THE ACTION OF SOME ANTIOXIDANT COMPOUNDS WITH FLAVONOLOGINANES (SILIMARIN AND LEGALON) ON THE RAT LIVER, INTOXICATED WITH N-NITROSO DIMETHYLAMINE (NDMA)

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ABSTRACT. The effects of drugs (compounds) with flavonolignanes (Silimarin and Legalon) on the liver of male Wistar rats, intoxicated with N-nitrosodimethylamine (NDMA) (produced by the administration of aminopyrine+Na nitrite), during 30 days, were investigated.

Biochemical parameters, including serum transaminase activity (GPT and GOT), the glycogen content; histoenzymological (lactate dehydrogenase, succinate-dehydrogenase, glutamate-dehydrogenase, steroid dehydrogenase, cytochromoxidase, Mg-dependent adenosine triphosphatase, glucose-6-phosphatase): histochemical (Sudan black for lipids) and histological examination (haematoxilin-eosine staining) of the liver were studied. Administrations of aminopyrine + Na nitrite produce in stomach of rat the N-nitrosodimethylamine. This has a very complex and toxic actions in rats' liver: decreases of survival, hepatocytolisis, necrosis, steatosis, proliferation of collagen, decreases of glycogen level, reduction of enzymatic activities, like cirrhotic modifications.

The utilization of Silimarin and Legalon has some positive effects such as: a smaller mortality, decreases of histological injury, reduction of hepatocytolisis, necrosis, steatosis and collagen proliferation; enzymatic activity is more increased than intoxicated rats. The rich content of Silimarin and Legalon in flavonolignanes, antioxidants, may be the cause of these positive effects.

Keywords: n-nitrosodimethylamine, intoxicated, liver rats, Silimarin, Legalon

INTRODUCTION

N-Nitrosamines are very toxically compounds for men and animals, about 90% of them have cancerous properties (Rusu et al., 1983; Bartisch and Spiegelhalder, 1996; Hecht, 1997; Brecher, 2002). They were found in food (meat and fish conserves, salami, meal, beef, wine) (Tricker and Preussman, 1991; Domanska and Kovalski, 2002). They were also found in drink water due to desinfection with chlorinated compounds (Najm and Trussell, 2001). They were found in food (meat and fish conserves, salami, meal, beef, wine) (Tricker and Preussman, 1991; Domanska and Kovalski, 2002). They were also found in drink water due to desinfection with chlorinated compounds (Najm and Trussell, 2001; Mitch et al., 2003; Gereke and Sedlak, 2003).

N-Nitrosamines are made from two precursors very dispersed in the environment: nitrates (nitrates) and amines (secondary and tertiary), amidases, generally into milieu acid. Nitrates and nitrites are utilized like fertilizations (Pennington, 1998) or generatess (nitrite is introduced in meat products). The nitrate may transform exogenous into nitrite by the action of microorganisms, or endogen by the gastric-intestinal passage. The other precursor is a compound that contains amines or amide group (Silla-Santos, 1996) and is found in many foods or even in drugs frequently utilized (antibiotics, analgesics, etc.).

Nitrosamines may exogenous form, respectively from precursors, under the action of some microorganisms (Coli, Clostridium, Pseudomonas) or by processing (fumigation, baking). Nitrosamines may form endogen, too, at the stomach level (into milieu acid) from nitrite and amines precursors. There are hundred of nitrosamines, one of the most toxica being N-nitrosodimethylamine (NDMA) with an evident hepatic tropism (Brecher, 2002) that may induce even death of men or animals.

Because DMNA has a special hepatic toxicity, we have proposed to try the effects of some hepatotrope medicines, with antioxidant properties obtained from Silybum marianum – Silimarin and Legalon, which principally contain flavonolignanes (Luper, 1998: Flora et al., 1998; Pares et al., 1998; Weiss and Fintelmann, 2000; Rusu et al., 2007). We have chosen NDMA endogen production in rat stomach from Na-nitrate + aminopyrine precursors (aminophenasone – amines group).

We studied the biochemical, histoenzymological and histochemical changes occurring in liver – a major target organ for NDMA – and the effects of some antioxidants compound – flavonolignanes, from Silimarin and Legalon drugs, in rats.

MATERIALS AND METHODS

Experiments were performed on albino male Wistar rats, weighing 200 ± 15 g. Rats were maintained under the following laboratory conditions: light on 06.00 – 18.00, 60% relative humidity; 22 ± 2 0C (room temperature, access to commercial food pellets, and tap water ad libitum).

