

Alcohol, Cannabinoids and Nicotine in Liver Pathophysiology

Manuela Radic^{*1,2}, Francesca Rappa¹, Rosario Barone¹, Francesco Cappello¹, Giuseppe Crescimanno³, Maurizio Casarrubea³, Massimo Perucci², Antonella Marino Gammazza^{*1}, Giuseppe Di Giovanni^{*2}

¹Department of Experimental Biomedicine and Clinical Neuroscience (BIONECA), Section of Human Anatomy, University of Palermo, Italy

²Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Msida, Malta

³Department of Experimental Biomedicine and Clinical Neuroscience (BIONECA), Section of Human Physiology, University of Palermo, Italy

Abstract. The liver can be affected by a wide range of therapeutic and environmental chemicals and here we want to provide a summary of the complex effects of alcohol, cannabinoids and nicotine on liver function. Alcohol is the most important agent that produces liver injury, manifesting as alcoholic fatty liver disease. In addition, it is one of the main etiologic agents for hepatocellular carcinoma development. Studies reviewed in this article regarding cannabinoids, show that $\Delta 9$ -THC does not produce any harmful effects on the liver, while cannabidiol has hepatoprotective effects in ischemia/reperfusion and alcohol-induced liver injuries. The liver is negatively affected by nicotine exposure, but surprisingly nicotine was shown to have a positive effect on the liver in the diet-induced obese animal model, which should be confirmed by future research.

Keywords: liver, alcohol, cannabinoids, nicotine

1 Introduction

Alcohol, cannabinoids and nicotine are three major drugs of abuse, widely used especially among the youth (Johnston, O'Malley, Miech, Bachman & Schulenberg, 2017). It is important to study their effects, considering that they are common causes of preventable morbidity and mortality (Johnston et al., 2017). In this work, we want to provide a concise summary of the complex effects of alcohol, cannabinoids and nicotine on liver functions (see Table 1). The liver is a major detoxifying and drug metabolizing organ, with an enormous functional reserve (Theise, 2013). It can be affected by a wide

range of therapeutic and environmental chemicals, directly or by immune mechanisms (Theise, 2013).

Fatty liver disease (alcoholic or nonalcoholic) is a broad term which includes three liver alterations that can be present in any combination: hepatocellular steatosis, steatohepatitis and steatohepatitis with fibrosis (Theise, 2013). Hepatocellular steatosis or fat accumulation can be microvesicular (small lipid droplets) or macrovesicular (large lipid droplets) (Theise, 2013). Steatohepatitis is characterized by hepatocyte ballooning, Mallory-Denk bodies (eosinophilic cytoplasmic inclusions in degenerating hepatocytes) and inflammatory infiltration (Theise, 2013; Mandrekar & Szabo, 2010). Finally, steatohepatitis with fibrosis starts as central vein sclerosis and the scarring gradually spreads to the portal tracts forming central-portal or portal-portal bridging fibrosis (Theise, 2013; Sakhuja, 2014). Then, if the injury continues, the fibrosis and hepatocytes regeneration involves a pseudolobular or Laennec cirrhosis in which a nodular morphology is present (Theise, 2013; Sakhuja, 2014).

Among the inducers of liver diseases, alcohol is the most important agent that produces toxic liver injury and 60% of chronic liver disease in Western countries is caused by excessive ethanol intake (Theise, 2013). The Monitoring the future (MTF) survey conducted in 2016 shows that 61% of students have consumed alcohol by the end of high school and 46% of 12th graders have reported being drunk at least once in their life, which makes alcohol the most widely used substance by today's teenagers (Johnston et al., 2017). Alcohol causes lipid metabolism changes, decreases export of

*Correspondence to: Manuela Radic (manuela.radic@outlook.com), Antonella Marino Gammazza (antonella.marino@hotmail.it), Giuseppe Di Giovanni (giuseppe.digiovanni@um.edu.mt)

Table 1: Liver changes induced by ethanol, cannabinoids and nicotine.

