AN EXPERIMENTAL STUDY REGARDING THE BIOLOGICAL EFFECTS OF MINERAL WATER FROM SPRING 3 IN BĂILE TUŞNAD ON SOME ORGANS AFTER ETHYL ALCOHOL ADMINISTRATION

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ABSTRACT

Hepatobiliary and renal disorders are currently on the increase, being favored by increasing environmental pollution, alcohol consumption and synthesis drugs. Mineral water from spring 3 in Băile Tuşnad, with a total mineralization of 3351.0 mg/l, is recommended in chronic liver, gallbladder, pancreas diseases, kidney diseases and stones. This study aimed to assess potential changes in the liver, kidney, pancreas and stomach following ethyl alcohol administration in rats, as well as to monitor anatomopathological differences between animals that drank tap water and those that drank Tușnad mineral water, after cessation of ethyl alcohol administration. The study was carried out on 25 white Wistar rats over a period of 100 days. The animals were divided into 3 groups: group I, negative control group – 5 animals; group II, positive control group – 6 animals; group III, experimental group – 14 animals. The animals of group I received tap water (50-75 ml/day/animal) throughout the experiment, and those of groups II and III were administered ethyl alcohol 12% (12-15 ml/day/animal) during the first 70 days. During the last 30 days of the experiment, the animals of group II received tap water (50-75 ml/day/animal), and those of group III were administered Tuşnad mineral water (50-75 ml/day/animal). On experimental day 70, 5 animals were euthanized (2 of group I, 1 of group II and 2 of group III), and on day 100, the rest of 20 animals were euthanized. Fragments in the form of 4 mm thick slices from the liver, kidneys, pancreas and stomach of the euthanized animals were collected for histological investigations. The only changes detectable by optical microscopy were present in the liver. The rest of the studied organs did not show lesion aspects detectable by optical microscopy. The structural changes found in the liver were represented by mild to moderate fibrosis around the centrilobular venule in about 50% of the lobules. In the outer third of the lobules with centrilobular venular fibrosis, lipid hepatosis aspects were present. The degree of liver involvement in group II animals both on day 70 and on day 100 of the experiment was comparable to that of group III animals on experimental day 70. On experimental day 100, the degree of liver involvement in group III animals was lower compared to day 70, supporting the fact that mineral water from spring 3 in Baile Tuşnad potentiated the regenerative capacity of the liver.

Key words: carbonated mineral water, optical microscopy, liver, experimental studies

INTRODUCTION

Hepatobiliary and renal diseases are currently on the increase, being favored by increasing environmental pollution, alcohol consumption and synthesis drugs. Balneotherapy is a treatment method that uses natural therapeutic factors (mineral/thermal water, mud, therapeutic gases – mofettes, solfataras) with researched and recognized curative properties, based on their chemical, mechanical and thermal effects on the organism, which are found in climatic spas [1].

Mineral waters are complex solutions of mineral salts or gases that cannot be reproduced in laboratory, which are formed in the earth's crust through the dissolving action of underground water on the rocks with which it comes in contact [1]. Mineral waters can be administered as internal or drinking treatment (crenotherapy) or external treatment (balneation). Crenotherapy is used in digestive, biliary, hepatic, metabolic diseases, diabetes mellitus, obesity, hyperuricemia, gout, renal disorders [2].

Over the years, many studies regarding the beneficial effects of mineral waters on the organism have been conducted. The study "The action of Covasna main spring water on gastric secretion" found that this water, which has a weakly mineralized, carbonated, chlorine, sodium, calcium, magnesium composition, exerts an excito-secretory action on the stomach of both animals and humans [3]. A study regarding the action of the Călimăneşti 8 sulfurous chlorinated mineral water on subacute experimental hepatitis induced by carbon tetrachloride administration showed less intense lesions in the group that drank mineral water after cessation of the toxic agent administration [4]. The Malnaş mineral water, which has a hypotonic, carbonated, calcium, sodium, magnesium composition, exerts a quantitative excito-secretory action on animals with Pavlov stomach. Through degasification, secretion and acidity are lower in animals with Pavlov stomach [5]. In an experiment on animals with biliary fistula, the alkaline-terrous, carbonated, hypotonic Biborţeni water had an intense manifest excito-secretory action, particularly within an hour of administration, diluting bile secretion [6].

