**Original article**

**UDC: 612.35.015.1**
**doi:10.5633/amm.2011.0206**

# ENZYMOCHEMICAL AND BIOCHEMICAL CHANGES IN THE LIVER OF RATS INDUCED BY FURFURAL

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In today's industrial expansion of the chemical products, the liver is becoming increasingly important. Furfural (C4C3OCHO) is a colorless liquid with pleasant aroma and it is partially soluble in water (8, 3% of weight). The elimination of furfural is done slowly through the kidneys and lungs, while the liver oxidizes it into pyromucic acid (C4C3OCOOH). Glucose-6-phosphate dehydrogenase (G6PD) is a multi-component system of gluconeogenesis. Biochemical parameters (AST, ALT, glucose, γ-GT and alkaline phosphatase) are important markers of liver damage.

The aim of our study was to analyze the function of hepatocytes using biochemical parameters and to show the dynamics and topography in the development of changes in enzyme activity.

The experiment was conducted on Wistar rats aged 6 weeks. The animals were divided into three groups. The control group received pure drinking water, the second group received a 50 mg/kg body weight (BW) dose of furfural for seven days and in the third group the dose was progressively increased after which the animals were sacrificed. Biochemical methods were used to determine the parameters of liver damage. Enzyme-histochemical tests were performed on 8nm WKF 1150 cryostat cross sections which were stained according to Pearse (1968). The results are presented tables and graphs.

The amount of enzymes and biochemical parameters in the control group were normal. In the group treated for 7 days, the activity of the enzymes was diffusely decreased while the biochemical parameters were increased. In the group of rats treated for 90 days, the periportal G6PD was constantly preserved. Biochemical parameters were different. The differences in all parameters were statistically significant ($p<0.05$) both in the group treated for 7 days and the group treated for 90 days. The same goes for the control group and the group treated for 7 days.

Acute treatment with furfural causes damage to liver functions. The synthetic liver function is restored in chronic tests. Acta Medica M*edianae 2011;50(2):34-38.*

***Key words:*** *furfural, rat, glucose-6-phosphate dehydrogenase, biochemical parameters*

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## Introduction

In today's industrial expansion of chemical products, as well as the striking ecological environment pollution, the liver has gained increasing significance because, in most cases, it is on the way of their processing (1). In order to expand the knowledge on the detoxification role of the liver, the metabolism of some substances was analyzed which has provided insight into some of its functional engagements (2). In this sense, furfural is interesting because it indirectly damages hepatocytes, i.e. through its oxidative product - pyromucic acid (3).

Furfural (furan-2-aldehyde, $C_4C_3OCHO$) was first extracted in 1840 through the bran distillation (Latin: furfur) by diluted sulfuric acid (4). Furfural is a colorless liquid with pleasant aroma and it is partially soluble in water (8, 3% of weight). It has a significant use as a selective solvent of mineral oil products, in chemistry, in the rubber industry, plastic surgery and polymer industry (5). It is also present in orange juice, brandy (6) and Japanese sake (7). Due to its volatility, tissue penetration and relatively easy accumulation, it causes headaches, nausea, increased secretion of saliva and tears, as well as laryngitis and allergic skin reactions (8). In the organism, furfural occurs by pentose dehydration (9), so that the appearance in urine is physiological.

Furfural is eliminated slowly and in unchanged form through the kidneys and lungs. The liver oxidizes it into pyromucic acid ($C_4C_3OCOOH$) which is toxic to hepatocytes, but it is conjugated with glycine and mostly excreted in urine (10,11).

Glucose–6–phosphate dehydrogenase (G6PD) is a multi-component system situated on the luminal side of endoplasmic reticulum and consists of five different polypeptides: glucose–6 –phosphate enzyme with $Ca^{2+}$ binding protein and three transport proteins ($T_1$, $T_2$, $T_3$). It is the major enzyme in gluconeogenesis which releases glucose for peripheral tissues or the synthesis of glycogen. Its greatest activity is periportal. G6PD is also an important link in pentose cycle, which produces pentoses needed for growth, as they are contained in DNA and RNA (12).

Liver transaminases, sGOT (AST) and sGPT (ALT) are important markers of liver damage and are routinely done in modern laboratories. Also, glucose, γ-GT and alkaline phosphatase (APh) are increased in cases where there is damage to liver parenchyma under the influence of many harmful noxas which are detoxified in the liver every day (13).

**Aim**

The aim of the study was to:

1. Monitor the activities of glucose–6–phosphate dehydrogenase by histochemical analysis of hepatocytes and to show the dynamics and topography of changes developing under the influence of furfural.

2. Determine the relationship between liver transaminases, γ-GT and glucose at various time intervals of treatment with furfural using conventional biochemical methods in the blood.

