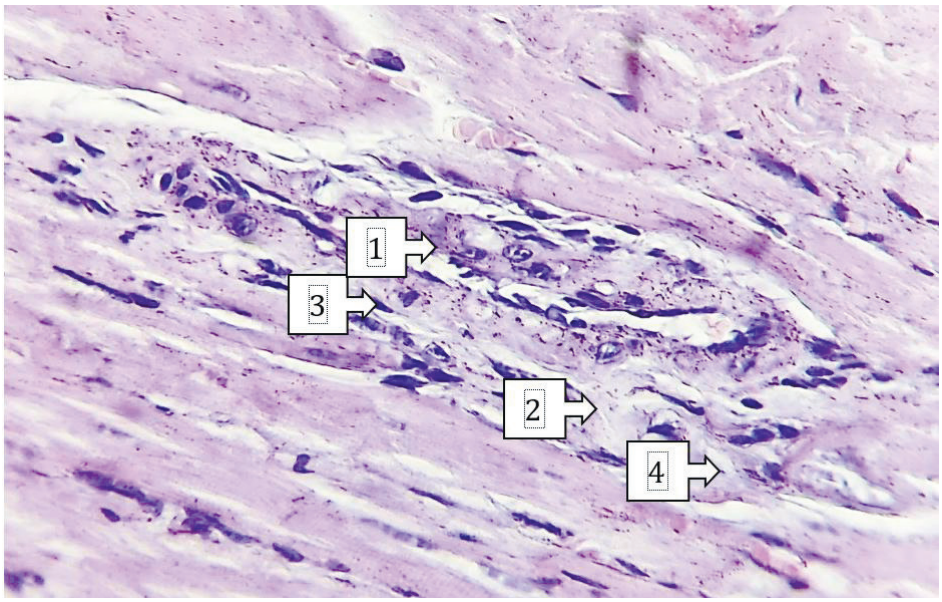


Figure 1. Histological view of the right atrial wall of rats in the 12-month control group

1. Blood vessels (congested/full-blooded).
2. Typical contractile cardiac muscle cell (Cardiomyocyte).
3. Oval or elongated, hyperchromatically stained cardiomyocyte nucleus.
4. Intercellular space between cardiomyocytes.Stain: Hematoxylin-eosin. Magnification: Obj. 10x, Oc. 10.

The thickness of the endocardial layer of the right atrial wall was recorded to range from 38.0 to 44.0 μm (mean 41.53 ± 0.49 μm). Specifically, the thickness of the endothelial layer of the endocardium ranged from 14.93 to 19.07 μm (mean 17.0 ± 0.34 μm), while the subendothelial layer ranged from 21.94 to 26.06 μm (mean 24.53 ± 0.34 μm).

In twelve-month-old rats poisoned with ethyl alcohol, the thickness of the epicardial layer of the right atrial wall was found to range from 19.97 to 27.02 μm (mean 23.07 ± 0.65 μm). The thickness of the outer plate of the epicardial layer ranged from 6.01 to 10.02 μm (mean 7.46 ± 0.37 μm), while the inner plate of this layer ranged from 13.96 to 18.04 μm (mean 15.69 ± 0.38 μm).

During this period of rat development, the thickness of the right atrial myocardial layer was observed to range from 501.0 to 639.0 μm, with a mean of 566.31 ± 12.70 μm (Figure 2).

