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Intermittent alcohol consumption changes the hepatocyte ploidy

O consumo intermitente de álcool altera a ploidia dos hepatócitos

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Abstract
Introduction: *Alcohol is mainly metabolised by hepatocytes in the liver. These cells are direct targets of stress, which can culminate in cellular adaptations earlier than alcoholic liver disease progression. Polyploidisation has been suggested as a genetic adaptation to hepatotoxic stress. This study aimed to investigate how intermittent alcohol administration could affect hepatocyte nuclear phenotypes.* ***Methodology:*** *rats were treated with 5% (v/v) and 20% (v/v) ethanol in drinking water overnight for 80 days. Thereafter, their liver was dissected and histologically processed. Histopathological analysis was performed using hematoxylin and eosin (H&E)-stained sections. Histomorphometric analysis of hepatocyte nuclei was performed in Feulgen-stained sections using ImageJ. The DNA content of nuclei was determined based on the integrated optical density values obtained from digital image analysis of liver sections stained with Feulgen stain.* ***Results:*** *severe congestion was observed in the group treated with 20% ethanol. No difference in fat accumulation in hepatocytes or infiltration of inflammatory cells was observed between the control and ethanol-treated groups. More nuclei with high DNA content (8C) were found in the ethanol-treated animals.* ***Conclusion:*** *it is concluded that alcohol induces hepatocyte polyploidisation, even at doses when typical signs of alcohol-induced liver disease, such as steatosis and hepatitis, are not significant.*
Keywords: *alcohol abuse; liver disease; hyperemia; polyploidy; hepatocytes.*

Resumo
Introdução: *o álcool é metabolizado principalmente pelos hepatócitos no fígado. Essas células devem ser alvos diretos de estresse, que pode culminar em adaptações celulares antes da progressão da doença hepática alcoólica. A poliploidização tem sido sugerida como uma adaptação genética ao estresse hepatotóxico. Este estudo teve como objetivo investigar como a administração intermitente de álcool poderia afetar os fenótipos nucleares dos hepatócitos.* **Metodologia:** *os ratos foram tratados com etanol a 5% (v/v) e 20% (v/v) em água potável durante a noite durante 80 dias. Posteriormente, o fígado foi dissecado e processado histologicamente. A análise histopatológica foi realizada em cortes corados com hematoxilina e eosina (H&E). A análise histomorfométrica dos núcleos dos hepatócitos foi realizada em seções coradas com Feulgen usando ImageJ. O conteúdo de DNA dos núcleos foi determinado com base nos valores de densidade óptica integrados obtidos a partir da análise de imagens digitais de cortes de fígado corados com Feulgen.* **Resultados:** *congestão severa foi observada no grupo tratado com etanol 20%. Nenhuma diferença no acúmulo de gordura nos hepatócitos ou infiltração de células inflamatórias foi observada entre os grupos controle e tratado com etanol. Mais núcleos com alto conteúdo de DNA (8C) foram encontrados nos animais tratados com etanol.* **Conclusão:** *esses resultados mostram que o álcool induz a poliploidização dos hepatócitos, mesmo em doses em que os sinais típicos da doença hepática alcoólica, como esteatose e hepatite, não são significativos.*
Palavras-chave: *abuso de álcool; hepatopatias; hiperemia; poliploidia; células hepáticas.*

INTRODUCTION

Alcohol use disorder (AUD) is a worldwide public health problem characterised by a behavioural disorder influenced by genetic, environmental, and psychosocial factors[1]. The global ethanol consumption is approximately 6.4 litres per person each year, and 43% of people are current drinkers[2]. These data support that the incidence of chronic alcohol consumption is high in the actual society and, therefore, may be the cause of a wide range of known systemic pathologies. Alcohol-induced liver disease (ALD) is an important cause of death due to excessive drinking[3].

Alcohol is oxidised to acetaldehyde through metabolic pathways, especially in the liver. Hepatocytes express high levels of ethanol-oxidizing enzymes and, thus, play a key role in alcohol metabolism. However,

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chronic exposure to alcohol causes hepatocyte injury. Accelerated alcohol metabolism generates a large amount of reactive oxygen species (ROS), which play an important role in liver pathology. Excess levels of ROS can damage DNA and induce mutations[4].

In healthy conditions, hepatocytes have a good and organised proliferative ability that helps to maintain liver homeostasis and regenerative capacity despite reversible injuries [5]. These cells do not form a homogenous population. During growth, polyploid populations emerge from diploid cells that undergo the cell cycle but fail to undergo cytokinesis. In adult and mature animals, the level of polyploidisation is high and is disturbed by metabolic overload, DNA damage and chemical-induced liver injury[6].

Hepatocyte polyploidisation was attributed as a genetic adaptation to hepatotoxic effects to prevent malignant transformation. This theory is based on the fact that polyploid cells have multiple copies of DNA, which can buffer alterations of tumour suppressor genes[7]. This effect must be related to a protective adaptation of the hepatic cells' genome during alcohol metabolism. A few studies have demonstrated that hepatocyte polyploidisation occurs in alcoholic-treated animals. This effect was always associated with steatosis and other liver injuries of ALD [8, 9].

This study analysed the nuclear phenotype of hepatocytes in an animal model of intermittent ethanol consumption and compared it to the histological conditions of the liver to better understand the effect of alcohol on hepatocytes in the tissue microenvironment.

METHODOLOGY

Ethical statement

This study was conducted in accordance with the ethical principles for animal experimentation adopted by the National Council for the Control of Animal Experimentation (CONCEA) in Brazil. It was approved by the local ethics committee for animal use (protocol 199/13). General procedures such as containment, anaesthesia, surgery, and euthanasia were performed in accordance with the 879/08 resolution of the Federal Council of Veterinary Medicine (CFMV, Brazil).

Animals

Thirty male Wistar rats (Rattus norvegicus; age: 60 days; weight 350–500 g) were used in the experiments. They were obtained from the bioterium of the Vale do Sapucaí University (UNIVÁS, Brazil) and were regularly evaluated and certified for their suitability for inclusion in the study.

