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https://doi.org/10.1016/j.toxlet.2024.07.493

P12 | Liver toxicology

P12-01
Moderate intake of beer improves nonalcoholic fatty liver disease (NAFLD) in a high fat diet (HFD)-induced mouse model

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Purpose: Both beer and some of its components, particularly polyphenols and iso-alpha-acids, have proven to be able to attenuate hepatic lipid accumulation or perturbed blood parameters in different rodent models through different putative mechanisms [1,2,3]. The current study was carried out within the NUTRATGE project (https://nutrage.it/) and aimed to evaluate the anti-steatotic capacity of beer in an HFD-induced NAFLD mouse model.

Methods: The beer was characterized for bioactive molecules content and individual phenolic compounds using UHPLC-ESI-MS/MS. In the *in vivo* study, forty-eight six-weeks-old male mice (C56BL/6) were randomly divided into four groups and supplemented daily during 10 weeks as follows: 1) normal diet (CTR); 2) a CTR diet and 0.14 ml/day beer (CTR+Beer); 3) a HFD (HFD); 4) a HFD and 0.14 ml/day beer (HFD+Beer). Prior to sacrifice, the weight of each animal was recorded, and blood was collected. We quantified liver lipids, performed histopathological evaluation using hematoxylin and eosin staining, and analyzed biomarkers of oxidative stress. Additionally, analysis of gene expression and DNA methylation of hepatic tissue was performed by RNA-Seq and Reduced Representation Bisulfite Sequencing.

Results: The beer displayed a good content in total phenols (25.01±1.27 mg GAE/100 ml), flavonoids (3.17±0.17 mg CE/100 ml) and flavonols (3.07±0.23 mg QE/100 ml). Among the single phenolic compounds, isoquercetin emerged as the predominant polyphenol (14.68±2.68 mg/100 ml). Compared to CTR, HFD group showed significantly higher levels of AST, ALT, TC, LDL-C, glucose, body weight and liver lipids, indicating the presence of steatosis, confirmed also by histological analysis. In HFD+beer group all the parameters returned to levels similar to those of CTR. All groups exhibited comparable levels of both protein carbonylation and lipid peroxidation in the liver, suggesting that our model represents an early stage of NAFLD with no oxidative stress. Analysis of transcriptomic and CpG methylation profile showed a clear separation between CTR and HFD groups. Beer consumption only partially affected gene expression whereas specifically changed the DNA methylation profile. RNA-Seq revealed 162 differentially expressed genes (DEGs) between CTR and HFD, whose biological function was related to cellular inflammatory processes and regulation of lipid metabolism. Beer consumption ameliorated the HDF effect (CTR*vs*HFD+beer, DEGs=43) showing alteration in the inflammatory response but not in the lipid homeostasis. RRBS profile identified 562 (CTR*vs*HFD), 429 (CTR*vs*HFD+beer), 469 (CTR*vs*CTR+beer) and 860 (HFD*vs*HFD+beer) differentially methylated cytosines (DMCs). DMCs target genes related to acyl glycerol and lipid biosynthetic process for CTR*vs*HFD+beer and insulin signaling for CTR*vs*CTR+beer comparisons. In summary, beer was capable to improve NAFLD likely due to the ability of polyphenols to modulate lipid metabolism.

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https://doi.org/10.1016/j.toxlet.2024.07.494

P12-02
effects of high-fat diet and streptozotocin-induced diabetes on CYP2E1 protein expression in rat liver

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The starting point of our study was the demonstration in various studies that CYP2E1 enzyme expression is affected by diabetes. As a toxicological concern, CYP2E1 is of interest because it metabolizes and activates a wide range of toxicologically significant compounds, including ethanol, carbon tetrachloride, acetaminophen, benzene, and halothane. Additionally, procarcinogens such as nitrosamines and azo compounds are among the substrates of CYP2E1 [1]. The metabolism of these compounds by CYP2E1 generates toxic intermediates and excessive levels of reactive oxygen species. As a consequence of its ability to produce reactive oxygen species at high levels, CYP2E1 has been linked to a wide range of pathological conditions, including diabetes, non-alcoholic steatohepatitis, and cancer [2]. All this information indicates that CYP2E1 is an important microsomal source of oxidative stress and lipid peroxidation [3]. For all of these reasons, our study examined the expression changes of CYP2E1 in liver tissues from Spraque-Dawley rats with type 2 diabetes caused by a high-fat diet combined with streptozotocin. On the other hand, we also highlight, for the first time, the effect of dapagliflozin, which is used to treat type 2 diabetes, on CYP2E1 expression. In our study, 32 maleSprague-Dawley rats were randomly divided into four groups: control, high-fat diet and streptozotocin-induced diabetes, dapagliflozin treated control, and dapagliflozin treated diabetes. In the microsomes obtained from the livers of these rats, the protein expression levels of CYP2E1 were determined by western blot. In our study, hepatic CYP2E1 expression level increased in control rats compared to the other three groups, but this increase is not statistically significant This result contrasts with previous studies reporting that hepatic CYP2E1 expression enhanced in diabetes [4]. Further research with a larger sample size is needed to clarify these conflicting results. From the result, hepatic CYP2E1 protein expression levels in the diabetic group treated with dapagliflozin were increased compared with in diabetic group. Although there was not statistically significant difference between two groups, this finding might indicate that increased CYP2E1 expression with the use of dapagliflozin under diabetic conditions may significantly affect impact