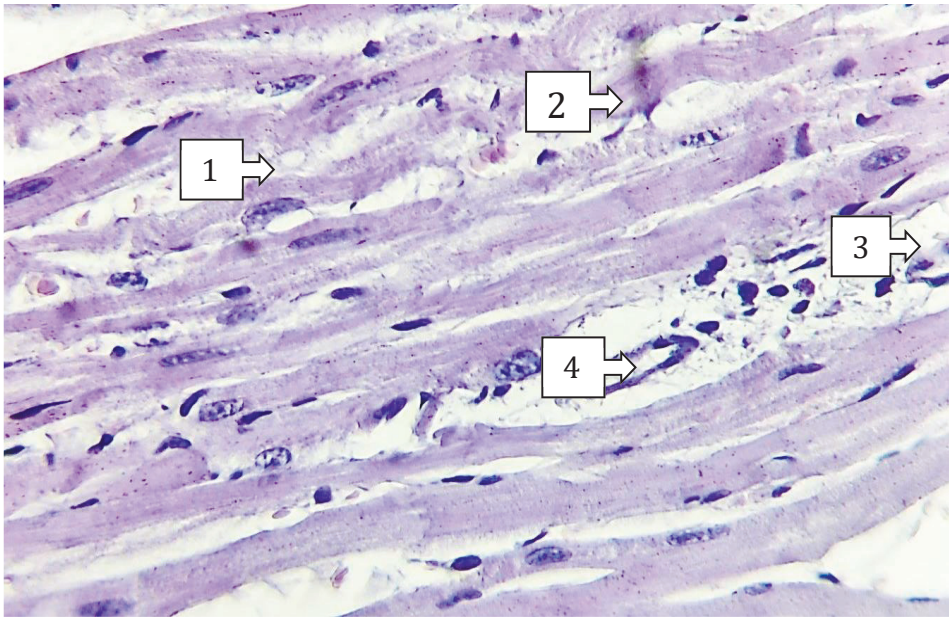


Figure 2. Histological view of the right atrial wall of 12-month-old rats poisoned with ethyl alcohol

1. Hypertrophy and fatty dystrophy of cardiomyocytes.
2. Focal atrophy and fragmentation of cardiomyocytes.
3. Edema in the interstitial space between cardiomyocytes.
4. Thickened blood vessel walls with edema in the intima.Stain: Hematoxylin-eosin. Magnification: Obj. 10x, Oc. 10.

The thickness of the endocardial layer of the right atrial wall was recorded to range from 35.0 to 40.0 μm (mean 37.08 ± 0.46 μm). The thickness of the endothelial layer of the endocardium ranged from 13.99 to 17.01 μm (mean 15.31 ± 0.28 μm), while the thickness of its subendothelial layer was observed to be between 19.98 and 23.02 μm (mean 21.69 ± 0.28 μm).

As a result of administering olive oil for one month to twelve-month-old rats poisoned with ethyl alcohol, the thickness of the epicardial layer of the right atrial wall was found to range from 21.89 to 28.0 μm (mean 24.53 ± 0.50 μm). The thickness of the outer plate of the epicardial layer

ranged from 7.0 to 11.0 μm (mean 7.93 ± 0.33 μm), and the thickness of the inner plate of this layer was 14.89 to 18.11 μm (mean 16.67 ± 0.26 μm).

In twelve-month-old rats, the thickness of the endocardial layer of the right ventricular wall was recorded to range from 37.78 to 48.05 μm (mean 43.92 ± 0.94 μm). The endothelial layer of the endocardium ranged from 15.83 to 20.17 μm (mean 18.36 ± 0.40 μm), while the subendothelial layer thickness was between 21.95 and 29.05 μm (mean 25.64 ± 0.65 μm).

During alcohol intoxication in twelve-month-old rats, the thickness of the epicardial layer of the right ventricular wall was found to range from 27.99 to 33.01 μm (mean 30.72 ± 0.46 μm). The thickness of the outer plate of the epicardial layer was 8.0 to 13.05 μm (mean 10.22 ± 0.46 μm), and the inner plate was 18.99 to 23.01 μm (mean 20.64 ± 0.37 μm).

During this stage of ethyl alcohol poisoning, the thickness of the right ventricular myocardial layer was observed to range from 1118.60 to 1300.50 μm, with a mean of 1209.85 ± 16.73 μm (Figure 3.25). Microscopic examination of the right ventricular myocardial layer in these rats revealed focal atrophy and fragmentation of cardiomyocytes, as well as congestion (hyperemia) of small-sized blood vessels.

During the correction period for ethanol-poisoned rats, the thickness of the right atrial myocardial layer was found to range from 500.0 to 700.0 μm, with a mean of 599.33 ± 16.40 μm (Figure 3).