All animals were housed individually in 49 × 34 × 16-cm polypropylene or polycarbonate cages (Beira Mar®, models GK 115 and GC 112) lined with wood. All animals received commercial feed (moisture: 130g/kg; Protein: 230g/kg; ethereal extract: 45g/kg; fibres: 50g/kg; minerals: 100g/kg) and water ad libitum. A 12-hour light and dark cycle was maintained with automatically controlled artificial lighting and all cages equally spaced from the light source. The air temperature and humidity were typical of the environment.

Experimental procedure

The experimental procedure was based on ethanol semi-voluntary intermittent intake for inducing mild to moderate alcohol dependence adapted from Macieira[10]. The animals were divided into three groups: control (0% ethanol, n = 10), 5% absolute ethyl alcohol (EtOH; 5% ethanol solution intake, n = 10), and 20% EtOH (20% ethanol solution intake, n = 10). All rats were allocated to the same shelf and subjected to the same ambient temperature and light conditions. All cages had the same environmental parameters, and all animals were housed individually. All animals were allowed free access to standard rodent chow.

Ethanol solutions (5% and 20% EtOH) were prepared by diluting EtOH (ISOFAR, Rio de Janeiro, Brazil) in water (v/v). These alcohol concentrations are similar to those in beer and enriched wine, respectively. Ethanol solutions were administered through spontaneous and intermittent ingestion as a part of the liquid diet. Animals received the solutions overnight (12–14 h). However, the ethanol solution was administered only on weekends (2 consecutive days), day and night. The volume of the ingested fluid was measured in the morning. In addition, body mass was monitored. The animals were subjected to the experimental conditions for 80 days. After this period, all animals were euthanised for collecting liver.

Liver mass and volume

All animals were sacrificed under general anaesthesia. Anaesthesia was induced by intramuscular injection of xylazine hydrochloride (dose: 5 mg/kg) and ketamine hydrochloride (dose: 50 mg/kg). Euthanasia was achieved by intracardiac injection of 1 mL potassium chloride solution (100 mg/mL). The liver and other constituent organs were collected and fixed in Carson's modified Millonig's phosphate-buffered formalin (pH 7.2) for 24 h.

The liver mass and volume were measured to calculate the density. The ratio between liver and body mass was also analysed. The largest lobe of the liver was transversely dissected to obtain a tissue specimen of approximately 3 mm, which was fixed in a 10% formalin solution for histological processing.

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Histological processing and histopathological analysis

The tissue specimens were treated with increasing concentrations of ethanol (70%, 95%, and 100%) for dehydration, followed by xylene for diaphanisation; after that, they were impregnated and embedded in paraffin. Histological sections of 5 µm thickness obtained using a rotary microtome (Leica, CasaLab, 3770, Belo Horizonte, MG, Brazil) were distended in a 50°C water bath and collected on clean glass slides.

Histopathological analysis was performed by staining the sections with hematoxylin and eosin. The sections were placed on clean, grease-free slides and incubated at 65°C. They were then dewaxed, hydrated, stained with Harris hematoxylin, and counterstained with alcoholic eosin. Finally, slides were dehydrated in ethanol-isopropanol, clarified in xylene, and a cover glass was mounted.

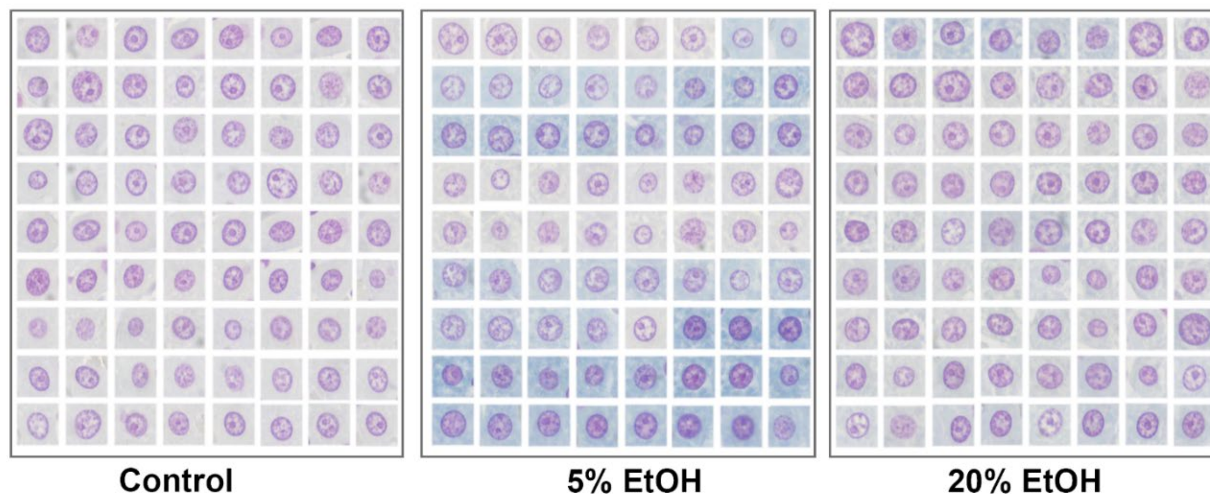
Histopathological analysis was adapted from Fogt and Nanji[9] (1996). The liver pathology was graded from 0 to 3, where 0 means no alteration, 1 (one) is mild, 2 (two) means moderate, and 3 (three) is severe. Steatosis was scored as the percentage of cells containing fat (1= <30% of cells containing fat, 2= 30-60%, 3= >60%). Chronic passive congestion was visually classified based on the extension of blood volume. Necrosis in the lobular region and inflammatory infiltration in centrilobular and periportal regions were scored as the number of focus/lobule (1= one focus/lobule; 2= two focus/lobule; 3= more than two focus/lobule).

DNA staining and nuclear morphometry of hepatocytes

DNA staining was performed using the Feulgen staining method for histochemical analysis. Briefly, sections placed on slides were deparaffinised, hydrated, and treated with a 5N hydrochloric acid solution for hydrolysis. Subsequently, the sections were incubated with the Schiff reagent for 60 minutes, washed under running water for 10 minutes and incubated in a distilled water bath two times. The slides were dehydrated, clarified, mounted, and protected from light afterwards.

Images were captured using a Nikon E-200 microscope at 400x magnification with a digital camera (Moticam® M1000) coupled to the eyepiece using the Motic Images Plus 2.0 software. Two photomicrographs were obtained for each section. Well-distinguishable whole hepatocyte nuclei were randomly selected for the calibration panel assembly (Figure 1). The range of area and circularity values of standard nuclei were obtained using the Image J software v1.47 (National Institute of Health, USA). These ranges were important for automatically selecting hepatocyte nuclei on all images using the software.

