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Histopathological assessment of C57Bl/J mice organs exposed to tannery effluents

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ABSTRACT

The effluent produced in tanneries can cause environmental damage and public health problems when disposed of improperly. However, few toxicological studies have evaluated the effects of the intake of tannery wastewater by mammals. The objective of this study is the histological assessment of organs of C57Bl/J mice exposed to the intake of different concentrations of raw tannery effluents, beginning with the hypothesis that these effluents can cause damage to the histological structure of the organs. The animals were divided into the following groups: control (0% effluent) and exposed to 0.1%, 1%, and 5% raw tannery effluent diluted in water, for a period of 120 days. For the histopathological evaluations, samples of the liver, kidney, spleen, heart, and lung were collected, fixed, and stained by the hematoxylin and eosin staining techniques. No anomalies were observed in kidney, spleen, heart, and lung fragments. Alterations were observed only in liver fragments. Moderate hydropic degeneration was detected in animals exposed to tannery effluents, mainly in the periportal space. A large number of necrotic hepatocytes ($p<0.05$) were detected, especially in animals exposed to higher tannery effluent concentrations. Further, the largest number of hepatocytes with karyomegaly ($p<0.05$) was observed in animals exposed to the highest effluent concentrations. Our study suggests that the observed alterations are related to the intake of tannery effluents and that these effluents cause changes that lead to an increase of free radical production and direct aggressions to hepatocyte membranes as well as to the appearance of hepatocellular karyomegaly.

Keywords: agro-industrial residues, animal model, histopathology, mice.

Avaliação histopatológica de órgãos camundongos C57Bl/6J expostos a efluentes de curtume

RESUMO

Os efluentes produzidos nas indústrias curtumeiras podem causar danos ambientais e problemas de saúde pública quando descartados de forma inadequada. Porém, poucos estudos avaliaram os efeitos da ingestão de efluentes de curtumes em modelos experimentais mamíferos. O objetivo deste estudo foi realizar uma avaliação histológica de órgãos de camundongos C57BL/6J expostos à ingestão de diferentes concentrações de efluentes de curtumes, partindo da hipótese de que estes efluentes poderiam causar danos à estrutura histológica dos órgãos escolhidos para o estudo. Os animais foram divididos nos seguintes grupos: controle (0% de efluente) e expostos às porcentagens 0,1%, 1% e 5% de efluente bruto diluído em água, por um período de 120 dias. Para as avaliações histopatológicas, foram coletadas amostras de fígado, rim, baço, coração e pulmões, fixados e corados pela técnica de coloração com hematoxilina e eosina. Não foram observadas anomalias nos fragmentos de rim, baço, coração e pulmões. Foram observadas alterações apenas em fragmentos de fígado. Degeneração hidrópica moderada foi detectada em animais expostos à efluentes de curtumes, principalmente no espaço periportal. Foi detectado um grande número de hepatócitos necróticos ($p<0,05$), em especial em animais expostos às concentrações mais elevadas de efluentes de curtume. Observou-se o maior número de hepatócitos com cariomegalia ($p<0,05$) em animais expostos às concentrações mais elevadas de efluentes. Nossos estudo evidência que as alterações observadas estão relacionadas com a ingestão de efluentes que podem levar ao aumento da produção de radicais livres e agressões diretas para membranas de hepatócitos, bem como o aparecimento de cariomegalia hepatocelular.

Palavras-chave: resíduos agroindustriais, modelo animal, histopatologia, camundongos.

1. INTRODUCTION

Industrial processes and human activities typically generate specific wastes that are composed of many different substances. Depending on the nature of these substances, such wastes can be harmful to the environment and to human health (Kraemer, 2006). In opposition to the amenities of modern society, the problems caused by such wastes are a serious threat to the current quality of life (Silva et al., 2012). The various types of waste generated include those that are produced by profitable industrial activities, such as bovine skin processing.

While this activity generates significant profits that contribute to the economic and social development of a country, it has been the subject of concern, primarily due to the production of a large amount of waste/effluent during bovine leather processing. As discussed by Gödecke et al. (2012), the leather tanning process requires several mechanical and chemical treatment processes that result in large amounts of waste as well as high concentrations of organic matter and various potentially toxic chemicals.