Rats were divided into the following experimental groups, consisting of 7-8 animals each, as follows: - a control group (C): - a group intoxicated with 10 mg aminopyrine + 10 mg sodium nitrite (AN); - a group intoxicated with 10 mg aminopyrine + 10 mg sodium nitrite. After 30 min. it was administered 30 mg of Silimarin (Biofarm) drug (ANS group); a group

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Article received: january 2009; published: march 2009
intoxicated with 10 mg aminopyrine + 10 mg sodium nitrite. After 30 min. it was administered 30 mg of Legalon (Madaus) drug (ANL group). Duration of experiment was for 30 days. Rats were killed, after a previous anesthesia according to UE ethical rules. Blood samples were immediately taken for transaminase activity determination (glutamo-oxalate-transaminase – GOT and glutamo-pyruvate-transaminase – GPT) (Fauvert, 1972).

Liver samples were removed for histology determinations (haematoxilin-eosine staining). Other liver fragments were frozen in nitrogen liquid and sectioned in a Shandon AS cryotome. By means of common histochemical and histochemical methods (Muresan et al., 1976; Chayen, Bitenski, 1991) were determined the activity of the following enzymes: lactate-dehydrogenase (LDH); succinate-dehydrogenase (SDH); glutamate-dehydrogenase (GtDH); steroid-dehydrogenase (StDH); Glucose-6-Phosphatasehydrogenase (G-6-P DH); cytochrome c oxidase (CyOx); Mg2+ dependent adenosine triphosphatase (ATP-ase); Glucose-6-Phosphatase (G-6-P-ase); and histochemical examinations (black Sudan, for total lipids). An IOR MC 5 microscope was used with an Exacta camera incorporated.

The biochemical data were statistically processed by means of Student’s “t” test. Aberrant values were eliminated by means of Chauvenet’s criterion. A probability value of p< 0.05 was considered. Appreciation of histoenzymological activity was semiquantitative.

RESULTS
Survival: is only of 25% at AN group; 45% at ANS group and 48% at ANL (Fig. 1).

Biochemical parameters:
- Glycogen: its concentration decreased with 62.73% in AN group, with 43.28% in ANS and with 39.5% in ANL.

- Serum transaminases: GPTS activity increased with 324% in AN group, with 55% in ANS and with 115% in ANL group; GOTS activity increased with 39% in AN, and in the others two groups it had no significant modification. (Fig.2).

Histological, histochemical and histochemical parameters
- Haematoxylin-eosine staining:
In the intoxicated group, the liver histology has changed: it may be observed isolation of the lobules and division into fragments. The histological changes are dependent on the hepatocytes position in the hepatic lobules. Thus, in the peribular area (zone I acinar, after Rappaport, 1958), there were found hepatocytes with increased volume (swollen hepatocytes, or macrohepatocytes), with hypertrophic nucleus and nucleolus. In the centrolobular area (zone III acinar), which is the most injured, there are many necrotic and steatosic cells, with picnotic nuclei and collagen proliferation.

- Histological changes (haematoxilin-eosine stain) in the intoxicated liver look like cirrhotic type (Fig. 3 a, b, c, d). There is a “chemical hepatectomy” (Rusu et al., 2005). Alterations are similarly both in intoxicated and treated ANS and ANL group, but more reduced.

- Enzymes activity - both oxide-reduction enzymes (SDH - Fig. 4 a, b, c, d; LDH, GtDH, StDH, G6PDH, CyOx) activities and hydrolytic enzymes (ATP-ase and G6P-ase) activities were more decreased in AN group comparative with C group. In ANS and ANL groups the decrease of enzymatic reactions is not as high as in AN group.

- Sudan black staining for lipids: in AN group, the lipid droplets number is very increased, especially in centrolobular area forming an extended hepatic steatosis. ANS and ANL group have a reduced hepatic steatosis than C group.

Histoenzymological and histochemical results are shown in Table 1.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>C</th>
<th>AN</th>
<th>ANS</th>
<th>ANL</th>
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<tbody>
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<td>GtDH</td>
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<td>G6PDH</td>
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<td>CyOx</td>
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<td>G6P-ase</td>
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<tr>
<td>Black Sudan</td>
<td>0,5</td>
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a = control group; b = AN group which received aminopyrine + sodium nitrite; c = ANS group which received aminopyrine + sodium nitrite + Silimar; d = ANL group which received aminopyrine + sodium nitrite + Legalon.

Intensity of enzymes reaction: 0 = negative reaction; 0.5 = reduced reaction; 1 = moderate reaction; 1.5-2 = intense reaction; 2.5 = very intense reaction
The action of some antioxidant compounds with flavono lignanes (Silimar in and Legalon) on the rat liver intoxicated with N-nitrosodimethylamine (NDMA)

DISCUSSIONS

Nitrosamines are bearing from those two precursors –nitrates + amine group, generally into an milieu acid. They are very toxic, even carcinogenic (Bartsch and Spiegehalder, 1996). One of most toxically nitrosamines is NDMA, which may induce centrolobular necrosis in animals. Toxic properties of NDMA were discovered by Barnes and Magee in 1954. NDMA is a priority pollutant (Mitch et al., 2003) in meat and fish cured with nitrite, beer, tobacco smoke, rubber products, in air, water, etc. Some researchers suggested that NDMA formed in polluted atmosphere could be responsible for increased urban cancer rates. US-FDA reduces the concentration of nitrite for curing meat to maximum 120 ppm. FAO recommend a tolerable daily dose of 30 mg nitrite/70 kg body weight for men.

