

## CHANGES OF MYOCARDIAL GLYCOGEN CONTENT IN RATS ADMINISTERED WITH MODERATE DOSES OF FURFURAL

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Furfural is produced by dehydration process when strong acids react with pentoses and formation of furfural occurs. It is used as a solvent for extracting mineral oils in many industrial branches and can also be found in orange juice or in brandy. Furfural is not toxic, but its oxidative by-product, pyromucic acid that is conjugated to glycine in the liver and excreted mostly in urine, has harmful effects.

The experiments were performed on 9-week old Wistar rats with body weight of about 259 gr. The animals were treated with furfuraldehyde  $C_4C_3OCHO$ , "Sigma chemical Co", as 1% solution in drinking water, first at a dose of 20mg/kg body weight for seven days, then the dosage was gradually increased for 45 days when the animals were sacrificed.

The analysis was performed on the myocardium of experimental animals. The methods of Hematoksilin-oesin staining (HE) and PAS (periodic acid Schiff) staining were used. Toxic changes were detected in myocardiocytes, showing partial loss of striation, sporadic discoloration of the nucleus and cytoplasm coagulation associated with the presence of expressed hyperemia and the massive loss of glycogen in cardiomyocytes as well. *Acta Medica Medianae* 2014;53(1):5-9.

**Key words:** *furfural, glycogen in cardiomyocytes*

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### Introduction

Furfural (furan-2-aldehyde,  $C_4C_3OCHO$ ) is a colorless liquid with pleasant aroma, it evaporates easily and is partially soluble in water (8,3% of weight). Its use as a selective solvent for extracting mineral oils, in chemical, rubber, plastic and polymer industries is of great importance. It is also present in orange juice concentrates, brandy (1,2) and in Japanese sake (3). In human body, furfural is produced by dehydration of the pentoses (4), so its occurrence in urine is physiological. The elimination of furfural is done slowly in unchanged form through the lungs and kidneys. The liver oxidizes it into pyromucic acid ( $C_4C_3OCOOH$ ) which is toxic to hepatocytes, so it is conjugated with glycine and mostly excreted in urine (5,6).

Glycogen is a nonliving component of cell inclusions or deposits (7,8). Glycogen is a long-chain polymer of glucose of molecular weight being 5 million or greater. It is stored in almost all body

cells, mostly in parenchymatous liver cells, but it is less accumulated in skeletal muscles, cardiac muscle cells and in adrenal cortex cells (7,9,10). Glycogen is accumulated in cells in the form of particles not bounded by membrane. They can be large (alpha particles) and spherical, rosette-like particles (beta particles) (9,11). Glycogen is depolymerized to glucose and is degraded in steps by a series of enzymes which may be impaired and lead to hyperglycemic states as a result of increased glycogen accumulation in cells. The amount of glycogen stored in liver cells is regulated by the blood glucose concentration (11-13).

Myocardium and the liver are vital organs that are exposed both to harmful effects of environmental substances and to derivatives of the degradation processes in the body. Since glycogen is one of the myocardiocyte cell components and is partially involved in glycogenolysis, it is interesting to observe furfural impact on myocardial glycogen content.

Out of these reasons we set the aim to test the effects of this substance on other parenchymatous organs. In this case we decided to test the effects of furfural on the myocardium since there are no literature available data on its effects upon cardiomyocytes and myocardium as a whole.

### Aim

The aim of our study was to examine furfural effects on cardiac muscle, primarily its

relation to glycogen content in cardiomyocytes and possible toxic unspecific effects on myocardium.

### Material and methods

The experiment was performed on thirty (30) 9-week-old Wistar rats with body weight of 150g-200g. Furfural (2 - furaldehyde  $C_4H_3OCHO$ ) made by "Sigma Chemical Co" was used for treatment. Furfural was administered to animals as 1% solution in drinking water, first at a dose of 20mg/kg body weight for seven days, then the dosage was gradually increased for 45 days when the animals were sacrificed.

Immediately after the rats were sacrificed by decapitation, all the parenchymatous organs and the heart were removed. The heart was cut horizontally at its widest region, along the middle part of the ventricle. In such a way, cross-section circumference allowed the mass of papillary muscle structures from epicardium to endocardium to be fully seen. Out of these structures, thin samples of up to 4 mm thickness were taken, fixed with 4% formaldehyde, placed into paraffin molds, and cross sections of 5 $\mu$  thickness were made and stained with HE and PAS methods (9,14).