**Material and methods**

Six weeks old Wistar rats (150g–200g) were used in the study as well as furfural (2 –furaldehyde $C_4H_3OCHO$) made by "Sigma Chemical Co.". Furfural was administered to the animals in drinking water.

The animals were divided into three groups:

- The first group was used as control and consisted of ten animals. They were given pure drinking water.
- The second group, containing 10 animals, was given 50mg/kg BW furfural in drinking water for seven days.
- The third group received furfural dose of 30 mg/kg body weight (BW) for the first 30 days. From day 31 to 60 the dose was increased to 40 mg/kg BW and then from day 61 to the end of the experiment the dose was increased to 50 mg/kg BW.

All animals from these groups were sacrificed by abdominal exsanguination one day following the last given dose. Immediately before the sacrifice, blood samples were taken from abdominal aorta. Conventional biochemical methods were used to determine AST, ALT, γ-GT and glucose in the blood. For enzyme-histochemical examination, fresh liver samples were used and series of 8nm sections were made in WKF 1150 cryostat at -20°C and stained according to Pearse (1968) in order to monitor the activities of zonal topography (peripheral, intermediate, central) of glucose -6- phosphate dehydrogenase.

The research results were systematized and presented in tables and graphs (Excel 2003, Word 2003) and were analyzed using descriptive statistics and quantitative analysis (SPSS 14.0 for Windows 2003). The frequency of attributive characteristics was determined by $\chi$2-test. Values of $p<0.05$ were interpreted as statistically significant.

**Results**

1. Control group

The activity of glucose-6-phosphate dehydrogenase was present diffusely in the cytoplasm of hepatocytes in all zones. The enzyme activity was mostly expressed in peripheral zone (+++). Therefore, the hepatocyte cytoplasm was rich in granular material in the central and intermediate zone areas, whereas the amount of this material was smaller towards peripheral zones (Figure 1).

The values of AST, ALT, γ-GT, alkaline phosphatase and glucose were within normal limits (Table1).

2. First experimental group (50mg/kg BW of furfural for 7 days)

G6PD activity was diffusely reduced. Its weak activity is present in the cytoplasm of hepatocytes with preserved contours (Figure 2).

The values of AST, ALT, γ-GT and glucose were dramatically increased, while alkaline phosphatase values were normal (Table 1).

3. Second experimental group (50mg/kg BW of furfural for 90 days)

The appearance of G6PD was multifocal, always periportal, often in the form of portoportal 'bridging'. Enzyme polymorphism was evident, ranging from strong (+++), over medium (++), weak (+) to total inactivity in the necrotic areas (Figure 3).

The activity of AST, ALT, glucose and AF and γ-GT was increased. Compared to the previous subgroup, the increase was small but it was significantly larger compared to the control group. The decrease in the values of glucose and AF was still present compared to the values in the previous groups (Table 1).

Comparing the values of parameters obtained from the blood, it was observed that there was statistically significant ($p<0,05$) difference between the parameters in the control group and the group treated with 50mg/kg furfural for 7 days (Graph 1).

The values in the groups treated for 7 days and 90 days showed a large decrease in chronic treatment with furfural (Graph 1). The difference was statistically significant for all parameters ($p<0.05$).

Comparing the results from the control group to chronic test, the difference was statistically significant ($p <0.05$) for AST, γ- GT and alkaline phosphatase, while this was not the case with glucose and ALT ($p>0.05$)

*Table 1.* The values of glucose, AST, ALT, γ-GT and alkaline phosphatase in the control and two experimental groups of animals

| | Control | After 7 days | After 90 days |
|---|---|---|---|
| | ±SD (mmol/l) | 50 mg/kg | 50mg/kg |
| Glucose | 6.2±1.1 | 10.4±1.7 | 6.9±0.8 |
| sGOT (AST) | 91.88±2.77 | 143±2.5 | 98±1.3 |
| sGPT (ALT) | 12.7±1.25 | 39±1.2 | 22±0.6 |
| γ - GT | 6.8±3.2 | 39±0.2 | 15±1.4 |
| AF | 124.2±15.4 | 124±0.5 | 111±1.6 |