We found no experimental literature studies that monitor by optical microscopy investigations the effects of mineral water from spring 3 in Băile Tuşnad on some organs in animals that were previously administered ethyl alcohol, so that this study is a national first.

This paper aimed to assess potential changes in the liver, kidney, pancreas and stomach following ethyl alcohol administration in rats, as well as to monitor anatomopathological differences between animals that drank tap water and those that drank Tuşnad mineral water, after cessation of ethyl alcohol administration.

Material and method

The animals were kept under standard vivarium conditions, according to European Directive 63/2010 and Law 43/2014 on the protection of animals used for scientific purposes, and they were euthanized by intramuscular injection of an anesthetic overdose (ketamine 10% and xylazine 2%). This experimental study was approved by the Ethics Commission of the University of Medicine and Pharmacy Cluj-Napoca no.533/23.12.2015.

Experimental animals: 25 white male Wistar rats with a weight of about 350 g.

Materials: ethyl alcohol 12%, mixed carbonated mineral water from spring 3 in Băile Tuşnad, tap water.

The natural mineral water from spring 3 in Băile Tuşnad has a total mineralization of 3351.0 mg/l, it contains sodium chloride (Cl ions 617 mg/l), HCO3 378.2 mg/l, traces of bromine, sodium 288 mg/l, potassium 76 mg/l, magnesium 35.5 mg/l, iron (Fe 17.3 mg/l), carbon dioxide (CO2 1647.0 mg/l), calcium (Ca 93.8 mg/l), boron, it is weakly iodinated, acidulated, with a pH of 6, and a temperature of

14.5°C. Water from spring 3 in Băile Tuşnad is recommended in chronic liver, gallbladder, pancreas diseases, kidney diseases and stones [2].

Experimental model

The study was performed on 25 white Wistar rats over a period of 100 days.

The animals were divided into 3 groups:

-group I – negative control group – 5 animals;

-group II – positive control group – 6 animals

-group III - experimental group – 14 animals.

The animals of group I received tap water (50-75 ml/day/animal) throughout the experiment, and those of groups II and III were administered ethyl alcohol 12% (12-15 ml/day/animal) during the first 70 days. During the last 30 days of the experiment, the animals of group II received tap water (50-75 ml/day/animal), and those of group III were administered Tuşnad mineral water (50-75 ml/day/animal).

On day 70 of the experiment, 5 animals were euthanized (2 of group I, 1 of group II and 2 of group III), and on day 100, the rest of 20 animals were euthanized. Fragments in the form of 4 mm thick slices from the liver, kidneys, pancreas and stomach of the euthanized animals were collected for histological investigations.

The collected fragments were fixed by immersion in Stieve mixture for 30 hours. At the end of fixation, the samples were dehydrated in ethyl alcohol (3 baths with a 70%, 96% and 100% concentration of 1 hour each) and cleared in butyl alcohol (2 baths of 24 hours each). Subsequently, these were infiltrated with paraffin at 56°C (3 baths of 1 hour each), cast in paraffin blocks, and after paraffin solidification, serial 5 µm thick sections were cut. The sections were displayed on slides and stained using the Goldner trichrome technique, the method of choice for collagen fibers, which stained green-blue [7,8]. The preparations were examined under the optical microscope (Olympus BX 41) and photographs were taken (Olympus E 330). Subsequently, the photos were processed in an image editing program (Adobe Photoshop CS 2.0.)

Results

In group I, the architecture of liver lobules has a normal appearance (Fig. 1). No inflammatory changes are seen inside the lobules or in perilobular spaces. Also, hepatocytes do not show degenerative or fat overload aspects.