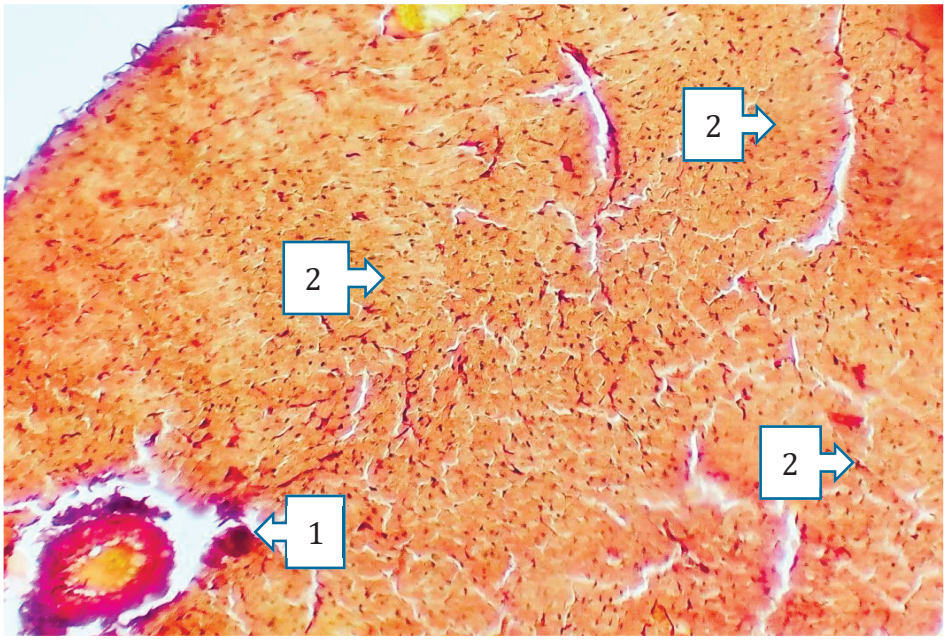


Figure 3. Morphological changes in the right atrial wall of 12-month-old rats following olive oil treatment after chronic alcohol intoxication under experimental conditions

1. Pathological processes in the blood vessel walls (fatty dystrophy) have been replaced by regenerative changes.

2. The proportionality of the interstitial spaces between cardiomyocytes has been restored.Stain: Van Gieson. Magnification: Obj. 4x, Oc. 10.

At the same time, the thickness of the endocardial layer of the right atrial wall was found to range from 37.0 to 41.11 μm (mean 39.07 ± 0.34 μm). The thickness of the endothelial layer of the endocardium ranged from 13.78 to 18.22 μm (mean 16.13 ± 0.36 μm), and the thickness of its subendothelial layer was 20.89 to 25.11 μm (mean 22.93 ± 0.35 μm).

As a result of administering olive oil for one month to twelve-month-old rats poisoned with

ethyl alcohol, the thickness of the epicardial layer of the right ventricular wall was recorded to range from 28.98 to 37.0 μm (mean 32.69 ± 0.66 μm). The thickness of the outer plate of the epicardial layer ranged from 8.99 to 14.01 μm (mean 10.81 ± 0.41 μm), while the inner plate thickness was 18.98 to 24.02 μm (mean 21.88 ± 0.41 μm).

During this stage of detoxification in ethanol-poisoned rats, the thickness of the right ventricular myocardial layer was found to be between 1100.0 and 1500.0 μm, with a mean of 1223.25 ± 32.80 μm.

Additionally, the thickness of the endocardial layer of the right ventricular wall ranged from 46.99 to 56.01 μm (mean 52.0 ± 0.74 μm). The endothelial layer of the endocardium was 18.99 to 24.01 μm (mean 21.75 ± 0.41 μm), and the subendothelial layer thickness was recorded to range from 27.98 to 33.02 μm (mean 30.31 ± 0.41 μm).

Conclusions. The study revealed that as a result of ethyl alcohol poisoning in twelve-month-old rats, the epicardial layer of the right atrial wall, including its outer and inner plates, thinned by 15.45% and 14.40%, respectively. Simultaneously, a 14.83% decrease in the thickness of the myocardial layer was observed. During this period of intoxication, the endocardial layer of the right atrial wall thinned by 10.91%, the endothelial layer by 9.94%, and the subendothelial layer by 11.58%. In chronic alcoholic poisoning, a generalized thinning of all cardiac layers is observed.

Histological investigations showed that after detoxification with olive oil, the epicardial layer of the right atrial wall in ethanol-poisoned rats showed recovery, with the inner plate increasing by 5.12% and the outer plate by 4.08%. Furthermore, a 5.08% recovery was recorded in the thickness of the myocardial and endocardial layers, including all sub-layers of the endocardium. Thus, it was determined that while ethyl alcohol poisoning leads to the thinning of the right atrial wall, olive oil facilitates the partial restoration of the chamber wall. A reduction in the single-layer squamous endothelial cells of the endocardium and a thickening of the connective tissue in the subendothelial layer were identified. Detoxification with olive oil restored the proportionality of interstitial spaces between cardiomyocytes. Additionally, an increase in the number of vascular anastomoses and small vessels was observed. These changes indicate an improvement in the nutrition of the cardiomyocytes.

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