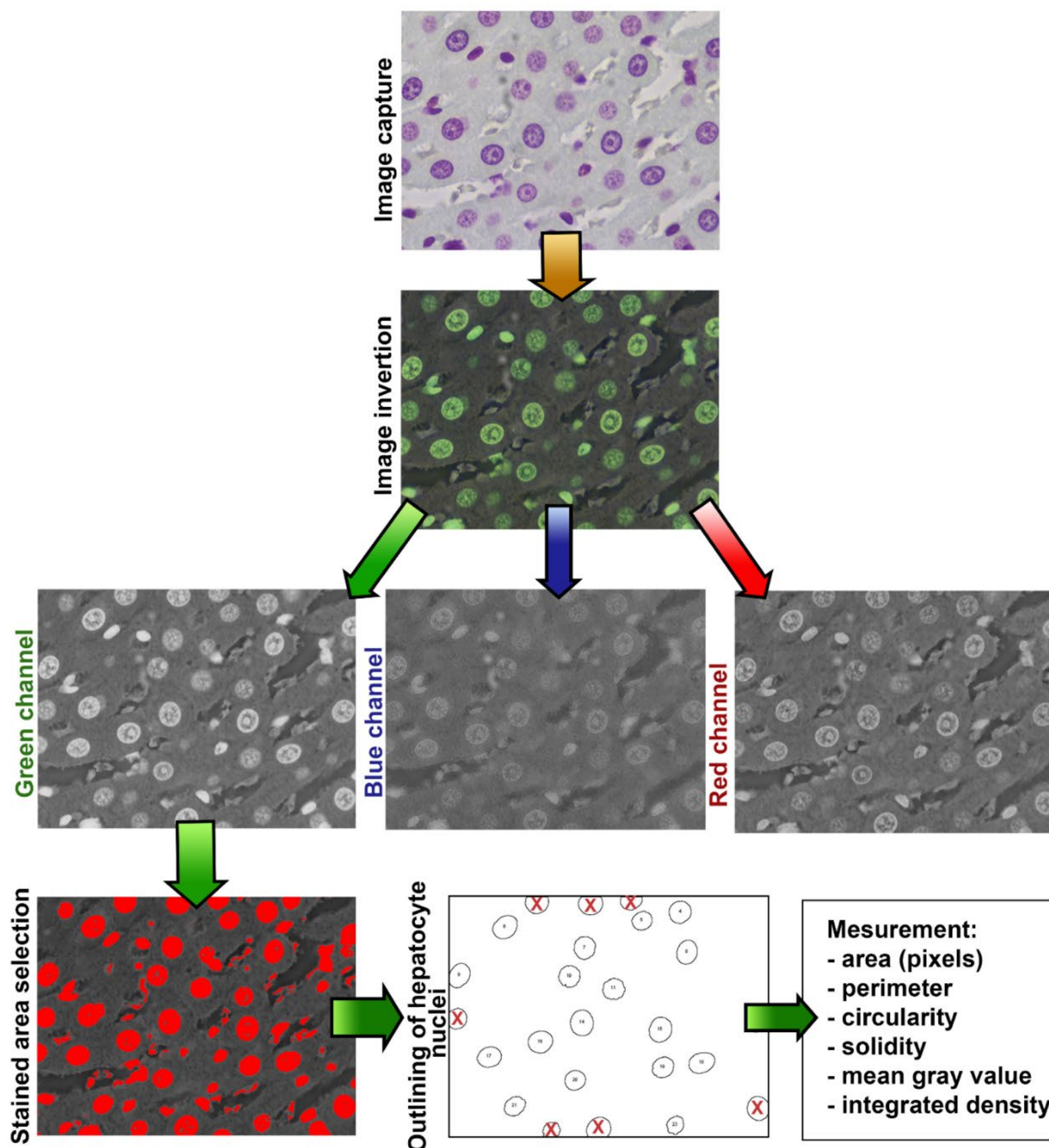
Figure 1 – *Images of the nuclear pattern of hepatocytes used for the determination of values to be used as an automatic selection parameter by the ImageJ software 400x magnification.*



Source: self-authored

The images were imported to the application using the RGB scale. An inverted filter was used to obtain reversed images. Colours were split to obtain the green channel, in which DNA is highly contrasted. The stained area was automatically selected using the Threshold function. Hepatocyte nuclei were measured using the Analyze Particles command. Ranges of circularity and area values obtained from standard images were used to outline only hepatocyte nuclei. Values from wrong and incomplete selections were excluded. The area (pixels), perimeter, circularity, solidity, mean gray value, and integrated density of hepatocyte nuclei were measured (Figure 2).

Figure 2 – *Morphometrical analysis of hepatocyte nuclei from Feulgen-stained liver sections using ImageJ software. Representative images at 400x magnification.*



Source: self-authored

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*The software calculated the circularity using the formula 4π*area/perimeter^2. The values ranged from 0.0 to 1.0, where the maximum value represented the perfect circle and the minimum value represented the elongated shape of the nuclei. Solidity is the ratio of the area to the convex area. The mean grey value is the sum of the grey values of all the pixels in the nuclei divided by the number of pixels. Considering the images were reversed, this value represented the mean optical density of pixels (absorbance).*

The integrated density is the sum of the grey values of the pixels in each nucleus. These values represent the amount of DNA in the nuclei of hepatocytes and are ex-

pressed in arbitrary units (AUs). The frequency distribution of DNA content in the nuclei was calculated and plotted on histograms using these values. Cell populations were distinguished by ploidy based on their peaks [11].