### Results

Macroscopically, the hearts of experimental animals were of appropriate size and slightly soft in consistency. Histologically, on HE specimens, subepicardial and subendocardial dilation of capillaries rich in blood is seen in large percentage. Such a dilation could be seen, although rarely, in the middle portion of the myocardium as well (Figure 1). Besides that, strong myocardial edema widely separating myocardiocytes bundles can occasionally be seen within the interstitial space (Figure 2). Strongly expressed cross-striation in cardiomyocytes was analyzed in not so small number of cases, but these cells also exhibit partial or complete loss of cross-striation, especially in the middle portions of the myocardium. In these portions, granular cytoplasm and myocardiocyte nuclei can be seen, and the nuclei of myocardiocytes are often pale and emptied, but with preserved nuclei margins.

PAS staining was used to identify the presence of glycogen found in the form of granules, rarely seen separately, but in clusters in the form of reddish-purple structures. In 90% of cases glycogen was not observed. In the remaining 10% of the myocardium, it was predominantly observed in the subepicardium cardiomyocytes, as well as in subendocardium, especially in papillary muscles, sporadically in ventricular central muscular mass and occasionally in some cells (Figure 3). It is interesting to note that glycogen is most commonly present in cardiomyocytes where cross-striation is well preserved (Figure 4); in the regions where striation is not so well preserved it occurs in the

form of sparse individual formations, or in the form of granular clusters. Glycogen is absolutely absent in muscle cells without cross-striation and in the cells with cytoplasmic clotting granules (Figure 5).

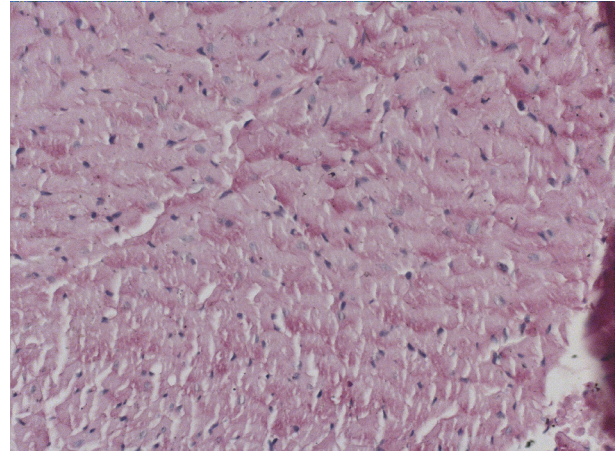


Figure 1. Notable dilation of capillaries overloaded with erythrocytes in ventricular myocardium, with myocardiocytes without cross-striation and with partly pale nuclei. 63x HE

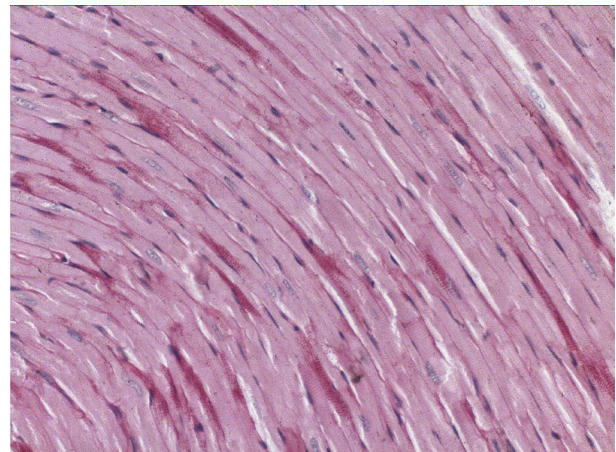


Figure 2. Strong myocardial edema with notable separation of muscular bundles and moderate hyperemia 10x HE

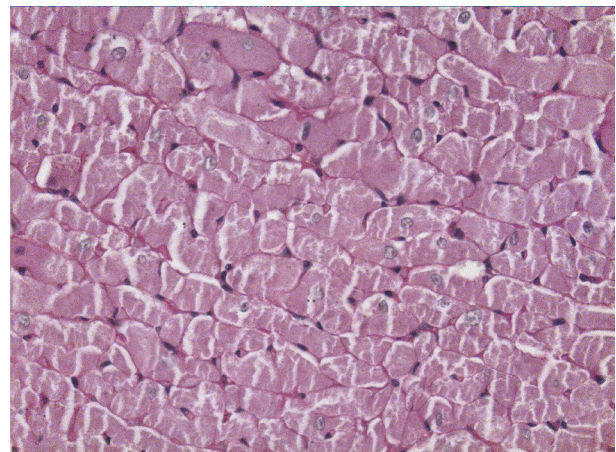


Figure 3. Scarce presence of glycogen in the form of partly involved cardiomyocytes, pale colour 40x PAS

