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Olive Oil Correction of Morphofunctional Cardiac Changes in Experimental Ethanol Intoxication of White Outbred Rats

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Abstract: The detoxifying and cardioprotective potential of olive oil against ethanol-induced cardiac injury was evaluated in white outbred rats during postnatal development. A total of 153 rats aged 3, 6, 9 and 12 months were divided into control, ethanol-intoxicated (40% ethyl alcohol, 7.0 g/kg, 30 days, intragastrically) and olive-oil-corrected (2 ml/kg, 30 days) groups. Organometric, morphometric, histological (haematoxylin–eosin, Van Gieson, Alcian blue), immunohistochemical (BCL2) and echocardiographic methods were applied. Olive oil partially restored the thickness of all heart-chamber wall layers, normalized the heart-mass and heart-volume indices and returned the conical heart shape that had disappeared under ethanol. Immunohistochemically, BCL2 expression in left-ventricular cardiomyocytes increased from 30% in the ethanol group to 40% after correction, indicating reduced apoptosis. Echocardiography showed that the ejection volume rose from $37.93 \pm 0.70\%$ (ethanol) to $40.93 \pm 0.96\%$, approaching the control value ($43.87 \pm 0.44\%$). The corrective effect was age-dependent and most pronounced in the early postnatal periods.

Keywords: ethanol intoxication, olive oil, alimentary correction, cardiomyocyte, BCL2, apoptosis, echocardiography, rat heart.

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Introduction

Alcohol remains one of the leading psychoactive and carcinogenic substances affecting public health worldwide. According to the World Health Organization, harmful use of alcohol causes about 3 million deaths each year, accounting for 5.3% of all deaths globally. Chronic ethanol consumption produces a wide spectrum of cardiac lesions that culminate in alcoholic cardiomyopathy, including thinning of the ventricular walls, cardiomyocyte atrophy, protein and fatty dystrophy, interstitial fibrosis and impaired contractility. At the cellular level, ethanol and its metabolite acetaldehyde damage mitochondria, increase the production of free radicals and activate apoptosis, which reduces the pumping capacity of the heart.[1][2]

Among dietary agents with cardioprotective properties, olive oil occupies a special place. It consists predominantly of monounsaturated fatty acids (omega-9 oleic acid) and contains more than thirty phenolic compounds, including hydroxytyrosol, oleuropein and tyrosol. These bioactive components exert antioxidant, anti-inflammatory, antiaggregant and endothelium-protective effects, reduce cardiac remodeling and improve the sensitivity of membrane receptors. Despite numerous studies on the cardiac effects of alcohol, the published data remain contradictory, and the alimentary correction of ethanol-induced cardiac injury has not been sufficiently investigated. The aim of the present study was to evaluate the detoxifying and corrective effect of olive oil on the morphofunctional changes of the heart caused by ethanol during the postnatal development of rats.[3][4][5]

Materials and Methods

The experiment was carried out on 153 white outbred rats aged 3, 6, 9 and 12 months. After a 21-day quarantine, the animals were divided into three groups: a control group (n=52) that received 0.5 ml of distilled water; an ethanol group (n=59) that received 40% ethyl alcohol at a dose of 7.0 g/kg of body weight for 30 days; and a correction group (n=51) of ethanol-intoxicated rats that received olive oil at 2 ml/kg for 30 days. All substances were administered intragastrically through a metal gastric tube. Ethanol intoxication was modelled according to the method of Sidorova (2002), and detoxification with olive oil was performed according to the method of Adetunji (2022). The distribution of animals by group is presented in Table 1.[6]

Table 1. Distribution of animals by group.

Age of rats	Control group	40% ethanol intoxication	Olive oil correction	Total
3 months	13	13 (5)	12	39
6 months	13	13 (2)	13	39
9 months	12	12 (1)	12	36
12 months	14	12 (1)	14	42
Total	52	50 (9)	51	153 (9)

Note: the number of animals that died during the experiment is given in parentheses.