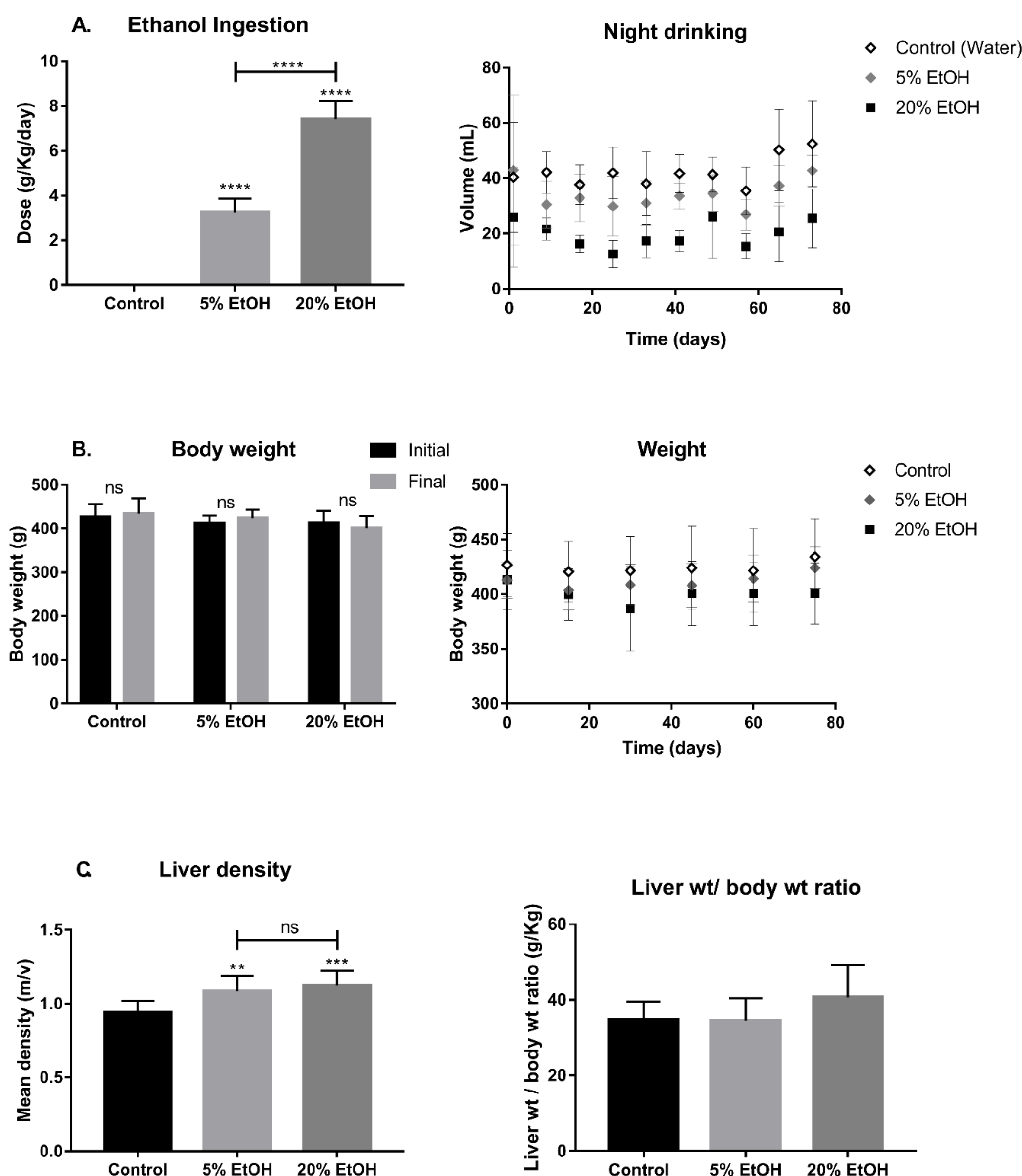
Statistical analysis

The values of alcohol consumption, body weight, liver mass and volume, and nuclear morphometry were imported into GraphPad Prism v.7.00. The mean and standard deviation obtained for each quantitative analysis are plotted automatically on graphs. The values were subjected to a one-way analysis of variance and Tukey's multiple comparison test. The differences between the groups were considered significant if the p-value was <0.05. The software automatically determined the frequency distribution of the integrated density of nuclei.

RESULTS

The ethanol intake amount was determined by calculating the mean dose between the groups. The average absolute ethanol consumption in the 5% EtOH group was 3.24±0.62 g/Kg/day, whereas that in the 20% EtOH group was 7.41±0.81 g/Kg/day (Figure 3A).

Figure 3 – *Calculated dose of ethanol intake (A), body weight (B), and liver density (C) in the three groups. ns: not significant, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, **** $p < 0.0001$.*



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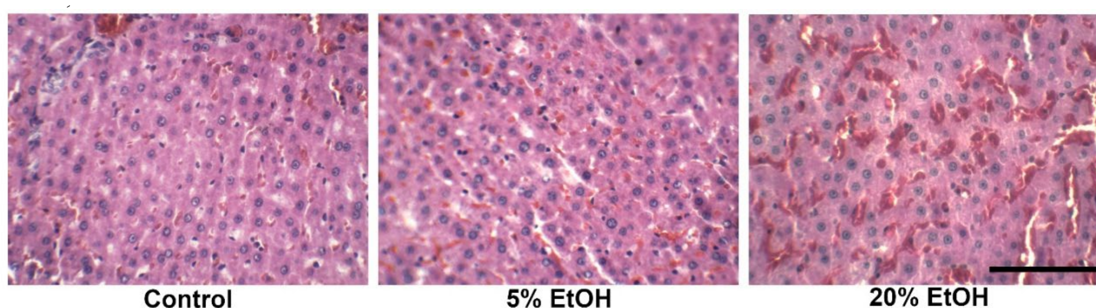
Differences of initial and final animals body weight were observed in control (from 426±28 to 434±35), 5% EtOH (from 412±19 to 424±19) and 20%EtOH (413±26 to 400±27) groups (Figure 3B). Statistical analysis did not indicate any correlation between weight and ethanol ingestion (p=0.660). The mass and volume of formalin-fixed livers showed no significant differences between the groups; however, a significant increase in liver density was evident in the 5% and 20% EtOH groups (Figure 3C).

The difference in the liver density between the 20% EtOH and control groups was higher (0.185 g/mL) than that between the 5% EtOH and control groups (0.144 g/mL). Liver and body mass ratio was not statistically different between groups, although a numerical rise in 20% EtOH-fed rats was observed (control: 34.62g/Kg; 5% EtOH: 34.49g/Kg and 20% EtOH: 40.67; p=0.199).