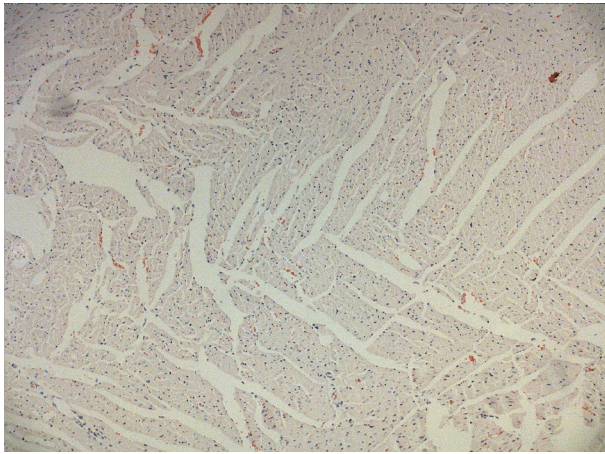


Figure 4. Relatively preserved glycogen content, especially in cardiomyocytes with preserved cross-striation. 40x PAS

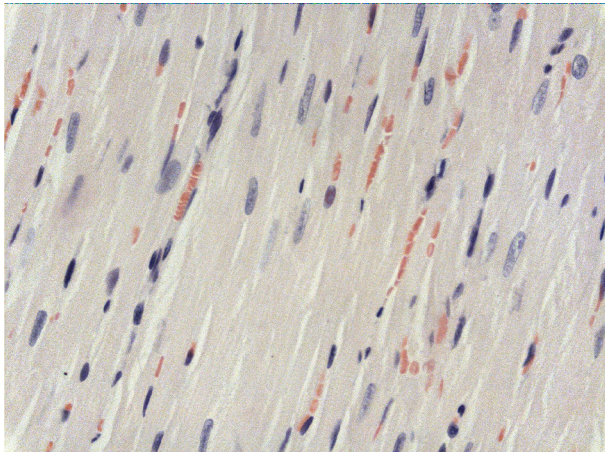


Figure 5. Muscle cells of the myocardium primarily with coagulated cytoplasm, mostly without nuclei, completely glycogen-depleted. 63x PAS

## Discussion

Furfural undoubtedly has toxic effects upon the myocardium, either directly or via its derivatives in tissues or in blood. This phenomenon is morphologically reflected as the presence of expressed dilation of capillaries, then as strong myocardial interstitial edema, focal or diffuse loss of cardiomyocyte cross-striations, granulomatous transformation of the cytoplasm and total or subtotal loss of glycogen. Furfural ( $C_5H_4O_2$ ) is unsaturated cyclic aldehyde and a well-known hepatotoxic substance (14-19).

Besides changes in activities of some enzymes, furfural in treated animals causes severe degenerative and necrotic alterations of hepatocytes, leading to cirrhosis development and hepatic carcinoma, considering anabolic, catabolic, detoxifying and excretory functions of the liver, as well as its functional relation with the pancreas (20).

On the other hand, it is known that furfural affects glycogenolysis and in applied experimental conditions glycogen depots are gradually reduced. At the same time, this substance considerably reduces detoxification properties of the liver, leading to an imbalance in the enzymatic system (21).

Therefore, in our study we managed to demonstrate furfural toxicity to cardiomyocytes, as well as considerable effects on their glycogenolyses, which definitely decreases functional ability of the myocardium.

## Conclusion

Furfural, administered to 30 rats which were sacrificed and treated with HE and PAS methods, showed definite toxicity to myocardium (notable dilation of the capillaries, edema, the loss of cross-striation of the muscle cells, clotting of their cytoplasm and subtoxic loss of cellular glycogen).