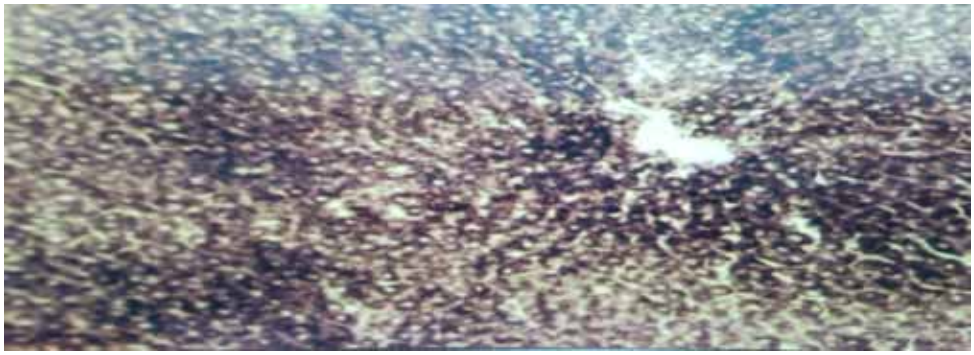


*Figure 1. G6PD activity in rat liver in the control group*

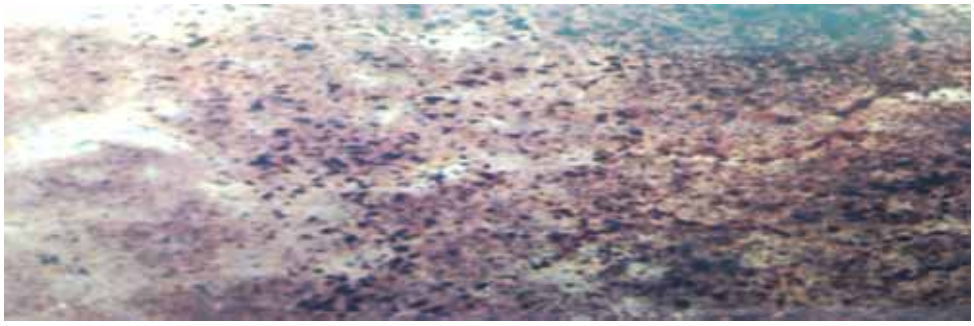


*Figure 2. G6PD activity in rat liver in the first experimental group*

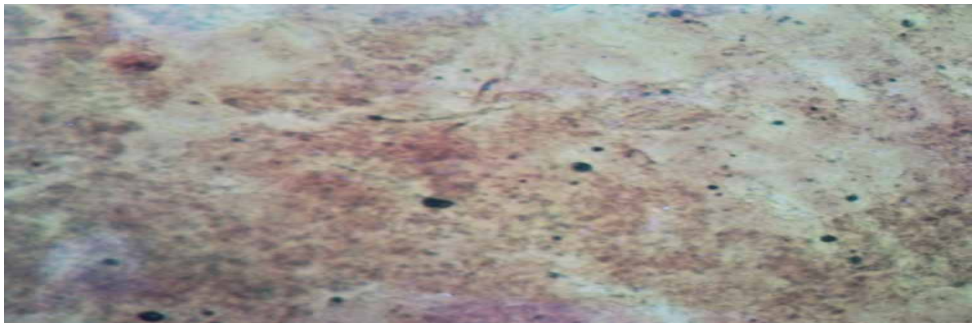
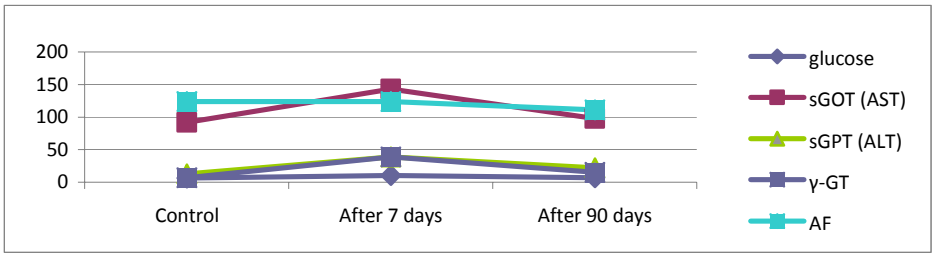


*Figure 3. G6Pdactivity in rat liver in the second experimental group*




*Graph 1. The values of biochemical parameters (glucose, AST, ALT, γ-GT, AF) in the blood of rats in the control, first and second experimental groups*

## Discussion

The liver has many important and different functions, including the synthesis of enzymes and structural proteins, detoxification of many internal and external products of the organism (14). For these functions, hepatocytes use enzyme systems. Generally, about 60% of these systems are localized in mitochondria. Furfural is not harmful. However, its by-product, pyromucic acid, has detrimental effect. Pyromucic acid is obtained by furfural oxidation and is later conjugated with glycine and excreted in the urine (15).

