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Supplementation of oat (*Avena sativa* L.) extract abates alcohol-induced acute liver injury in a mouse model

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ABSTRACT

Dietary supplementation of oats has been associated with reduced risk of cardiovascular disease, diabetes, and gastrointestinal disorders. The role of oat extract as prophylactic in treating acute liver injury is not thoroughly established. We, therefore, hypothesized that oat extract would exert protective effect against alcohol-induced acute liver injury in a mouse model. To test this hypothesis, male C57BL/6 mice were pretreated with phenolic-enriched ethyl acetate (EA) fraction of oats (prepared by fractionating aqueous ethanolic extract with solvents of increasing polarity) at dosages of 125 and 250 mg kg^{-1} d^{-1} for 12 consecutive days. Acute liver injury was induced by administering 5 doses of 50% ethanol intragastrically (10 g/kg body weight) to mice at an interval of 12 hours. The alcohol-induced liver injury was evaluated by measuring serum levels of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, antioxidant parameters, mitochondrial function, and histology of liver tissue. Our results demonstrated that pretreatment with EA fraction at 250 mg kg^{-1} d^{-1} significantly ($P < .001$ for aspartate aminotransferase, alanine aminotransferase, and thiobarbituric acid–reactive species and $P < .01$ for lactate dehydrogenase and nitrites) reduced the levels of liver injury markers and significantly ($P < .001$ for glutathione reductase and glutathione S-transferase; $P < .01$ for catalase, superoxide dismustase, and vitamin C; $P < .05$ for reduced glutathione and NAD(P)H quinone dehydrogenase 1) increased the levels of antioxidant defenses. Furthermore, EA-pretreated mice showed mechanistic inhibition of nuclear factor κB signaling pathway through decreased phosphorylation and degradation of IκBα. We conclude that phenolic-enriched EA fraction of oats has immense potential to serve as dietary intervention against alcohol-induced liver damage.

Abbreviations: ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); ALD, alcoholic liver disease; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; AVN, avenanthramide; BSA, bovine serum albumin; BUT, *n*-butanol; CAT, catalase; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EA, ethyl acetate; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione S-transferase; HEX, hexane; HPLC, high-pressure liquid chromatography; IC_{50}, 50% inhibitory concentration; IκB, inhibitor of κB; IL-6, interleukin 6; LDH, lactate dehydrogenase; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADH, β-nicotinamide adenine dinucleotide hydrate; NF-κB, nuclear Factor-κB; NO, nitric oxide; NQO1, NAD(P)H:quinine oxidoreductase 1; OD, optical density; PEG, polyethylene glycol; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TBARS, thiobarbituric acid–reactive substances; TNF-α, tumor necrosis factor-α; TPC, total phenolic content; WAT, water.

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1. Introduction

Alcohol consumption ranks among the top 5 risk factors leading to disease, disability, and death throughout the world [1,2]. Approximately, 3.3 million deaths in 2012 (5.9% of all deaths) were estimated to have been caused merely by alcohol consumption worldwide [2]. An excessive consumption of alcohol over a length of time damages nearly every organ in the body. However, liver is the primary site of ethanol metabolism and sustains the earliest and the maximum degree of tissue injury from excessive drinking [3]. The development of alcoholic liver disease (ALD) is a consequence of heavy drinking, and consensus exists on the point of view that there is a clear-cut relationship between the amount of alcohol a person takes and the plausibility of the liver being seriously affected in the case of the particular individual [4,5]. Considerable evidence has shown that women drinkers are at greater risk of ALD than men and are more likely to develop acute liver failure from excessive alcohol use [6,7]. Moreover, women are at the additional risk of developing ALD at approximately half the levels of alcohol consumption when compared with men, along with the rapid progression of the disease [8]. According to National Institute on Alcohol Abuse and Alcoholism guideline, low-risk drinking is no more than 20 g/d for women and 40 g/d for men [9]. Alcoholic liver disease comprises an overlapping spectrum of pathological processes that include alcoholic fatty liver, alcoholic hepatitis, fibrosis, alcoholic cirrhosis, and hepatocellular carcinoma [10-12]. Several studies have reported that reactive oxygen species (ROS) viz, mainly hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and lipid peroxides, generated due to alcohol metabolism are prime causes of acute liver injury [13-15]. Under the above-mentioned conditions, the rate of ROS generation exceeds the liver's capacity to neutralize them with natural enzymatic and nonenzymatic antioxidants [16]. Although considerable progress has been achieved in understanding the pathogenesis of ALD, to date there has been no effective therapy as such [17].