DRUG	ANIMALS	ADMINISTRATION ROUTE	DOSE	DURATION	LIVER CHANGES	REFERENCES
Ethanol	Albino rats	Drinking water	2 ml (0.5 g)/100 g body weight per day of 30% v/v of an aqueous solution	8 weeks	↑ALT, ↑GGT, ↑liver weight, ↑liver volume, ↑hepatocyte size, large number of cytoplasmic vacuoles, pyknotic nuclei, periportal inflammation	Habib-ur-Rehman, Tahir, Lone and Sami (2011)
Ethanol	Wistar rats	Drinking water	Weekly increase in concentration to a 40% v/v	Up to 29 weeks	Steatosis, inflammation, hepatocyte necrosis, pericentral sclerosis	Keegan, Martini and Batey (1995)
Ethanol	Mice (ICR - Institute for Cancer Research)	Drinking water	Concentration: 5% (first week), 10% (next 8 weeks), 15% ethanol thereafter ad libitum for 60 and 70 weeks	70 weeks	Several larger nodules (5-22 mm) → trabecular HCC (eosinophilic and vacuolated cells)	Tsuchishima et al. (2013)
Δ9-THC	C57BL/6J mice	Intraperitoneal injection	10 mg/kg body weight	10 days	No harmful effects on: lipid peroxidation, protein carbonylation or DNA oxidation	Pinto, Moura, Serrão, Martins and Vieira-Coelho (2010)
Cannabidiol	Sprague-Dawley rats (exposed to ischemia/reperfusion liver injury)	Intravenous injection	5 mg/kg	1-hour following the procedure and 24 hours thereafter for two consecutive days	↓TNF-α elevation, ↓NO elevation and ↓ALT, induced by ischemia/reperfusion injury + histology comparable to the control group	Fouad and Jresat (2011)
Cannabidiol + Ethanol	C57BL/6 mice	Cannabidiol: intraperitoneal injection. Ethanol: gavage	Cannabidiol: 5 mg/kg, every 12 h, 30 min before each ethanol gavage. Ethanol: 30% v/v in saline, 4 g/kg, every 12 h	5 days	Cannabidiol prevented the ethanol-induced ↑AST and hepatic triglycerides, reversed the ethanol-induced ↓hepatic ATP levels. Cannabidiol ↓basal triglycerides levels	Yang et al. (2014)
Nicotine	Fischer 344 rats	Intraperitoneal via implanted osmotic minipumps	9 mg/(Kg d)	2 weeks	↑ALT, ↑AST, ↑ALP, ↑biliary proliferation and fibrosis	Jensen et al. (2013)
Nicotine	Sprague-Dawley rats (diet-induced obesity)	Subcutaneous injection	2 mg/kg every 12 h	17 days	↓liver steatosis, ↓inflammation, ↓ER stress	Seoane-Collazo et al. (2014)
Nicotine + Ethanol	C57BL/16 mice	Nicotine: intraperitoneal injection. Ethanol: liquid ethanol diet	Nicotine: 1 mg/kg body weight, Ethanol: from 10% gradually to 35% of total calories	3 weeks	Nicotine ↓ethanol-induced steatosis. Nicotine+Ethanol: ↑hepatic contents of collagen type I	Lu, Ward and Cederbaum (2013)

lipoproteins, and causes cell injury, which manifest as fatty liver disease (Theise, 2013). Hepatotoxicity of ethanol has been shown in experimental animals (Habibur-Rehman, Tahir, Lone & Sami, 2011; Keegan, Martini & Batey, 1995; Tsuchishima et al., 2013). Habibur-Rehman and co-workers (2011) treated albino rats with ethanol for 8 weeks and found functional and structural liver changes, such as increase in the serum alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) levels, periportal inflammation, liver weight and volume increase (Habibur-Rehman et al., 2011). Another group induced alcoholic liver injury by delivering ethanol in the drinking water (Keegan et al., 1995). They found evident hepatotoxic histological changes (i.e., steatosis, inflammation, hepatocyte necrosis and pericentral sclerosis) while maintaining a nutritionally adequate food intake (Keegan et al., 1995).

In addition, ethanol consumption is one of the main etiologic agents for hepatocellular carcinoma (Theise, 2013). For example, in the study conducted by Tsuchishima and co-workers, male mice were administered ethanol through drinking water for 70 weeks (Tsuchishima et al., 2013). 50% of those mice had several larger nodules (5–22 mm) in their liver, histologically verified as trabecular HCC composed of eosinophilic and vacuolated cells (Tsuchishima et al., 2013) (see Figure 1). The authors conclude that the tumorigenesis could occur as a result of proto-oncogenes and/or oncosuppressor genes mutations, due to repeated regeneration processes, although further studies are necessary (Tsuchishima et al., 2013).

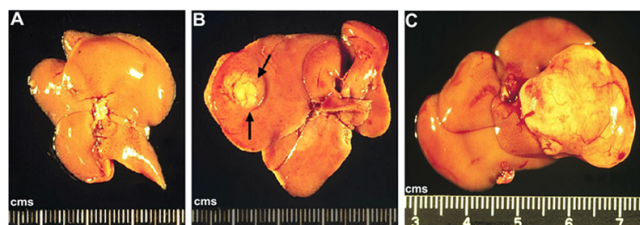


Figure 1: Stereoscopic images of the liver tissue of ethanol administered mice at weeks 60 and 70. (A) Control group (60 W): no tumor. (B) Ethanol group (60 W): hepatic tumor was observed. (C) Ethanol group (70 W): large hepatic tumor measuring 22 mm was present. Adapted from Tsuchishima et al. (2013).

