Effectiveness of antioxidant treatments on cytochrome P450 2E1 (CYP2E1) activity after alcohol exposure in humans and in vitro models: A systematic review

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ABSTRACT
This systematic review aimed to describe the effects of antioxidant treatments after alcohol exposure in humans and in vitro models. A systematic review of quantitative studies to identify the effectiveness of antioxidant treatments on CYP2E1 activity after alcohol consumption. Multiple databases were searched from 2010 to May 2020 for studies in English, using MeSH terms and text words relating to antioxidant treatments, CYP2E1 and alcohol consumption. The protocol is registered on PROSPERO CRD420211226123. Study quality was assessed using the NICE methodology. The review is reported according to PRISMA. A total of eight original articles were analyzed. All papers collected and reported quantitative data. Antioxidant treatments could be beneficial after alcohol exposure to improve oxidative stress by decreasing CYP2E1 activity and increasing GSH levels. Our results suggest that antioxidant treatments could be beneficial and effective during ethanol exposure in human or in vitro models.

Introduction
It has been described that excessive consumption of alcohol promotes the pathogenesis of many fatal diseases, including cancer, liver cirrhosis cardiovascular diseases, diabetes, and neuropsychiatric disorders. In effect, causing liver injury in humans due to increased oxidative stress has been shown. Ethanol metabolism produces alcoholic fatty liver, alcoholic hepatitis, or cirrhosis. The major pathway of oxidative metabolism of ethanol in the liver involves alcohol dehydrogenase (ADH) present in the cytosol. Both ADH, CYP450 2E1 (CYP2E1), and catalase (CAT) are responsible for the oxidative metabolism of ethanol, while via non-oxidative metabolism, fatty acid ethyl esters (FAEE) synthase produces FAEEs.

During alcohol intoxication, the CYP2E1-dependent microsomal monooxygenase system and the microsomal respiratory chain are the main sources of reactive oxygen species (ROS) within the hepatocytes. Cytochrome P450 2E1 is of special interest because of its ability to metabolize and activate numerous hepatotoxic substrates in the liver such as ethanol, carbon tetrachloride, acetaminophen, and N-nitroso dimethylamine, to more toxic products. In this sense, induction of cytochrome CYP2E1 by ethanol appears to be one of the central pathways by which ethanol generates...
a state of oxidative stress. In addition, oxidation of ethanol by CYP2E1 produces acetaldehyde, a highly reactive compound that may contribute to the toxic effect of ethanol.\textsuperscript{[11]}

Acetaldehyde has been identified as the primary component responsible for the development of alcohol-mediated liver injury, extracellular matrix alterations, and inflammation.\textsuperscript{[12,13]} Its effects are produced through the formation of ROS and an imbalance in the redox potential (NAD/NADH). It also forms protein aggregates in hepatocytes, reducing the secretion of proteins and promoting hepatomegaly, with dopamine to generate salsolinol, which can contribute to alcohol dependency, and binds with the DNA to form carcinogenic products like 1,2-propane-2ʹ-desoxyguanosine.\textsuperscript{[14,15]}

There are numerous evidence for alcohol-mediated oxidative stress and toxicity in animal models and in vitro studies involving ethanol inducible CYP2E1 mediated oxidative stress and toxicity,\textsuperscript{[16,17]} where laboratory findings have stimulated investigations exploring novel pathophysiological targets that could be used for ALD therapy. The effects produced by alcohol in the liver can be counteracted by the antioxidant defense of the hepatocyte, which induces enzymatic mechanisms like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase,\textsuperscript{[18–20]} as well as other non-enzymatic mechanisms. Although the effectiveness of several antioxidants, including silymarin, vitamin E, N-acetylcysteine, and S-adenosylmethionine (SAMe), has been evaluated in recent clinical trials, conflicting outcomes were reported.\textsuperscript{[18,19,21,22]} Therefore, the specific question addressed in this systematic review was: could the antioxidant treatments improve CYP2E1 activity after alcohol exposure in humans and in vitro models?