Histopathological analysis revealed considerable chronic passive congestion of the liver in rats that ingested 20% EtOH. It was characterised by the high blood volume observed in liver sections of all animals of the 20% EtOH group (Figure 4). Moderate to severe congestion was detected in 5 of 10 animals of this group. Interestingly, discreet hepatic congestion was noted in five 20% EtOH-fed animals, four 5% EtOH fed-animals and one animal of the control group. The statistical comparison revealed a highly significant difference between the 20% EtOH group and the control (p < 0.0001) and the 5% EtOH groups (p = 0.0001).

Figure 4 – *Liver chronic passive congestion found in the 20%EtOH group. HE stained sections of 0%, 5% and 20% EtOH.*



Source: self-authored

Weak signs of steatosis (discrete) were observed in two animals of control (n=2/10), two animals of 5% EtOH group (n=2/10) and four animals of 20% EtOH (n=4/10). No fat was detected in the liver of other animals. Differences between groups were not statistically significant. No remarkable sign of liver injury was histologically identified. Just rare necrotic cells were found in liver sections of one animal of control (n=1/10) two animals of the 5% EtOH group (n=2/10), and none in the 20% EtOH. No sign of necrosis was observed in other animals. Few inflammatory cells were identified in liver sections of some animals at periportal and centrilobular regions of hepatic lobules (Periportal: Control n=5/10, EtOH 5% n=5/10, EtOH 20% n=2/10; Centrolobular: Control n=2/10, EtOH 5% n=0/10, EtOH 20% n=0/10), but no moderate or severe inflammation was observed. Statistical analysis did not support significant differences among means of groups for steatosis, necrosis and inflammation (Table 1).

Table 1 – *Histopathological parameters in different groups. The ethanol-treated (5% and 20% EtOH) groups were statistically compared with the control group.*

	Steatosis	*Congestion*	*Necrosis*	*Inflamatory cells*	
				Periportal	*Centrolobular*
Control	*0.2 ±0.42*	*0.1 ±0.32*	*0.1 ±0.32*	*0.5 ±0.53*	*0.2 ±0.42*
Ethanol 5% (v/v)	*0.2 ±0.42*	*0.4 ±0,52*	*0.2 ±0.42*	*0.5 ±0.53*	*0.0 ± 0.0*
Ethanol 20% (v/v)	*0.4 ±0.52*	***1.7 ±0.42*******	*0.0 ± 0.0*	*0.2 ±0.42*	*0.0 ± 0.0*

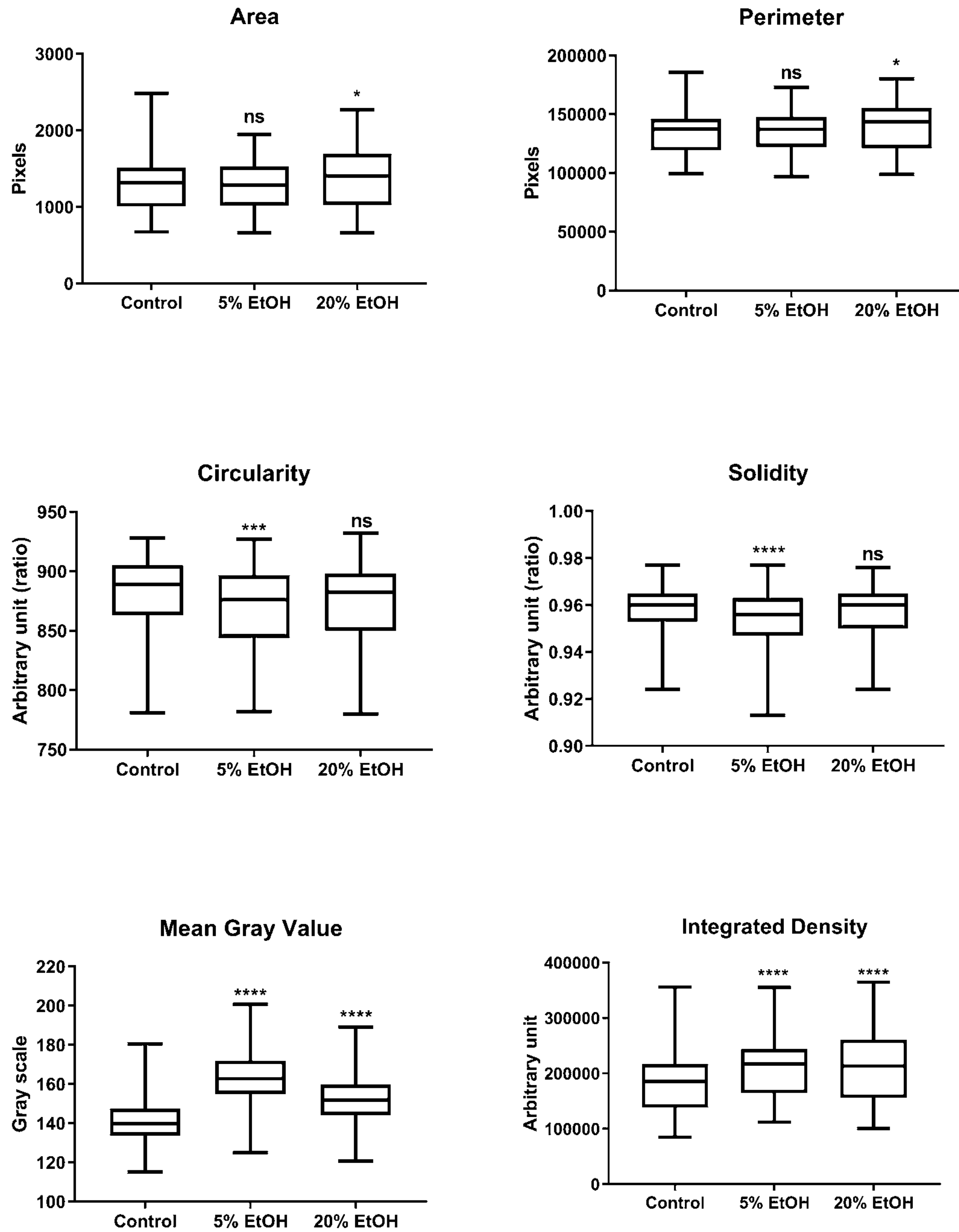
***** p<0.0001*
0: normal
1: weak
2: moderate
3: severe

Source: research data

Feulgen staining and morphometric analysis were performed to determine the area, perimeter, circularity, solidarity, average, and integrated optical density of hepatocyte nuclei (Figure 5). The 20% EtOH solution administration significantly increased hepatocyte area and nuclear perimeter. On the other hand, the circularity

and solidity values were lower in the 5% EtOH group. The optical density was higher in the ethanol-treated groups (5% and 20% EtOH groups) than in the control group, indicating the presence of larger amounts of nuclear DNA.