Nutritional intervention and off-label use of various pharmacotherapies are aimed at the possibility of an alternative treatment of ALD [18]. The oxidative stress caused by alcohol is attributed to a decrease in antioxidant defense mechanisms, thereby resulting in hepatocyte damage [19-21]. Recent studies have demonstrated that administration of phenolic phytochemicals has increased the antioxidant defense response against alcohol-induced liver damage in mice [22-26].

For a long period, oats (*Avena sativa L.*) have been considered a healthy dietary supplement owing to higher nutritional value and fiber content [27]. This is because oats contain numerous bioactive phytochemicals such as phenolic acids, flavonoids, carotenoids, vitamin E, and phytosterols [28,29]. In addition, oats produce 2 unique types of biologically active phytochemicals, avenanthramides (AVNs) [30] and steroidal saponins [31]. An earlier study in this aspect has suggested that the consumption of AVN-enriched oat extract enhanced antioxidant potential in healthy human beings [32]. Furthermore, the consumption of oats attenuated exercise-induced production of ROS [33], prevented coronary heart disease [34], and showed an improvement in symptoms related to diabetes and obesity [35]. The intake of oat bran phenol–rich extract exhibited prophylactic and/or curative effect on low-density lipoprotein oxidation in humans and hamsters [36], D-galactose–induced oxidative stress in mice [37], and hyperlipidemic diet–induced oxidative stress in rats [38]. Based on in vitro studies, the AVNs showed the antiproliferative effect on vascular smooth muscle cells [39] and colon cancer cells [40]. Furthermore, the ethyl acetate (EA) fraction of oat extract, inhibited oleic acid–induced hepatic steatosis in HepG2 cells [41], and supplementation of oats prevented alcohol-induced gut leakiness and inflammatory bowel disease in rats [26,42].

Taking into consideration the numerous beneficial effects of oats, our study proposes the hypothesis that EA fraction of oat extract may exert a prophylactic effect on alcohol-induced acute liver injury in a mouse model. In the present study, to test our hypothesis, we investigated the prophylactic effect of EA fraction of oat extract on alcohol-induced changes in relative liver weight, serum liver injury markers, oxidative stress markers, mitochondrial function, inflammatory markers, and histopathology to acquire an insight into the underlying protective mechanisms through antioxidant and anti-inflammatory properties.

2. Methods and materials

2.1. *Chemicals and reagents*

Gallic acid; Folin-Ciocalteau reagent; 1,1-diphenyl-2-picrylhydrazyl (DPPH); 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt; potassium persulfate; sodium nitroprusside; sulfanilamide; *N*-(1-naphthylethylenediamine); Bradford reagent; silymarin (purity >97%); catalase (CAT); β-nicotinamide adenine dinucleotide 3-phosphate reduced form; β-nicotinamide adenine dinucleotide hydrate (NADH); 2,6-dichlorophenolindophenol; trifluoroacetic acid; reduced glutathione (GSH); 1-chloro-2,4-dinitrobenzene; 5,5-dithio-bis (2-nitrobenzoic acid); 2-thiobarbituric acid; Halt protease inhibitor cocktail (Pierce Biotechnology, Rockford, IL, USA); bovine serum albumin (BSA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); cytochrome c; superoxide dismutase (SOD) assay kit; and cytochrome c assay kit were purchased from Sigma (Sigma-Aldrich Co, St Louis, MO, USA). Orthophosphoric acid, dimethyl sulfoxide (DMSO), absolute ethanol (99.9%), high-pressure liquid chromatography (HPLC) grade acetonitrile, and methanol were purchased from Merck Life Science Pvt Ltd (Mumbai, India). Synthesized AVN standards, *N*-[4′-hydroxy-(*E*)-cinnamoyl]-5-hydroxyanthranilic acid (AVN-A), *N*-[4′-hydroxy-3′-methoxy-(*E*)-cinnamoyl]-5-hydroxyanthranilic acid (AVN-B), and *N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-5-hydroxyanthranilic acid (AVN-C) were kindly provided by Dr Mitchell L. Wise, Crop Research Unit, US Department of Agriculture as gratis. Kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) estimation were purchased from Siemens Diagnostic Ltd, Mumbai, India. Bicinchoninic acid protein assay kit and radioimmunoprecipitation assay buffer were purchased from Pierce Biotechnology. Antibodies against