**Methods**

A systematic review of quantitative articles studying the effectiveness of antioxidant treatments on CYP2E1 activity after alcohol exposure. The protocol is registered on the PROSPERO database, CRD42021226123.\textsuperscript{[23]} The review is reported according to PRISMA.\textsuperscript{[24]}

**Search strategy and selection criteria**

**Search strategy**

Multiple databases (MEDLINE, EMBASE, Scopus, and Web of Science) were searched from 2010 to May 2020 for original articles, primary quantitative studies in English, using MeSH terms (“alcohol consumption” AND “CYP2E1” AND “antioxidant treatment”) and text words relating antioxidant treatments, CYP2E1 and alcohol consumption according to the research question (Supplemental Appendix 1). The searches were part of broader searches for a series of reviews covering a range of health parameters, as biochemistry parameters and molecular biology analysis. Additionally, the reference lists of included studies and relevant reviews were searched.

**Identification of relevant studies**

Two reviewers screened titles, abstracts, and papers for inclusion. Differences between reviewers’ results were resolved by discussion with another reviewer.

**Types of study and design**

The human studies the specific inclusion criteria were: 1. primary quantitative studies or mixed methods studies with a quantitative component (using descriptive or inferential statistics methods, with parametric or non-parametric methods); cross-sectional studies or randomized controlled trials; which report antioxidant treatments type used, CYP2E1 effects and alcohol consumption patterns and; 2. studies in English. Research studies were excluded if they: 1. systematic reviews; 2. conference abstracts; 3. editor letters; 4. were not an original investigation published in full; 5. did not provide or specify numerical data; 6. studies on population older than 80 years old and 7. studies not focused on the effectiveness of antioxidant treatments on CYP2E1 activity after alcohol exposure.
**Population/settings**

People aged between 15 and 80 years old, living in the community; including healthy participants with no pre-conditions for later ill health such as diabetes, insulin resistance, high blood pressure, or high cholesterol. People on medication or studies that primarily focused on populations with ill health e.g. stroke, coronary heart disease, and mental health conditions were excluded. For *in vitro* studies, HepG2 or SK-Hep-1 cells were exposed to ethanol and protective molecules to evaluate their protective capacity on alcohol-induced liver disease.

**Quality assessment/risk of bias**

Methodological quality was assessed using the NICE methodology for quantitative studies by one reviewer and checked for accuracy by a second reviewer.\[25\] Differences between reviewers were resolved by discussion. No studies were excluded based on quality.

**Data extraction and synthesis**

Data relating to the population and study characteristics of the included studies were extracted by one reviewer (CC) and checked by another reviewer (DC) (Table 1). To identify information relevant to variables involved in the effectiveness of antioxidant treatments on CYP2E1 activity after alcohol exposure, one researcher (CS) examined the results and discussion sections of each text, line by line, to identify data relating to antioxidant treatments, CYP2E1 activity, and alcohol exposure. Interpretation and concepts of the study authors were also included if they were developed from the original data. The text was then further examined and reorganized into themes (Table 2). Further interpretation and analysis about possible treatments on CYP2E1 activity after alcohol exposure in identified from the texts.

**Results**

Figure 1 illustrates the flow chart for the study selection process from eight papers were identified.\[26–33\] A summary of included studies, and the population/settings and context in which they were conducted is shown in Table 1.

**Description of included studies**

From the primary studies, four papers were conducted in Korea, two in China, and two in Indonesia. A total of eight original articles were analyzed. All papers collected and reported quantitative data through randomized controlled trials (Table 1). Six studies were realized in cell culture (*in vitro* models);\[26–29,31,32\] and two in human models.\[30,33\] Five studies were conducted in HepG2 cells,\[26,27,29,31,32\] one in SK-Hep-1 cells\[28\] and two in healthy adults.\[30,33\] Details of antioxidant treatment of each study where available are shown in Table 1. Two studies included *S. quelpaertensis* Nakai leaf extract,\[31,32\] one fermented sea tangle,\[27\] one baicalin,\[29\] one corn-peptides,\[30\] one use phytochemicals,\[28\] one in reduced-glutathione (GSH)\[26,31\] while one including a standardized polyphenolic extract of clove buds (Clovinol).\[33\]