IARC classifies NDMA in A group (probably carcinogen for men). NDMA pass through placenta from mother to fetus, and also in maternal milk. Several meat processors add reducing agents like ascorbic acid, to quench nitrosating agents, and minimize in vivo NDMA formation (Bogovski, 1980; Mitch et al., 2003).

In these conditions we made an experiment in which NDMA is endogen produced (in rat’s stomach) from aminopyrine and sodium nitrite precursor. It was a chronic intoxication, for 30 days. We studied biochemical, histological, histoenzymological and histochemical changes occurring in the liver, a major target organ for NDMA, and the effects of drugs Silimar in and Legalon containing flavonolignans compounds (Basaga, 1997) with antioxidant property. These flavonoides are part of the great group of polyphenols with multiple actions on the body (Leng-Pescholv, 1996; Dehmolow et al., 1996). The most evident proofs for DMNA production in rat body are the results obtained. Thus, survival in AN group is of 25%. The glycogen level decreases very much, and GPTS activity increases with very high values, which means that a very intense process of hepatocytolisis has taken place.

The liver histology (structure, architecture) is very changed in the intoxicated group. A process of mosaic hepatocyte injury depending on position within the hepatic lobules (acinus) is observed. In the centrolobular area the hepatocytes are most affected. Changes occurring in the centrolobular area are more intense than in perilobular area. A result of this hepatic parenchyma injury was the isolation of lobules and division into fragments and collagen proliferation. These modifications correspond to a cirrhotic-type alteration. Also it is observed a true chemical hepatectomy (Rusu, 2004; Rusu et al., 2005).

Structural alterations of the hepatocytes in intoxicated rats are accompanied by modifications of the activities of the majority studied enzymes. Thus, these oxide-reduction and hydrolytic enzymes with diverse localization: mitochondrial, membranal, cytoplasmatic, etc, are involved in vital metabolically processes (Krebs cycle, terminal respiration, and carriage through membrane, etc). Diminution of their activities proves their injurious effects on the hepatic metabolism, as well as the NDMA complex toxic action. The decrease of enzymatic activity is determined both of parenchyma injuries, when...
sometimes remain only isle of live hepatic tissue and of enzymes activity inhibition in remaining parenchyma. In NMDA intoxicated group is manifested an important hepatical injury generally characterized by: increased mortality, a large hepatocitolisis, destruction of hepatic architecture, diminution of some important enzymes activity, etc. In the case of NDMA used in our experiments, a highly reactive free radical, results, diazomethane, that can methylate and destroy hepatic DNA (Kamendulis and Corcoran, 1995; Souliotis et al., 2002), and affect other cellular organelles.
The action of some antioxidant compounds with flavonolignanes (Silimarlin and Legalon) on the rat liver, intoxicated with N-nitrosodimethylamine (NDMA)

Administration of those two drugs, Silimarlin and Legalon that contain silimarlin had evident positive effects: a more increased survival rate, a hepatocitolyis reduced several times, less destroyed hepatic architecture, less reduced enzymatic activity. Silimarlin is a strong antioxidant that contains many flavonoides (flavonolignanes) with antioxidant activity, antifibrotic, necrotrop, lipotrop, and membrane protection, obstruction of some free radicals and xenobiotics access in cells (Luper, 1998; Kropacova et al., 1998; Bode, 1999; Rusu et al., 2005, 2007).

We consider that positive effects of Silimarlin and Legalon in NDMA hepatic toxicosis are a result of more factors:
- reduction of nitrozation process in rat’s stomach, even though those two drugs were administered in short time after NDMA precursors administration;
- Due to antioxidant properties of flavonolignanes, are partially neutralized the free radicals resulted from DNMA metabolisation.
- Cellular membrane protection by flavonolignanes limitetes the access of toxins in hepatic cells.
- Due to Silimarind antifibrotic activity, the collagen infiltrations are reduced.

CONCLUSIONS
Silimarin and Legalon treatment in intoxication with aminopyrine + Na nitrite conditions due to NDMAS formation, which produced grave structurally and functional hepatic injuries, had certain hepatoprotective qualities, concerning more parameters:
- A greater survival rate for rats;
- Glycogen level closer to control group;
- reduced hepatocytolysis;
- Reduction of hepatic steatosis;
- Reduction of hepatic architecture injuries comparative with intoxicated group; keeping an enzymatic activity closer to control group;
- It doesn’t seem to be any important modification between the actions of those two drugs, Silimarin and Legalon.

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