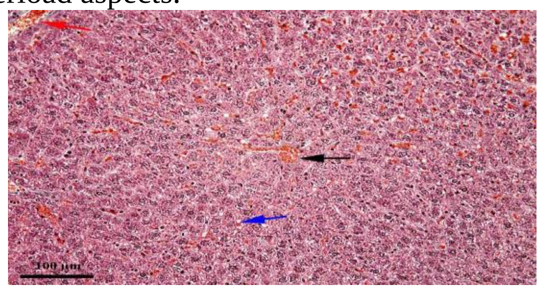

Fig. 1 Liver in group I , Goldner trichrome stain
black arrow – centrilobular venule, red arrow – portobiliary space, blue arrow – sinusoid capillary

In the kidneys of group I, no changes in nephrons (Fig. 2) or collecting tubules (Fig. 3) are seen. In renal corpuscles, Bowman's space appears to be normally sized, and in nephron tubules and collecting tubules, no aspects suggesting renal failure are observed. Also, there are no congestive-hemorrhagic aspects in the renal parenchyma.

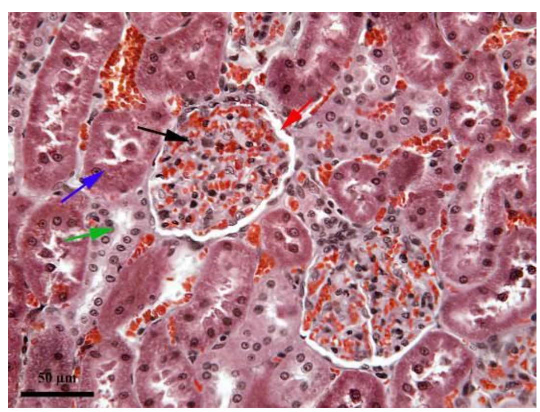

Fig. 2 Kidney in group I – cortical labyrinth, Goldner trichrome stain
black arrow – renal glomerulus, red arrow – Bowman's space, blue arrow – proximal convoluted tubule, green arrow – distal convoluted tubule

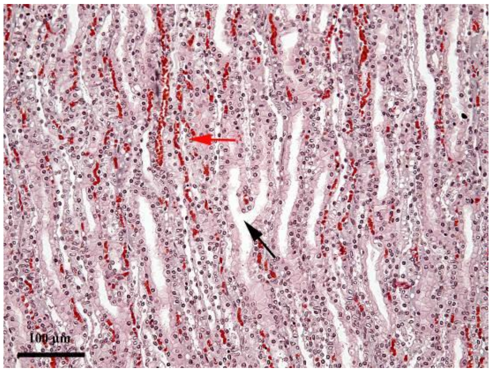

Fig. 3 Kidney in group I – Malpighi pyramid, Goldner trichrome stain
black arrow – collecting tubule, red arrow – renal interstitium

In both the exocrine and endocrine component of the pancreas in group I rats (Fig. 4), there are no pathological aspects suggestive of pancreatic failure.

The stomach of rats in the control group has a normal appearance. The surface and glandular epithelium of the gastric mucosa (Fig. 5) shows no erosions or ulcers.

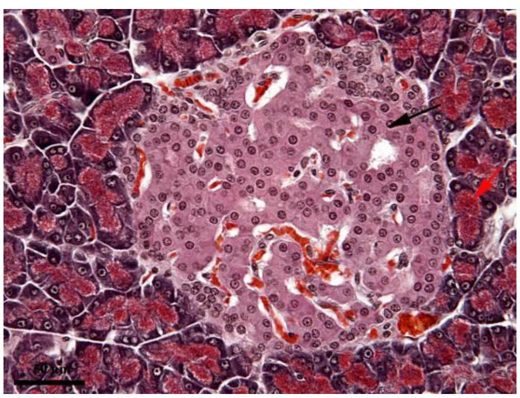

Fig. 4 Pancreas in group I, Goldner trichrome stain
black arrow – islet of Langerhans, red arrow – serous acinus