Figure 5 – *Morphometric values of hepatocyte nuclei obtained by image analysis using the software. The minimum and maximum values are plotted on the graphs. ns: not significant, * p < 0.05, **p < 0.005, **p < 0.0005, ****p < 0.0001.*



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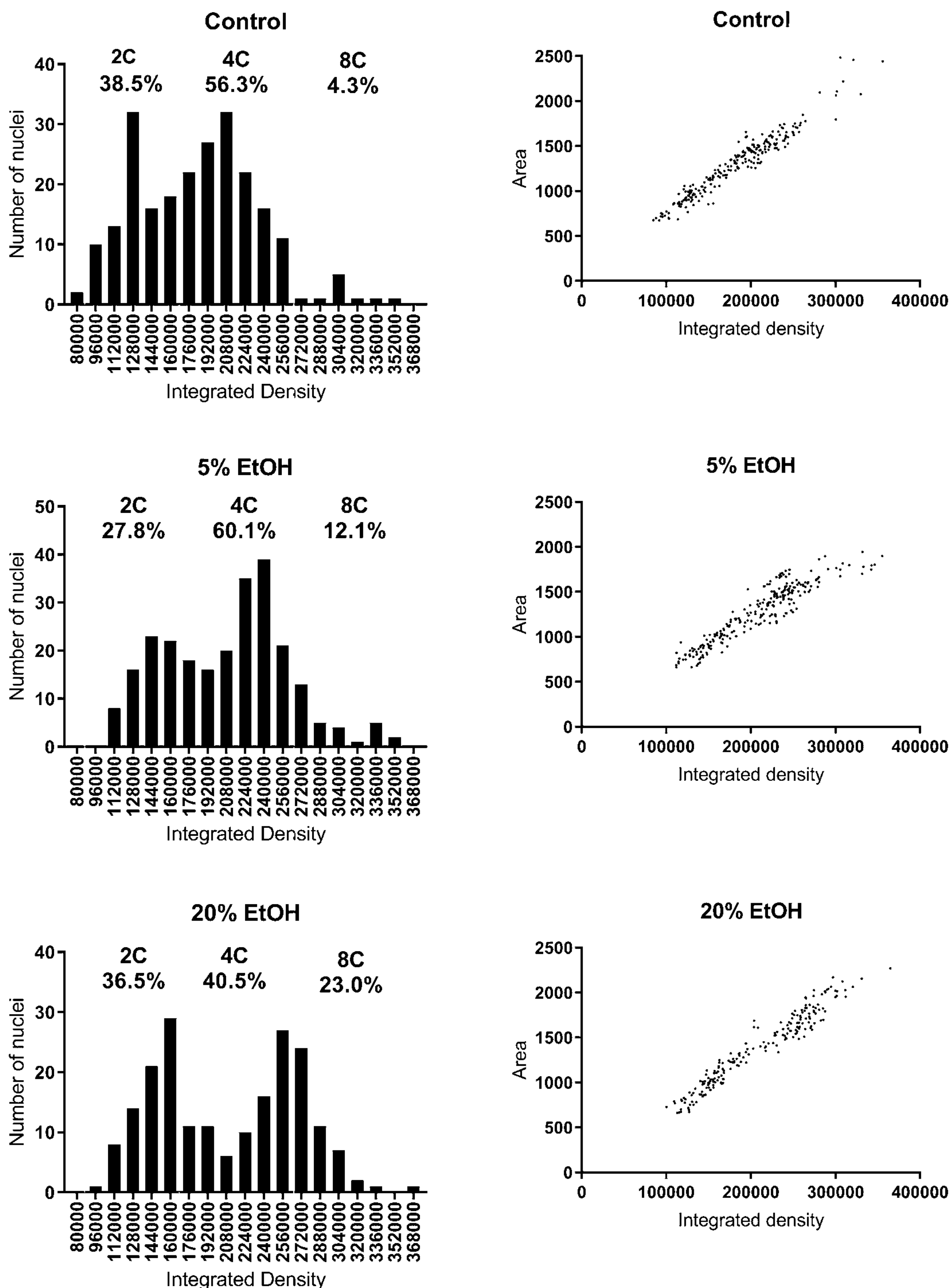
Nuclei were counted based on the DNA content determined by the optical density of Feulgen-stained cells. The frequency distribution of the nuclei was plotted on a histogram (Figure 6). These clearly distinguishable peaks indicated the ploidy of cell populations.

Figure 6 – *Frequency distribution of nuclei according to DNA content (optical density).*



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In the control group, the majority of cells peaked at approximately 1200 and 2000 AU, defined as diploid (2C) and tetraploid (4C) cells, respectively. A small number of cells peaked at approximately 3000 AU, defined as octaploid (8C) cells. Based on the histogram peaks of the control group, the ranges of the integrated optical density of each ploidy population were visually estimated. These defined ranges were applied to all groups to determine the percentage of 2C, 4C, and 8C cells.

Most of the cells were tetraploid in all groups. These nuclei accounted for >60% of the 5% EtOH group, clearly indicating a slight reduction in the diploid population. The number of octaploid nuclei increased in the ethanol-treated groups (5% and 20% EtOH groups) compared with

the control group. These cells constituted approximately 4.3% of the cells in the control group and were three and five times more frequent in the 5% and 20% EtOH groups compared with the control group.

DISCUSSION

The accumulation of fat in hepatocytes is an early response to chronic ethanol ingestion[12]. This alteration was not considerably observed in ethanol-treated groups in this study, although discrete signs of steatosis were detected in some animals, especially of 20%EtOH. Except for hepatic passive congestion, no other histopathological alteration was statistically considered for ethanol-feeding groups. These data suggest that ethanol consumption was not sufficient to cause liver damage.

In many ALD experiments, ethanol solution is the only animal drinking source. Although, it must be considered that an intermittent alcohol-feeding model was proposed. Ethanol solutions were not given continuously, except on weekends. The maximum amount of ethanol consumption was approximately 7 g/kg, unlike that in other studies (12–18 g/kg), which could effectively induce liver steatosis [13].