In our experimental conditions, glycogen depots are gradually reduced, so that in chronic treatment they are almost completely absent. Loss of glycogen implies depression of the detoxification function of hepatocytes (16). These changes in glycogen values lead to an imbalance in the enzymatic system (17). Namely, the progressive reduction of G6PD in hepatocytes, from the periphery to the center lobulus, with longer treatment time may be linked to depletion of the amount of glucagon (13,18). These results of other researchers are in keeping with the results of our study that the perivenular activity of G6PD is the strongest due to the proximity of blood vessel whereas this is not the case with periportal hepatocytes (19). Our research indicates a diffuse decrease in G6PD in the first experimental group, while in the group of rats treated for 90 days, there are non-activity areas that are more pronounced in the periportal zones. These changes in the production of energy are referred to as functional insufficiency of the liver (20), which proves the rapid restoration of the enzyme examined.

An important indicator of liver damage is the appearance of increased enzyme activity in serum (21,22). Changes occurring in the serum levels of certain laboratory parameters (glucose, AST, ALT, γ-GT, alkaline phosphatase) can be interpreted in the following way: first, by increased decomposing process in hepatocytes, and second, by the failure of the liver enzyme needed to oppose increased metabolic needs (23, 24). The first group shows a significant increase in transaminases, γ-GT, alkaline phosphatase, glucose. The increase was induced by increased permeability of cell membrane because of its damage (25). In chronic treatment there is mild normalization of the results compared to the values of acute treatment. This recovery is the result of the preserved reparative mechanisms of hepatocytes (26).

## Conclusion

Furfural is not toxic, but its derivative pyromucic acid ($C_4H_3OCOOH$) has toxic effects. Pyromucic acid in the liver is conjugated with glycine and mostly excreted in the urine.

Decrease of glucose-6-phosphate dehydrogenase in the periportal area of the liver is explained by the proximity of blood vessels, since this area first comes into contact with furfural and performs detoxification. There is a decline in the levels of glycogen in the permanently functional zone (peripheral zone) which is explained by the process of energy recovery of liver cells, and the creation of glycine required for the detoxification of furfural metabolites.

In the treatment with a 50mg/kg of BW dose of furfural, there is a damage of normal liver function, which is proved by an increase in biochemical parameters of liver damage and significant decline in G6PD activity.

The synthetic liver function is recovered when furfural doses are gradually increased from 30 to 50 mg/kg BW during the period of 90 days. This is manifested by the normalization of biochemical parameters.

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# ENZIMOHEMIJSKE I BIOHEMIJSKE PROMENE U JETRI PACOVA INDUKOVANE FURFURALOM

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U današnje vreme industrijske ekspanzije hemijskih proizvoda uloga jetre u organizmu je sve više značajna. Furfural ($C_4C_3OCHO$) je bezbojna tečnost prijatnog mirisa koja je delimično rastvorljiva u vodi (8, 3% od težine). Eliminacija furfurala se vrši sporo i u nepromenjenom obliku, putem bubrega i pluća, dok se u jetri oksidiše u piromucinsku kiselinu ($C_4C_3OCOOH$). Glukozo-6-fosfat dehidrogenaza (G6PD) je multi komponentni sistem glukoneogeneze. Biohemijski parametri (AST, ALT, glukoza, γ-GT i alkalna fosfataza) bitni su markeri oštećenja jetre.

Cilj našeg istraživanja bila je analiza funkcije hepatocita preko biohemijskih parametara, sa prikazom dinamike i topografije nastajanja promena u enzimskoj aktivnosti.

Eksperiment je sproveden na pacovima Wistar soja starim 6 nedelja. Životinje su podeljene u tri grupe. Kontrolna je dobijala čistu pijaću vodu, druga grupa je sedam dana dobijala po 50 mg/kg TT furfurala a u trećoj su doze progresivno povećane. Nakon ispitivanja, životinje su žrtvovane. Biohemijskim metodama određeni su paremetri oštećenja jetre. Za enzimohistohemijska ispitivanja pravljeni su preseci debljine 8nm na kriostatu WKF 1150 a bojeni prema Pearsu (1968). Rezultati su prikazani na tabelama i grafički.

U kontrolnoj grupi su količina enzima i biohemijski parametri normalni. Kod grupe tretirane 7 dana aktivnost enzima je difuzno smanjena, biohemijski parametri su povećani. U grupi pacova tretiranih 90 dana, G6PD je uvek periportalno očuvana. Biohemijski parametri su promenjeni. Razlika je statistički značajna za sve parametre ($p<0,05$) između grupe tretirane 7 dana i 90 dana. Isto je i kod kontrolne grupe i one tretirane 7 dana.

Kod akutnog tretiranja furfuralom dolazi do oštećenja funkcije jetre. Kod hroničnog testa dolazi do obnavljanja sintetske funkcije jetre. *Acta Medica Medianae 2011;50(2): 34-38.*

***Ključne reči:*** *furfural, pacov, glukozo 6 fosfat dehidrogenaza, biohemijski parametri*