## References

1. Castellino N, Elmino O, Rozaga G. Experimental research on toxicity of furfural. Arch Environ Health 1963; 547-82. [[PubMed](#)]
2. Kaminska O, Gruszecka B. Morphological and histochemical changes in the rat liver in chronic furfural poisoning. Med Pr 1977; 28(5): 377-91. [[PubMed](#)]
3. Higashi T. ON some constituents of Sake. Sci Pap Inst Phys Chem Res 1937; 33: 1-129.
4. Petković M. Hemija biomolekula III. Nis: Univerzitet u Nisu; 1990.
5. Kuznetsov PJ. Toxicological characteristics of furfural. Parts I and II. Tr Omak Med Inst 1966; 69: 38-44.
6. Janzowski C1, Glaab V, Samimi E, Schlatter J, Eisenbrand G. 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. Food Chem Toxicol 2000;38(9):801-9. [[CrossRef](#)] [[PubMed](#)]
7. Ross MH, Romrell LJ, Kaye GI. Histology: A Text and Atlas. Baltimor: Williams and Wilkins; 1995.
8. Krstić RV. Illustrated Encyclopedia of Human Histology. Berlin: Springer – Verlag; 1984. [[CrossRef](#)]
9. Radenković M. Promet materije i energije. U: Veličković D, urednik. Fiziologija. Nis: Galaksija; 2013: 163-79.
10. Rubin E, Farber JL. Cell Injury, 1-32. In: Rubin E, Farber JL, editors. Pathology. J.B. Lippincott Co, Philadelphia: . J.B. Lippincott Co;1988.
11. Zaitsev AN, Simonian TA, Pozdniakov AL. Hygienic standards for hydroxymethylfurfural in food products. Vopr Pitan 1975;(1):52-5. [[PubMed](#)]
12. Nada MS. Izbor biohemijskih pokazatelja za dijagnostikovanje akutnog koronarnog sindroma. Jugoslav Med Biohem 2005; (24): 1–13.
13. Dixon K. Principles of some tinctorial and cytochemical methods. In: Champion RH, Gilman T, Rook AJ, Sims RT, editors. An Introduction to the Biology of Skin. Blackwell: Oxford; 1971: 1070.
14. Godfrey VB, Chen LJ, Griffin RJ, Lebetkin EH, Burka LT. Distribution and metabolism of (5-hydroxy methyl) furfural in male F344 rats and B6C3F1 mice after oral administration. J Toxicol Environ Health A 1999 ;57(3):199-210. [[CrossRef](#)] [[PubMed](#)]
15. Lake BG, Edwards AJ, Price RJ, Phillips BJ, Renwick AB, Beamand JA, et al. Lack of effect of furfural on unscheduled DNA synthesis in the in vivo rat and mouse hepatocyte DNA repair assays and in precision-cut human liver slices. Food Chem Toxicol 2001;39(10):999-1011. [[CrossRef](#)] [[PubMed](#)]
16. Surh YJ, Liem A, Miller JA, Tannenbaum SR. 5-Sulfooxymethylfurfural as a possible ultimate mutagenic and carcinogenic metabolite of the Maillard reaction product, 5-hydroxymethylfurfural. Carcinogenesis 1994; 15(10):2375-7. [[CrossRef](#)] [[PubMed](#)]
17. Racine-Samson L, Scoazec JY, D'Errico A, Fiorentino M, Christa L, Moreau A. The metabolic organization of the adult human liver: a comparative study of normal, fibrotic and cirrhotic liver tissue. Hepatology 1996; 24: 104-11. [[CrossRef](#)] [[PubMed](#)]
18. Junqueira LC, Carneiro J. Basic histology. Text and Atlas. Lange. International edition, 2003.
19. Cekić S, Filipović, Jović Z, Nešić M, Ćirić M, Veljković S, et al. Imunocitohemijske karakteristike insularnih D ćelija pankreasa kod pacova tretiranih furfuralom. Acta biologica iugoslavica - serija C: Physiologica et pharmacologica acta. 2004; 40(1-3):125-32.
20. Veličković D, Milenković S, Stojanović D. Enzimo hemijske i biohemijske promene u jetri pacova indukovanog furfuralom. Acta Medica Medianae 2011; 50(2): 34-38. [[CrossRef](#)]

## PROMENE SADRŽAJA GLIKOGENA U MIOKARDU PACOVA KOD PRIMENE UMERENIH DOZA FURFURALA

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Furfural nastaje procesom dehidracije, kada delovanje jakih kiselina na pentoze izaziva njegovo stvaranje, a koristi se kao rastvarač mineralnih ulja u mnogim delovima industrije ili se može naći u oranž džusu ili brendiju. Furfural nije toksičan, ali njegov oksidativni produkt, piromucinska kiselina jeste i ona se u jetri konjuguje sa glicinom i uglavnom se izbacuje urinom.

Ispitivanja su vršena na 30 pacova Wister soja starosti 9 nedelja, telesne težine oko 250gr. Životinje su tretirane furfuraldehidom  $C_4H_4O$ , "Sigma chemical Co", u vidu 1% rastvora datog u vodi za piće, u početku 20 mg po kilogramu telesne mase u toku sedam dana. Doza je postepeno povećavana do žrtvovanja životinja posle 45 dana.

Istraživanje je izvršeno na miokardima eksperimentalnih životinja i bojenjem na hematoksil-eozin (HE) i PAS (periodic acid Schiff) metodom. Konstatovane su toksične promene na miokardiocitima, koje se ogledaju parcijalno gubitkom poprečne ispruganosti, mestimično diskoloracijom jedra, kao i zgrušnjavanjem citoplazme, uz prisustvo naglašene hiperemije, kao i masivnim gubitkom glikogena u kardiomiocitima. *Acta Medica Medianae 2014;53(1):5-9.*

**Ključne reči:** furfural, glikogen u kardiomiocitima