Together with alcohol, marijuana, made from leaves of the *Cannabis sativa* plant, is the most widely used illegal drug (Kumar, Abbas & Aster, 2013) and it is a popular drug of choice among young people which is mainly due to its easy availability and low cost. The MTF survey shows that one in seventeen 12th grades smoke marijuana daily (Johnston et al., 2017). Pinto and co-workers showed that Δ -tetrahydrocannabinoid (Δ 9-THC), marijuana's most psychoactive cannabinoid, does not produce any harmful effects on the liver of

healthy mice, when chronically administered (Pinto, Moura, Serrão, Martins & Vieira-Coelho, 2010). Mice were given intraperitoneal injections of Δ 9-THC, in a total daily dose of 10 mg/kg body weight, for 10 days and this treatment did not produce any significant changes in the hepatic redox state (Pinto et al., 2010).

An increased expression of cannabinoid receptors type 1 (CB1) in human cirrhotic liver samples has been observed and it has been shown that CB1 signalling has a profibrogenic effect (see Parfieniuk & Flisiak, 2008). Julien and co-workers showed that cannabinoid receptors type 2 are expressed in cirrhotic human liver, predominantly in hepatic fibrogenic cells, but not in normal liver, and when activated endogenously they counteract liver fibrogenesis (Julien et al., 2005). Cannabidiol, the major non-psychoactive cannabis component, ameliorates ischemia/reperfusion-induced liver damage (Fouad & Jresat, 2011). Indeed, cannabidiol treatment resulted in significant reduction of ischemia/reperfusion-induced elevations of tumour necrosis factor- α and nitric oxide in liver homogenates, as well as the serum level of ALT (Fouad & Jresat, 2011). Furthermore, the hepatoprotective effect of cannabidiol was also shown on the histopathological examination, where the histological picture of the ischemia/reperfusion cannabidiol-treated group was comparable to the control group (Fouad & Jresat, 2011). Yang and co-workers showed that cannabidiol protects mouse liver from acute alcohol-induced steatosis (Yang et al., 2014). Cannabidiol prevented ethanol-induced serum aspartate aminotransferase (AST) increase and significantly attenuated the hepatic triglycerides elevation (Yang et al., 2014) (see Figure 2). Furthermore, cannabidiol lowered basal triglycerides levels and completely reversed the ethanol-induced decline in hepatic ATP levels (Yang et al., 2014).

Cigarette smoking represents another factor that increases risk or susceptibility for a lot of diseases and for liver disease as well. In particular, cigarette smoking by teenagers and young adults leads to immediate and serious health problems including respiratory and non-respiratory effects, addiction to nicotine, and the associated risk of other drug use. Several studies have found nicotine to be addictive in ways similar to heroin, cocaine, and alcohol. In fact, nicotine is a highly addictive alkaloid, found in tobacco leaves, responsible for the acute effects of smoking (i.e., increased heart rate, blood pressure, cardiac contractility and output) (Kumar et al., 2013). Thirty-day prevalence of cigarette use, for 12th graders, was 11% in 2016 (Johnston et al., 2017).

It is continuously declining from 1997 (37%), due to increases in disapproval and perceived risk (Johnston et al., 2017). A retrospective follow-up study of a 10-year interval was conducted on a total of 3,365 sub-