This problem is intensified by the fact that in many tanneries wastes/produced effluents are discharged into waterways incorrectly, causing a high risk of environmental contamination (Pacheco, 2005; Pinheiro, 2005; Ganem, 2007; Gödecke et al., 2012). Even after treatment in a treatment plant, tannery contains considerable organic and inorganic fillers such as acids, phenols, sulfates, and sulfides and toxic elements such as chromium, which is used during the tanning process (Batista e Alovisi, 2010).

To assess the impact of these effluents on organisms, toxicological studies have demonstrated teratogenicity in sea urchin species, the reduction of growth in microalgae, and a variety of toxic effects in micro-crustaceans (Oral et al., 2007). However, it is important to

consider that while these organisms are suitable for determining lethality, toxicity must be evaluated in other ways because other signs and/or symptoms can be found in mammals. This limitation hinders the extrapolation of the obtained results to human beings. With respect to the effects of exposure to tannery effluents in experimental mammalian models, we highlight the work of Siqueira et al. (2011) and Moysés et al. (2014). In the first study, the authors demonstrated that Swiss mice that were exposed to different concentrations of tannery effluent diluted in water for 21 days exhibited a state of anxiety. However, Moysés et al. (2014) studied the neurotoxicity and hepatotoxicity induced by chronic exposure to tannery effluents and anxiety behaviors that are predictive of depression and memory changes. Those authors failed to observe changes in the evaluated parameters in rats.

Moreover, the analysis of the health of workers exposed to organic solvents and heavy metals associated with tannery effluents or wastes is widely discussed in the field of occupational toxicology (Shahzad et al., 2006; Salazar, 2008; Cuberos et al., 2009; Rumin et al., 2013); however, few basic studies investigated the effects of such wastes in an animal model.

Thus, the objective of this study was the histological assessment of different C57BL/6J mice organs exposed to water containing various raw tannery effluent concentrations, beginning with the hypothesis that these effluents can cause damage to the histological structure of the organs. We are not aware of any such study in the literature, and therefore this paper is a contribution to research on the effects of tannery effluents in the homeostasis of an organism.

2. MATERIAL AND METHODS

Male C57BL/6J mice obtained from the animal house of the Tropical Pathology and Public Health Institute of Goiás (Goiânia, GO, Brazil) were maintained in the Animal Laboratory of Biological Research of the Federal Institute of Goiás – Campus Urutaí (Urutaí, GO, Brazil). The animals were exposed to a normal light/dark cycle, and food and liquids were available *ad libitum*. A total of 32 animals, which were 21 days old, were divided into four groups: the control group, in which the animals received only drinking water that contained 0% tannery wastewater (see characterization in Table 1), and groups 0.1%, 1% and 5%, which received raw tannery effluent diluted to the indicated concentrations in water. The animals were chronically exposed to the indicated concentrations of the raw tannery effluent, whose chemical characterization is presented in Table 1. The methodology of this study was consistent with the principles for ethical animal experimentation and was approved by the Ethics Committee for Animal Use of the Federal Institute of Goiás, GO, Brazil (protocol n. 18/2014).

The tannery effluent used in this study was obtained from a tannery located in Pires do Rio, GO, Brazil. The effluent provided by the tannery did not contain the element chromium because the effluent was removed from the steps of river operations (i.e., the steps prior to the tanning phase of bovine leather, which uses chromium salts). We chose to use this type of effluent because many tannery industries dispose of these wastes directly into the waterways that border the properties.

The animals in groups 0.1%, 1%, and 5% were chronically exposed to raw tannery effluents diluted in water for 120 days. After this period, the animals were euthanized. They were anaesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital, followed by cervical dislocation.

For the histopathological evaluations, the liver, kidney, spleen, heart, and lung were collected and fixed in 10% buffered formalin, embedded in paraffin blocks, and thin-sliced to 5 µm thickness (Luna, 1968). The hematoxylin and eosin staining technique (HE) was used,

following Behmer et al. (1976). The thin-session descriptions were carried out using a bright-field Carl Zeiss® optical microscope, model Jenaval, in order to compare the tissue structures of the organs removed from animals of the different experimental groups.

Table 1. Characterization of the raw tannery effluent, which was diluted in water at different concentrations and offered to C57BL/6J mice.