In a previous study, rats consumed 12% ethanol ad libitum for 42 days, which resulted in pronounced congestion of the liver[14]. This hemodynamic disorder is caused by heart failure, which increases hepatic veins and sinusoid pressure[15]. Excessive alcohol consumption has been considered to be cardiotoxic[16]. In this context, the present results indicate that rats ingested higher ethanol concentrations (20% EtOH) probably had a heart condition that promoted liver congestion. This must also explain the numerical increase of liver-to-body mass observed in the 20% EtOH group due to the blood volume.

Although this finding was prominent only in histopathological analysis, morphometric changes in hepatocytes were observed in both the alcoholic groups. Nuclei morphometry revealed interesting alterations in the ethanol-treated groups, suggesting changes in the cell population. Previous studies have investigated nuclear morphometry of hepatocytes and reported changes in the area and perimeter in pathological conditions[17,18], including those that were experimentally induced[19,20]. A size increase in hepatocyte nuclei in Wistar rats was observed after short-term treatment with toxic compounds [11].

Nuclei content can be measured by optical density (mean grey value) to characterise and compare cells[21]. Considering that hepatocytes were stained for DNA with Feulgen stain, the measured optical density was proportional to the chromatin density and volume. The optical density was increased in the ethanol-treated groups, which supports the hypothesis that ethanol causes alterations in the hepatocyte population. In general, we can claim that alcohol increases DNA content. Interestingly, some toxic treatments reduce DNA content because of oxidative damage[22].

These data corroborate the findings of previous studies, which reported that alcohol feeding increases the DNA content of hepatocytes because of polyploidization[8,9]. Analysis of hepatocyte distribution based on DNA content revealed the presence of three cell populations: diploid, tetraploid, and octaploid. In normal adult rats, the majority of cells are tetraploid, and a minority of the cells are octaploid[6]. This pattern seems to correspond to the distribution of nuclei found in the control group. However, alcohol induced an increase in 8C nuclei, indicating increased DNA content levels in the ethanol-treated groups.

These results show the DNA content in isolated nuclei; however, some hepatocytes are binucleated. Tetraploid hepatocytes have one 4C nucleus or two 2C nuclei. Similarly, octaploid hepatocytes are mononucleated with a single 8C nucleus (1 × 8C) or binucleated with two 4C nuclei (2 × 4C)[23]. In fact, the nuclei populations identified in these studies do not completely correspond to the ploidy population of hepatocytes. In the control group, 38% of nuclei were estimated to be diploid, although the same should constitute binucleated tetraploid cells (2 × 2C).

Octaploid nuclei are mononucleated (1 × 8C) or binucleated (2 × 8C) hepatocytes. Cell sorting of rat hepatocytes has revealed that only a small portion of octaploid hepatocytes are mononucleated (1 × 8C), with large nuclei[24]. At this point, the present results indicate that alcohol increases this population, and once the 20% EtOH group had larger hepatocyte nuclei, it was significantly more 8C.

Polyploidization must occur from an abnormal cell cycle through endoreplication, mitotic slippage, or cytokinesis failure. Except for this last, mononuclear polyploid cells are formed. In endoreplication, DNA is replicated in the S phase, and cells move from the G2 to the G1 phase without going through the M phase. This results in cells with doubled DNA content. The metaphase-anaphase transition is perturbed in mitotic slippage, and cells fail to conclude division[25].

Ethanol-induced hepatic polyploidisation has been previously discussed as a protective mechanism against mutations induced by oxidative agents from alcohol metabolism 9. Ethanol produces reactive species in the liver through cytochrome P450 CYP2E1 metabolism, which causes oxidative DNA lesions[26].

In addition, it has been proposed that oxidative stress induces DNA damage response in hepatocytes, which causes cell cycle G2/M checkpoint activation through ATR/p53/p21 pathway in a non-alcoholic fatty liver disease (NAFLD) study[27]. This mechanism prevents cell division and forms polyploid cells. Other studies must be performed to elucidate if this mechanism is also involved in ethanol-induced polyploidisation due to alcohol's potential to cause DNA oxidative damage.

Hepatocyte polyploidisation has been associated with normal liver homeostasis and pathological conditions. Hepatic polyploidy presents an impact on liver metabolism and hepatocellular carcinoma, although many studies

are controversial about the role of polyploid cells in the protection or development of diseases[25]. Therefore, it is impossible to establish the consequences of 8C population growth induced by ethanol here. At the same time, it should not be ruled out that there may be some relation to the development of ALD.

CONCLUSION

It is concluded that alcohol interferes with hepatocyte ploidy, even without developing relevant histopathological signs of ALD. The animal model used did not consume sufficient alcohol to evolve into steatosis or hepatitis. However, alcohol induces hepatocyte polyploidisation even at low doses, probably as a response to oxidative stress. At this stage, heart disorders may cause passive chronic congestion of the liver, which was observed as cumulative blood cells in the sinusoids of rats that ingested higher doses of ethanol. These data contribute to the understanding of the effects of alcohol on the liver.

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REFERENCES

1. Wurst FM, Tabakoff B, Alling C, Aradottir S, Wiesbeck GA, Müller--Spahn F, et al. World Health Organization/International Society for Biomedical Research on Alcoholism Study on State and Trait Markers of Alcohol Use and Dependence: Back to the future. Alcohol Clin Exp Res. 2005;29(7):1268–75. doi: 10.1097/01.alc.0000171483.93724.96

2. WHO. World Health Organization. Global status report on alcohol and health 2018. Global status report on alcohol. 2018;

3. Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. J Hepatol. 2013 Jul;59(1):160–8.

4. Osna NA, Donohue TM, Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. Alcohol Res. 2017;38(2):147–61.

5. Chen F, Jimenez RJ, Sharma K, Luu HY, Hsu BY, Ravindranathan A, et al. Broad Distribution of Hepatocyte Proliferation in Liver Homeostasis and Regeneration. Cell Stem Cell. 2020;26(1):27-33.e4. doi: 10.1016/j.stem.2019.11.001

6. Wang MJ, Chen F, Lau JTY, Hu YP. Hepatocyte polyploidization and its association with pathophysiological processes. Cell Death Dis. 2017;8(5):e2805.