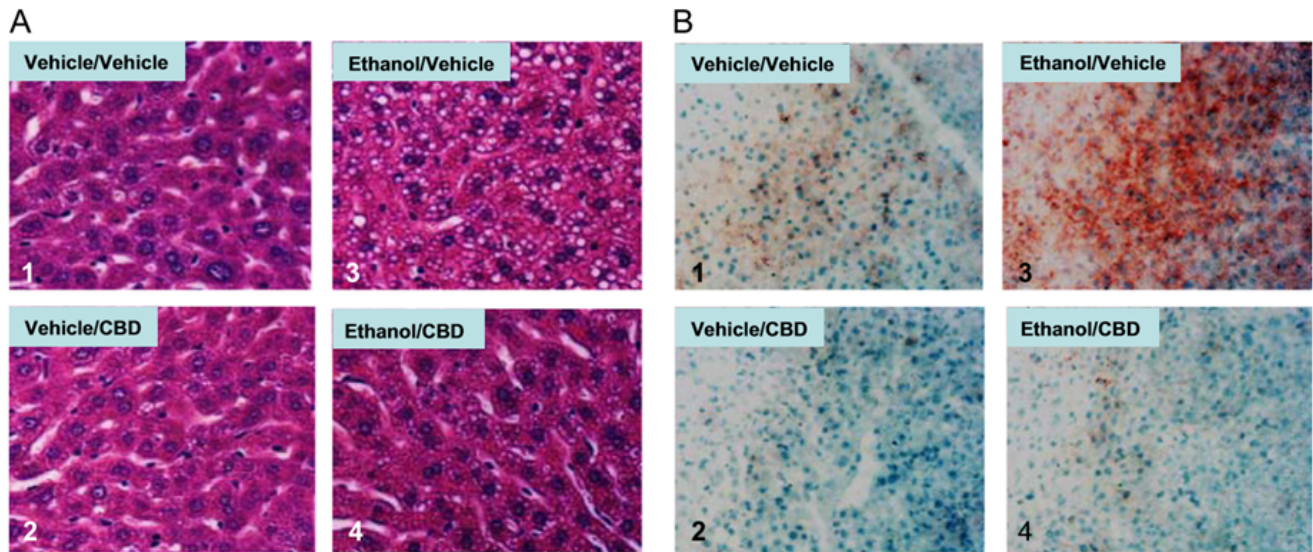


Figure 2: (A) H&E staining and (B) oil red O staining showing increased lipid accumulation in mouse liver after binge alcohol treatment. CBD decreases this lipid accumulation (image 4 compared to image 3 in (A) and (B)). Adopted from Yang et al. (2014).

jects, to assess the effect of cigarette smoking on the development or cure of nonalcoholic fatty liver disease (Hamabe et al., 2011). This study showed that cigarette smoking is an independent risk factor for nonalcoholic fatty liver disease development (Hamabe et al., 2011). Jensen and co-workers (2013) showed that chronic nicotine exposure induces a significant increase in ALT, AST and alkaline phosphatase (ALP) levels in rats, as well as an increase of biliary proliferation and fibrosis, which may play a role in the pathogenesis of cholangiopathies (Jensen et al., 2013) (see Figure 3). On the other hand, an interesting study was conducted by Seoane-Collazo and co-workers (Seoane-Collazo et al., 2014). They found that nicotine reduced liver steatosis, inflammation and ER stress in diet-induced obese male rats (Seoane-Collazo et al., 2014). Moreover, this effect was produced independently of nicotine's anorectic action (Seoane-Collazo et al., 2014). Finally, Lu and co-workers (2013) showed that nicotine treatment alone does not induce a necro-inflammatory response nor steatosis, while it enhances ethanol-induced steatosis (Lu, Ward & Cederbaum, 2013). In addition, nicotine and ethanol when given alone increase hepatic contents of collagen type I and this effect is enhanced by a nicotine and ethanol combination (Lu et al., 2013).

2 Conclusion

In conclusion, ethanol treatment deteriorates liver's function which can lead to cancer development. Nicotine exposure was shown to induce liver injury and to promote fibrosis, while its positive effect in diet-induced obese animal models should be evaluated by further research. Regarding cannabinoids, Δ^9 -THC was not

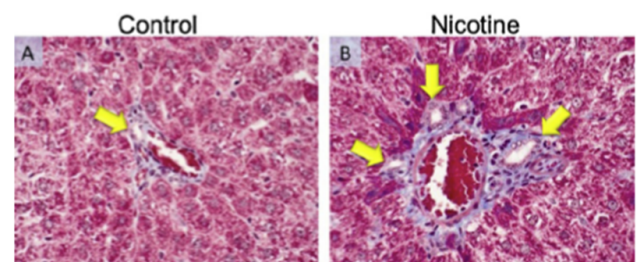


Figure 3: Evaluation of collagen deposition by Masson's trichrome staining in liver sections from control and nicotine-treated rats. Masson's trichrome staining in control (A) and nicotine-treated rats (B). Chronic administration of nicotine stimulated an increase in collagen deposition (blue staining) around the portal areas of nicotine-treated (B) compared with normal control rats (A). Bile ducts are indicated with yellow arrows. Adopted from Jensen et al. (2013).

shown to produce any harmful effects on the liver, while cannabidiol showed hepatoprotective effects in ischemia/reperfusion and alcohol-induced liver injuries. Therefore, cannabinoid signalling modulation could potentially be a new therapeutic approach in the liver fibrosis management.

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