Parameters[1]	Tannery effluent	Potable water
pH at 25ºC (UpH)	8.19	7.19
Turbidity (NTU)	382.00	<1.00
Turbidity ammonia nitrogen ($mg.L^{-1}$)	2.10	0.01
Total Nitrogen ($mg.L^{-1}$)	110.00	1.20
Nitrate ($mg.L^{-1}$)	23.00	0.30
Electrical conductivity at 25º C ($\mu S.cm^{-1}$)	72.10	52.00
Total phosphorus ($mg.L^{-1}$)	33.61	0.11
Orthophosphate ($mg.L^{-1}$)	77.09	0.26
Biochemical oxygen demand (BOD) ($mg.L^{-1}$)	9,333.33	0.50
Total solids ($mg.L^{-1}$)	82,190.00	30.00
Dissolved Copper ($mg.L^{-1}$)	<0.01	0.04
Dissolved Manganese ($mg.L^{-1}$)	<0.10	ND
Dissolved iron ($mg.L^{-1}$)	1.91	0.09
Zinc ($mg.L^{-1}$)	<0.01	1.06
Sodium ($mg.L^{-1}$)	5,680.00	5.01
Magnesium ($mg.L^{-1}$)	243.20	1.21
Calcium ($mg.L^{-1}$)	2,805.00	4.00
Sulfur ($mg.L^{-1}$)	833.33	1.00
Potassium ($mg.L^{-1}$)	122.00	1.60
Total organic carbon (TOC) ($mg.L^{-1}$)	**93.32**	**8.20**

[1]The analysis of the raw tannery effluent and water followed the methodology recommended by the American Public Health Association (APHA et al., 2005). All analyses were performed in a commercial laboratory located in Goiania, GO, Brazil.

The alterations in affected organs were analyzed quantitatively. Specific counts for these alterations were carried out under the microscope using ten randomly chosen fields per thin section for each animal and 40x objective and 10x ocular lenses (magnitude 400x). The images were captured using the image analysis system TSView version 7.1.

Quantitative data were analyzed by analysis of variance (one-way ANOVA) with Tukey's test at 5% probability. The residual normality was verified using the Shapiro-Wilk test, and Bartlett's test was used to check the remaining homoscedasticity, using the R version 3.0.3 software (R Core Team, 2014).

3. RESULTS AND DISCUSSION

No anomalies were observed in the kidney, spleen, heart, and lung fragments (images not shown). Alterations were observed only in liver fragments. Moderate hydropic degeneration was detected, mainly in the periportal space, in animals that drank water containing 5% of tannery effluent (Figure 1).

Studies focusing on histopathological alterations in mice exposed to the intake of tannery effluents are lacking in the literature, which makes the discussion of the data difficult. However, we believe that this alteration is related to varied factors but mainly to agents or elements contained in the effluents that damage the hepatocyte membranes and organelles by means, for example, of the generation of free radicals. The high sodium concentration in the effluent used (Table 1) may have contributed to the retention of the element in the organism, increasing hepatocyte intracellular osmotic pressure, allowing water to enter the cytoplasm, and causing cell isosmotic expansion.

Moysés et al. (2014) analyzed the parameters related to the hepatic oxidative stress, by means of the quantification of free radicals and analysis of lipid peroxidation in the livers of Wistar rats exposed to effluent intake. The authors did not observe any alteration in these parameters. However, it is worth mentioning that in the study of Moysés et al. (2014), the animals were exposed to effluent intake for only 30 and 45 days, which differs from our study, in which the animals were exposed to the effluents for 120 days. Also, the type of tannery effluent used by Moysés et al. (2014) as well as the rodent species and lineage was different from that used here. On the other hand, studies involving microalgae (Ajayan e Selvaraju, 2012) and fish (Prabakaran et al., 2007) corroborate the hypothesis that tannery effluents can significantly affect the production of species that react with oxygen and consequently the production of free radicals, damaging the structure and function of vital organs.

Another aspect observed here was the high number of necrotic hepatocytes, mainly in animals exposed to higher tannery effluent concentrations (Figures 2 and 3), which can also be related to the alterations that lead to the increase in the production of free radicals and direct aggressions to the hepatocyte membranes, creating hydrophilic channels through which the cell loses electrolytes and dies. It is known, for example, that Kupffer cells also generate free radicals in answer to liver damage (Friedman e Arthur, 1989) and, in this case, such cells can have contributed to necrotizing phenomena that occurred in these animals. Additionally, a high number of hepatocytes with karyomegaly were observed in animals exposed to the highest tannery effluent concentrations (Figures 2 and 3). It is emphasized that single hepatocytes or groups of hepatocytes with strikingly enlarged nuclei and frequently with prominent nucleoli were evaluated as karyomegaly.