Animal handling and the experimental protocol were approved by the local ethics committee. Heart morphometric parameters – thickness, length (from the origin of the aorta to the apex) and width (at the level of the ventricular bases) – were measured with an anatomical ruler and a calliper graduated to 0.05 mm using the method of Avtandilov (1990).[7] The heart shape was determined by the Babich formula (width/length $\times$ 100%) and classified as conical (up to 65%), elliptical (65–75%) or spherical (above 75%). The heart-mass index was calculated as organ weight/body weight $\times$ 100%, and the heart-volume index as organ volume/thoracic-cavity volume $\times$ 100%. Histological sections were stained with haematoxylin–eosin, by Van Gieson and with Alcian blue.[8] Immunohistochemical analysis of the apoptosis inhibitor BCL2 was performed on a Leica Bond system. Echocardiography was carried out on a SonoScape SSI-5000 device with a 2.5 MHz sector transducer in anatomical M-mode. Statistical processing was performed with IBM SPSS Statistics v.23.[9]

Results

Administration of olive oil to ethanol-intoxicated rats was accompanied by a partial restoration of the morphometric and functional parameters of the heart. The heart-mass index, which had decreased under ethanol, and the heart-volume index, which had increased, returned towards control values; in 6-month-old rats the mass index rose to $0.58 \pm 0.012\%$ and the volume index fell to $25.22 \pm 1.31\%$. The conical heart shape, which had disappeared completely in the ethanol group, reappeared after correction (2% of cases at 6 months), while the proportion of spherical hearts decreased. The corrective effect was clearly age-dependent and was most pronounced in the early postnatal periods (3 and 6 months), where all three heart shapes were restored.[10][11]

Morphometry of the chamber walls showed a recovery of the thinned layers in all heart chambers. In 3-month-old rats the layers of the left-ventricular wall recovered by up to 5.93% compared with the corresponding ethanol group, and in 6-month-old rats the right-atrial wall recovered by up to 5.52%. Histologically, the protein and fatty dystrophy regressed: droplet-like protein and fat inclusions and lipofuscin disappeared from the cardiomyocyte sarcoplasm, the myofibrils thickened, the regularity of the intercellular spaces was restored, the number of adipocytes between muscle fibres decreased, and

regenerative changes replaced the pathological processes in the vascular walls, with reduction of perivascular oedema and infiltration.[12]

Discussion

Immunohistochemical analysis with the BCL2 marker confirmed the antiapoptotic effect of the correction. In the control group, membrane expression of BCL2 in the left-ventricular cardiomyocytes was positive in 70% of cells; under ethanol intoxication it fell to 30%, reflecting enhanced apoptosis; after one month of olive oil it partially recovered to 40%. Echocardiography of 6-month-old rats demonstrated that the reduced ejection volume of the ethanol group ($37.93 \pm 0.70\%$) increased to $40.93 \pm 0.96\%$ after correction, approaching the control value ($43.87 \pm 0.44\%$), while the elevated heart rate decreased from 800.0 ± 34.8 to 520.0 ± 17.4 beats per minute. The principal comparative indicators are summarized in Table 2.[13][14][15]

Table 2. Key cardiac parameters of 6-month-old rats by group.

Parameter (6-month rats), M±m	Control	Ethanol	Olive oil
Heart mass index, %	0.57 ± 0.012	0.54 ± 0.022	0.58 ± 0.012
Heart volume index, %	24.84 ± 1.22	25.99 ± 1.22	25.22 ± 1.31
Ejection (stroke) volume, %	43.87 ± 0.44	37.93 ± 0.70	40.93 ± 0.96
Heart rate, beats/min	513.33 ± 17.4	800.0 ± 34.8	520.0 ± 17.4
BCL2 expression (LV myocardium), %	70	30	40

Conclusion

Olive oil administered at 2 ml/kg for 30 days exerts a pronounced detoxifying and cardioprotective effect on the ethanol-damaged heart of rats. It partially restores the thickness of all heart-chamber wall layers, normalizes the heart-mass and heart-volume indices, returns the physiological heart shape, reduces cardiomyocyte apoptosis (increase of BCL2 expression) and improves the ejection volume towards control values. The efficacy of the bioactive components of olive oil depends on the developmental period of the organism and is most expressed in the early postnatal stages. The obtained results may be recommended for use in cardiology practice and in the development of effective methods for the correction of alcohol-induced cardiac injury.

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