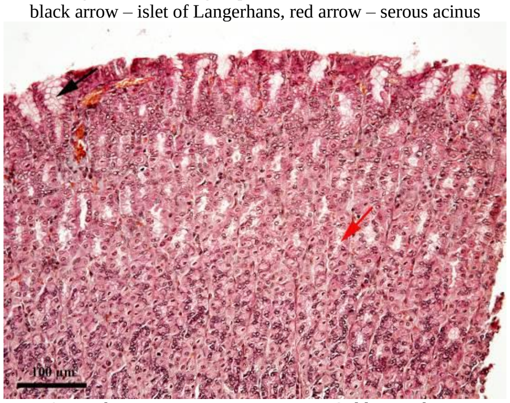

Fig. 5 Stomach in group I – gastric mucosa, Goldner trichrome stain
black arrow – gastric crypt, red arrow – fundic glands

In rats of groups II and III on experimental day 70, as well as in rats of group II on day 100, the architecture of liver lobules is comparable to that of rats in the control group, but about 50% of the lobules show mild (Fig. 6) to moderate (Fig. 7) fibrosis around the centrilobular venule. The connective tissue proliferated around the centrilobular venule is young, because proliferated collagen in this area is represented by very thin fibers. The fibrosis process does not seem to be completed, because in the connective tissue, some euchromatic nuclei that seem to be specific to fibroblasts can be seen. The presence of young connective cells (fibroblasts) indicates that the connective proliferation and consolidation process has not ended. The relatively small number of fibroblasts shows that the rate of this fibrosis process is not very high.

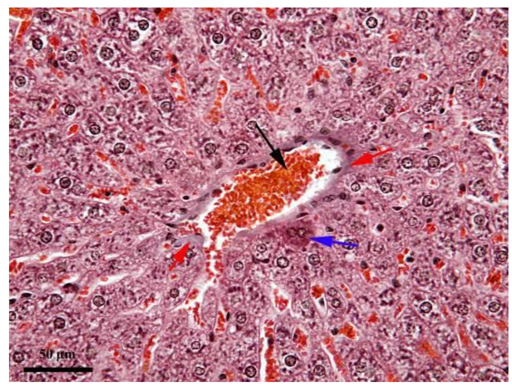

Fig. 6 Liver in group II, Goldner trichrome stain
black arrow – centrilobular venule, red arrow – fibrosis area, blue arrow – apoptotic hepatocyte

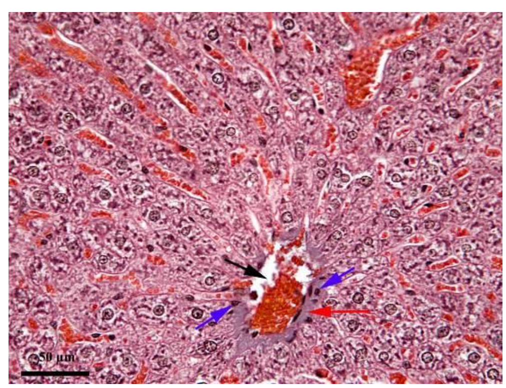

Fig. 7 Liver in group II, Goldner trichrome stain
black arrow – centrilobular venule, red arrow – fibrosis area, blue arrow – fibroblasts

Also, in lobules with more marked fibrosis in their outer third, lipid hepatosis is seen (Fig. 8).

Lipid droplets are well defined and variable in size, which gives the cytoplasm a foamy appearance. These aspects, i.e. overloading of hepatocytes, have led to an eccentric dislocation of the nuclei of the affected hepatocytes. The degree of hepatocyte involvement is somewhat directly proportional to the degree of centrilobular venular fibrosis.

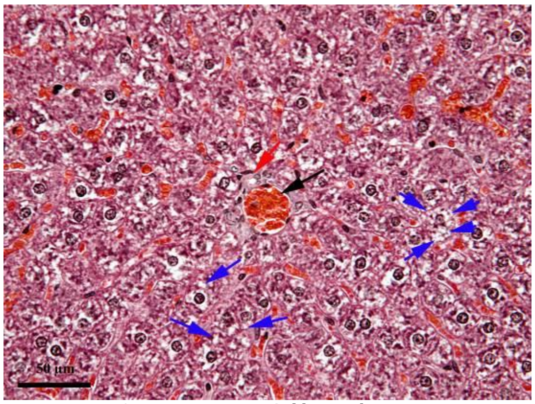

Fig. 8 Liver in group II, Goldner trichrome stain
black arrow – perilobular venule, red arrow – bile canaliculus, blue arrow – lipid inclusions in hepatocytes

Inside the lobules and in perilobular spaces, there are no polymorphonuclear or mononuclear inflammatory aspects, and the fibrosis process is not accompanied by necrotic lesions. Rare apoptotic hepatocytes are seen, without exceeding the normal rate of apoptotic cells.