Until now, we have not found any study in the literature that reports the presence of hepatocytes with karyomegaly in rodents exposed to tannery effluents. However, some studies have shown that the presence of karyomegaly in renal tubule cells has been frequently associated with chemical carcinogenicity in rodents, no matter the genotoxic potential of the administered compounds (Kitaura et al., 1999; Lock e Reed, 2006; Inoue et al., 2008; 2009). Kitaura et al. (1999), analyzing Long-Evans rats, observed that the karyomegaly is a typical histological characteristic observed in (kidney) proximal tubular epithelium before tumor development. More recently, Enzmann et al. (2013) showed that different carcinogens (diethylnitrosamine, DEN, 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone, NNK), evaluated in turkey eggs for *in ovo* carcinogenicity assay (IOCA), were responsible for the appearance of different hepatic alterations, including the hepatocellular karyomegaly. Therefore, these data signal the potential of tannery effluents to present carcinogenic characteristics (i.e., they are able to cause tumors in the liver) when animals are exposed to chronic and longer intake of such effluents.

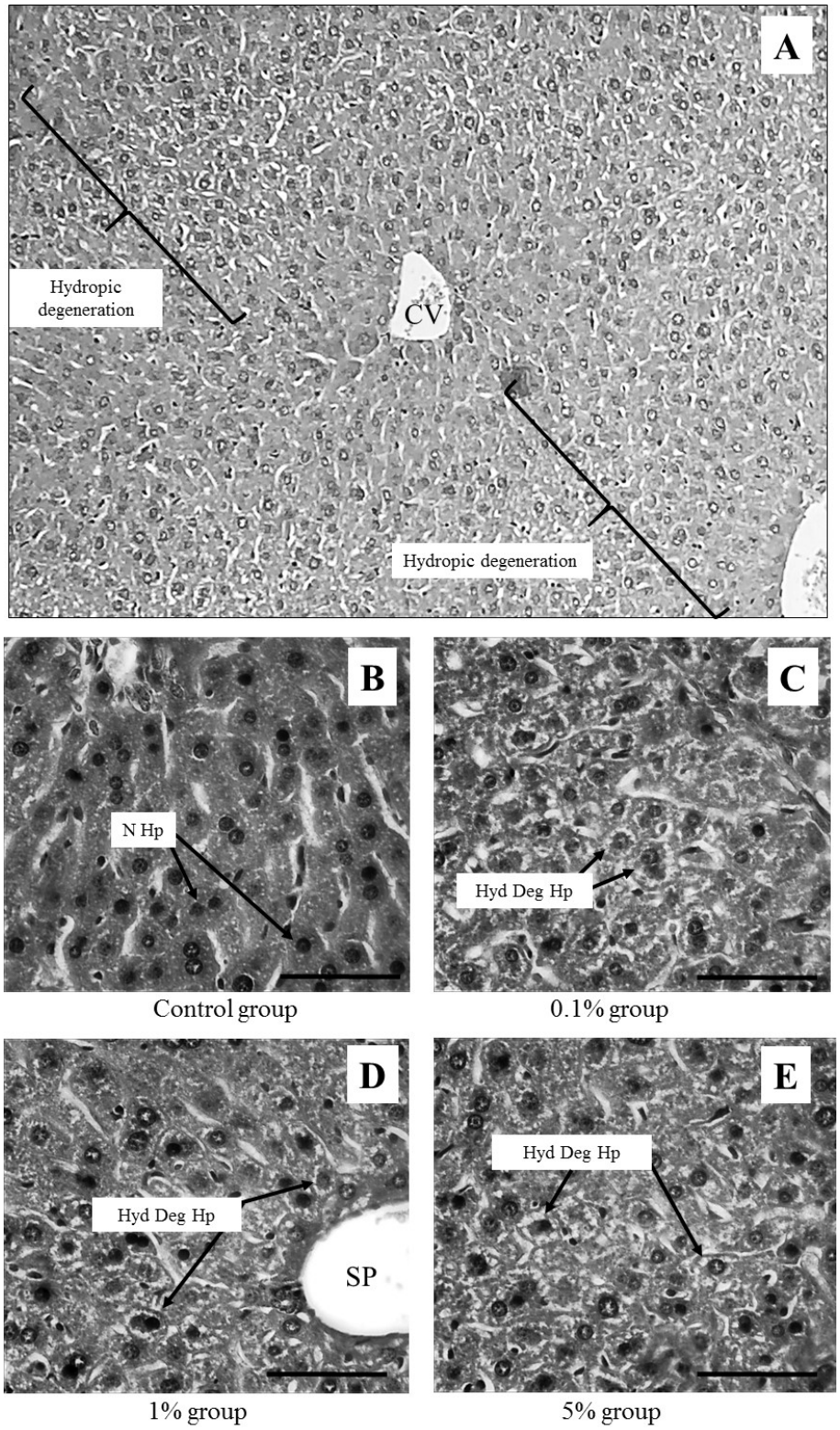

Figure 1. Photomicrographs of distinct liver regions (A-E) of C57BL/6J mice exposed to intake of tannery effluent in different concentrations diluted in filtered water. In A, a marked hydropic degeneration in the mediozonal and periportal region is observed (animal from group 5% of tannery effluent diluted in water). N Hp: Normal hepatocyte; Hyd Deg Hp: hepatocyte with hydropic degeneration; CV: centrilobular vein; SP: space port. Bar=100 µm. HE stain.