7. Sladky VC, Eichin F, Reiberger T, Villunger A. Polyploidy control in hepatic health and disease. J Hepatol. 2021;75(5):1177–91. doi: 10.1016/j.jhep.2021.06.030

8. Gaub J, Fauerholdt L, Keiding S, Kondrup J, Petersen P, Wantzin GL. Cytophotometry of liver cells from ethanol-fed rats: ethanol causes increased polyploidization and protein accumulation. Eur J Clin Invest. 1981;11(3):235–7. doi: 10.1111/j.1365-2362.1981.tb01846.x

9. Fogt F, Nanji AA. Alterations in nuclear ploidy and cell phase distribution of rat liver cells in experimental alcoholic liver disease: Relationship to antioxidant enzyme gene expression. Toxicol Appl Pharmacol. 1996;136(1):87–93. doi: https://doi.org/10.1006/taap.1996.0010

10. Macieira MS, Almeida WG, Silva EA, Schenberg LC, Nakamura--Palacios EM. Alcohol dependence induced in rats by semivoluntary intermittent intake. Braz J Med Biol Res. 1997;30(9):1107–11. doi: https://doi.org/10.1590/S0100- 879X1997000900009

11. Gil R, Callaghan R, Boix J, Pellin A, Llombart-Bosch A. Morphometric and cytophotometric nuclear analysis of altered hepatocyte foci induced by N-nitrosomorpholine (NNM) and aflatoxin B1 (AFB1) in liver of Wistar rats. Virchows Arch B Cell Pathol Incl Mol Pathol. 1987;54(1):341–9.

12. Seitz HK, Bataller R, Cortez-Pinto H, Gao B, Gual A, Lackner C, et al. Alcoholic liver disease. Nat Rev Dis Primers. 2018;4(1).

13. Lamas-Paz A, Hao F, Nelson LJ, Vázquez MT, Canals S, del Moral MG, et al. Alcoholic liver disease: utility of animal models. World J Gastroenterol. 2018;24(45):5063–75.doi: 10.3748/wjg.v24.i45.5063

14. Radic I, Mijovic M, Tatalovic N, Mitic M, Lukic V, Joksimovic B, et al. Protective effects of whey on rat liver damage induced by chronic alcohol intake. Hum Exp Toxicol. 2019;38(6):632–45.

15. Hilscher MB, Kamath PS. The Liver in Circulatory Disturbances. Clin Liver Dis. 2019;23(2):209–20. doi: 10.1016/j.cld.2018.12.004

16. Whitman IR, Agarwal V, Nah G, Dukes JW, Vittinghoff E, Dewland TA, et al. Alcohol Abuse and Cardiac Disease. J Am Coll Cardiol. 2017;69(1):13–24. doi: 10.1016/j.jacc.2016.10.048

17. Mathan G, Fatima G, Saxena AK, Chandan BK, Jaggi BS, Gupta BD, et al. Chemoprevention with aqueous extract of Butea monosperma flowers results in normalization of nuclear morphometry and inhibition of a proliferation marker in liver tumors. Phytother Res. 2011;25(3):324–8. doi: 10.1002/ptr.3249

18. Wen CH, Huang MS, Wang SL, Su YC, Yang SF, Chai CY. Diagnostic value of computerized nuclear morphometry for the prediction of malignancy in liver fine needle aspiration cytology. Acta Cytol. 2009;53(1):77–82. doi: 10.1159/000325087

19. Gatiatulina ER, Sheina EA, Nemereshina ON, Popova EV, Polyakova VS, Agletdinov EF, et al. Effect of Zn Supplementation on Trace Element Status in Rats with Diet-Induced Non-alcoholic Fatty Liver Disease. Biol Trace Elem Res. 2020;197(1):202–12. doi: 10.1007/s12011-019-01985-z

20. Lima IR, Silva IB, Lima RML, Silva TMS, Maia MBS, Leite SP. Hepatoprotective efficacy of methanolic extract of indigofera suffruticosa (Mill) on paracetamol-induced liver damage in mice. Arq Gastroenterol. 2019;56(4):333–8. doi: https://doi.org/10.1590/S0004-2803.201900000-62

21. Rothmann C, Barshack I, Kopolovic J, Malik Z. Spectrally resolved morphometry of the nucleus in hepatocytes stained by four histological methods. Histochem J. 1998;30(8):539–47. doi: 10.1023/A:1003235201563

22. Mossa ATH, Heikal TM, Omara EAA. Physiological and histopathological changes in the liver of male rats exposed to paracetamol and diazinon. Asian Pac J Trop Biomed. 2012;2(Suppl 3):S1683–90. doi: https://doi.org/10.1016/S2221- 1691(12)60478-X

23. Donne R, Saroul-Aïnama M, Cordier P, Celton-Morizur S, Desdouets C. Polyploidy in liver development, homeostasis and disease. Nat Rev Gastroenterol Hepatol. 2020;17(7):391–405.

24. Katsuda T, Hosaka K, Matsuzaki J, Usuba W, Prieto-Vila M, Yamaguchi T, et al. Transcriptomic Dissection of Hepatocyte Heterogeneity: Linking Ploidy, Zonation, and Stem/Progenitor Cell Characteristics. Cmgh. 2020;9(1):161–83. doi: 10.1016/j.jcmgh.2019.08.011

25. Kim JY, Choi H, Kim HJ, Jee Y, Noh M, Lee MO. Polyploidization of Hepatocytes: Insights into the Pathogenesis of Liver Diseases. Biomol Ther (Seoul). 2022;30(5):391–8. doi: 10.4062/biomolther.2022.070

26. Bradford BU, Kono H, Isayama F, Kosyk O, Wheeler MD, Akiyama TE, et al. Cytochrome P450 CYP2E1, but not nicotinamide adenine dinucleotide phosphate oxidase, is required for ethanol-induced oxidative DNA damage in rodent liver. Hepatology. 2005;41(2):336–44. doi: 10.1002/hep.20532

27. Gentric G, Maillet V, Paradis V, Couton D, L'Hermitte A, Panasyuk G, et al. Oxidative stress promotes pathologic polyploidization in nonalcoholic fatty liver disease. J Clin Invest. 2015;125(3):981–92. doi: 10.1172/JCI73957