In rats of group III on experimental day 100, aspects are similar to those described in groups II and III at 70 days, but it seems that both centrilobular venular fibrosis (Fig. 9) and the degree of overloading of hepatocytes with lipids (Fig. 10) in the outer third of the lobules are more reduced.

The rest of the studied organs do not show structural changes detectable by optical microscopy, the aspects being similar to those of the control group.

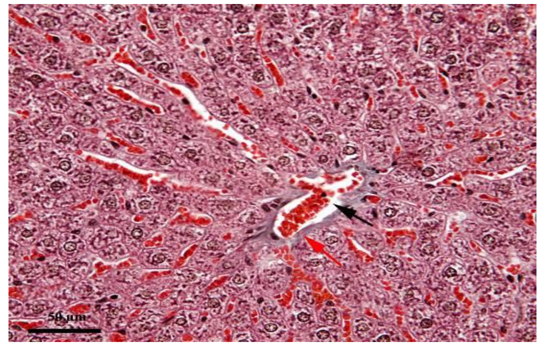

Fig. 9 Liver in group III, Goldner trichrome stain
black arrow – perilobular venule, red arrow – fibrosis area

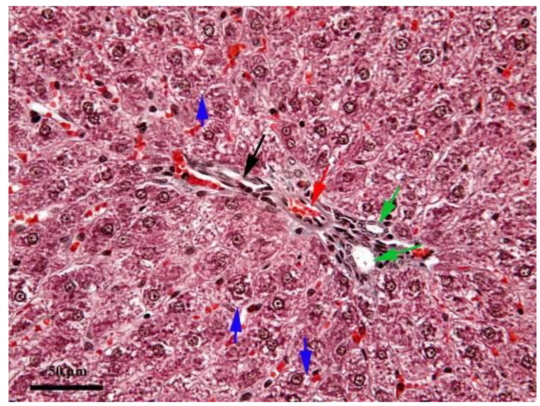

Fig. 10 Liver in group III, Goldner trichrome stain
black arrow – perilobular venule, red arrow – perilobular arteriole, green arrow – bile canaliculus, blue arrow – lipid inclusions in hepatocytes

Discussions

The lesion aspects identified in the liver of rats in the experimental groups are partially similar to those described by Keegan et al. (1995) [9] in rats and by Song et al. (2005) [10] in mice, but without the presence of inflammatory and necrotic aspects in the liver tissue. Hepatic steatosis accompanied by moderate inflammatory reaction was also reported by Kerai et al. (1999) [11], Liedtke et al. (2013) [12], Louvet and Mathurin (2015) [13] in animals that consumed alcohol. Hepatic steatosis developed following ethyl alcohol consumption was reported in rats by electron microscopy investigations [14].

Conclusions

The only changes detectable by optical microscopy were present in the liver. The rest of the studied organs did not show lesion aspects detectable by optical microscopy.

The structural changes identified in the liver consisted of mild to moderate fibrosis around the centrilobular venule in about 50% of the lobules.

In the outer third of lobules with centrilobular venular fibrosis, lipid hepatosis aspects were present.

The degree of hepatocyte involvement in the outer third of the lobules was directly proportional to the degree of centrilobular venular fibrosis.

Centrilobular venular fibrosis and lipid hepatosis were not accompanied by necrotic and inflammatory processes.

The degree of liver involvement in group II animals on experimental days 70 and 100 was comparable to that of group III on experimental day 70.

On experimental day 100, the degree of liver involvement in group III rats was more reduced compared to day 70, which supports the fact that mineral water from spring 3 in Baile Tuşnad potentiated the regenerative capacity of the liver.

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