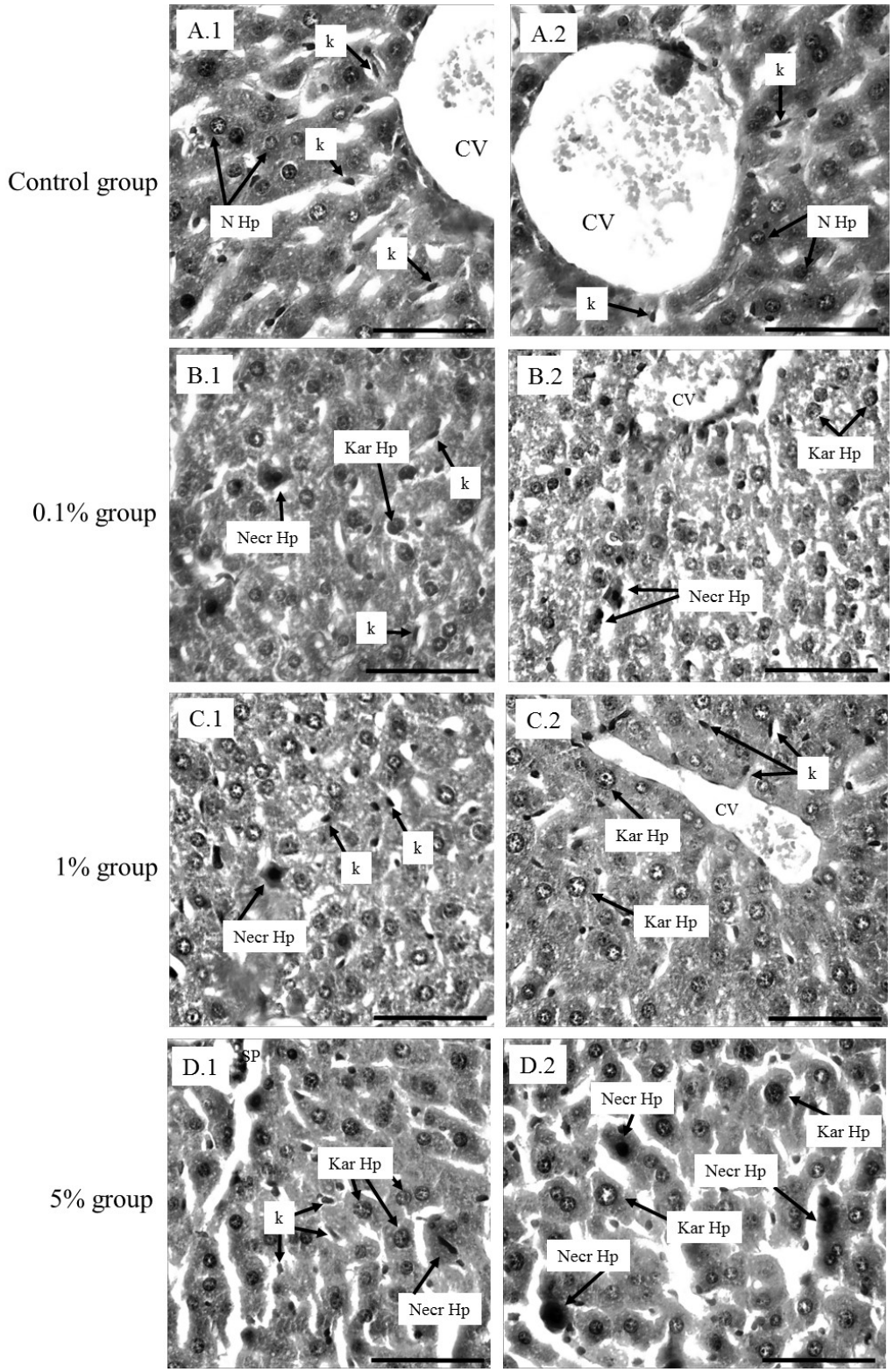

Figure 2. Photomicrographs representing distinct liver regions (A.1-D.2) of C57BL/6J mice exposed to intake of tannery effluents in different concentrations diluted in filtered water. A.1-A.2: control group; B.1-B.2: 0.1% group; C.1-C.2: 1% group; D.1-D.2: 5% group. N Hp: Normal hepatocyte; Kar Hp: karyomegaly hepatocytes; k: Kupffer cell; Necr Hp: Necrosis in hepatocyte; CV: centrilobular vein; SP: space port. Bar=50 µm. HE stain.

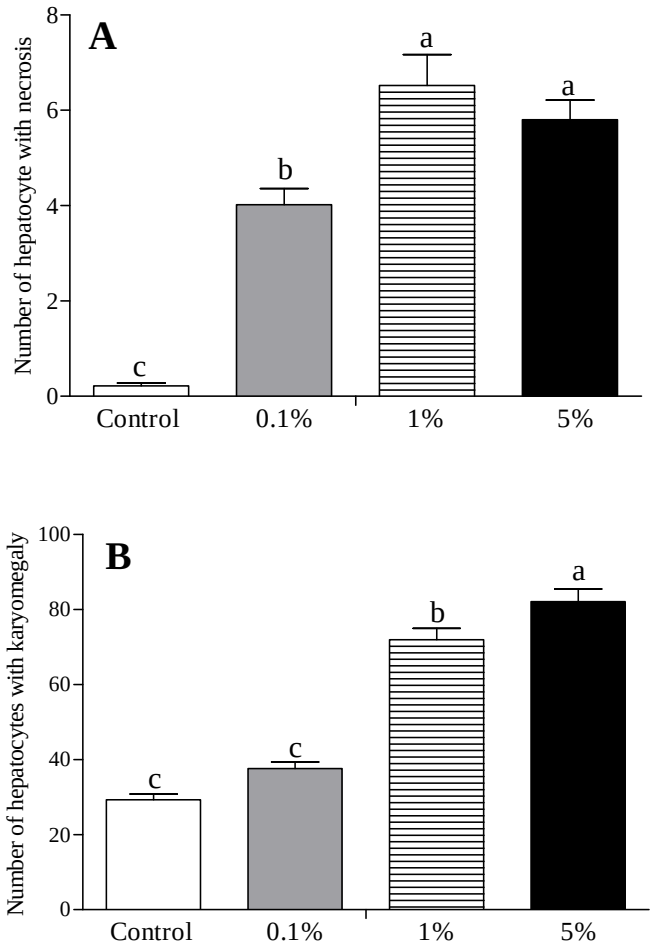

Figure 3. (A) Number of hepatocytes with karyomegaly and (B) with necrosis in liver of C57BL/6J mice exposed to intake of tannery effluents in different concentrations diluted in filtered water. Distinct letters indicate significant differences among the experimental groups by the analysis of variance (one-way ANOVA) at 5% probability. Bars indicate mean ± standard deviation (n=5).

4. FINAL CONSIDERATIONS

Our study is a contribution to the knowledge on how the intake of tannery effluents can affect mice hepatocytes. Our results suggest that the alterations we observed are linked to changes that lead to the increase of free radical production and direct aggressions to hepatocyte membranes as well as the appearance of hepatocellular karyomegaly. On the other hand, studies that analyze the effects of effluent intake in mammal experimental models are rare; therefore, this opens the perspective of research to elucidate the biologic mechanisms related to the physical, physiological and anatomopathological effects in these animals.

Concerning histopathological alterations, a field of interest would be to better understand and influence the species that react with oxygen such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals in the etiology of damage, especially if we consider that they are related to the toxicity of several xenobiotics and to the development of diseases.

5. ACKNOWLEDGMENTS

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