**Quality assessment**

Quality assessment results and assessment criteria of individual studies are shown in Supplemental Table 1. Overall, the quality of studies was generally high or moderate for internal and external validity. No studies were excluded based on quality.
| Author/year | Country | Population or sample | Inter details | Other treatment | Investigated outcomes | Study aims | Results before Inter | Results after Inter |
|-------------|---------|----------------------|---------------|-----------------|-----------------------|------------|----------------------|---------------------|
| Chandrasekaran et al. [26] | | HepG2 or VL-17A cells | Addition of 100 mM EtOH. | NA | Cell viability, caspase 3 activity, DNA analysis, analysis of apoptosis, analysis of the mitochondrial membrane potential, measurement of intracellular reactive oxygen species, measurement of lipid peroxidation, measurement of protein carbonyl adducts, staining for 4-hydrohynonenal adduct, measurement of GSH, ADH and CYP2E1 catalytic activity, protein expression of ADH and CYP2E1. | Investigates the role of GSH, an important cellular antioxidant in protecting liver cells against ethanol mediated toxicity and apoptosis. | Cell viability: 85%; caspase 3 activity: 250 pNA; DNA analysis: +; analysis of apoptosis: ++; analysis of the mitochondrial membrane potential: +; measurement of intracellular reactive oxygen species: a 3.8-fold increase in ROS level as compared with untreated HepG2 cells; measurement of lipid peroxidation: did not show an increase in basal lipid peroxidation as compared with untreated HepG2 cells; measurement of protein carbonyl adducts: did not show an increase in protein carbonyl formation as compared with untreated HepG2 cells; staining for 4-hydrohynonenal adduct: +++; measurement of GSH: No significant increase as compared with untreated HepG2 cells; ADH activity: No changes was found in the activity of ADH as compared with untreated HepG2 cells; and CYP2E1 catalytic activity: No significant increase in the level of CYP2E1 level as compared with untreated HepG2 cells. | Cell viability: 47%; caspase 3 activity: 500 pNA; DNA analysis: +++; analysis of apoptosis: +++; analysis of the mitochondrial membrane potential: ++; measurement of intracellular reactive oxygen species: a 3.8-fold increase in ROS level as compared with untreated VL-17A cells; measurement of lipid peroxidation: a 3-fold increase in lipid peroxidation as compared with untreated VL-17A cells; measurement of protein carbonyl adducts: a 2.2- and 3.8-fold increases in protein carbonyl formation as compared with untreated VL-17A cells; staining for 4-hydrohynonenal adduct: +++; measurement of GSH: a 3-fold increase in GSH content as compared with untreated VL-17A cells; ADH activity: a 1.3-fold increase in the activity of ADH as compared with untreated VL-17A cells; and CYP2E1 catalytic activity: a 2.6-fold increase in the level of CYP2E1 as compared with untreated VL-17A cells. |
Table 1. (Continued).

| Author/year | Country | Population or sample | Inter details | Other treatment | Investigated outcomes | Study aims | Results before Inter | Results after Inter |
|-------------|---------|----------------------|---------------|----------------|-----------------------|------------|----------------------|---------------------|
| Kang et al. [27] | KR | HepG2 cells. | Addition of 1.0 M EtOH. | | | Cell viability, determination of intracellular GSH, GGT activity and CYP2E1 expression. | Evaluate the protective effects of FSTJ against ethanol-induced cytotoxicity in HepG2 cells by assaying levels of GSH content, GGT activity, and CYP2E1 expression. | Cell viability: cells appear to be quite resistant to ethanol up to 0.5 M but concentrations from 1.0 to 2.0 M result in a severe loss of cell viability; intracellular GSH activity: GSH concentration decreased to 44.3% of control; intracellular GGT activity: 100% activity; and intracellular CYP2E1 expression: Not reported. | Cell viability: pretreatment of the cells with FSTJ significantly attenuated the loss of cell viability induced by 1.0 M ethanol in a dose-dependent manner; intracellular GSH activity: increased GSH levels in relation of untreated cells; intracellular GGT activity: decreased in cells treated with FSTJ; and intracellular CYP2E1 expression: completely inhibited by 25 μg/mL FSTJ treatment. |
| Author/year  | Country | Population or sample | Inter details | Other treatment | Investigated outcomes                                                                                                                                                                                                                                                                                                                                 | Study aims                                                                                                                                                                                                                                                                                                                                 | Results before inter | Results after inter                                                                                                                                                                                                 |
|-------------|---------|----------------------|---------------|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Lee et al. [28] | KR      | SK-Hep-1 cells.      | Addition of 100 or 200 mM EtOH. | Addition of 5 μM of individual phytochemicals. | Cell proliferation, evaluation of cytotoxicity, determination of reactive oxygen species and lipid hydroperoxide, measurement of mitochondrial membrane potential and protein oxidation; determination of intracellular GSH content, antioxidant enzyme activities and caspase-3 activity.                                                                 | Examine the cytoprotective effect of phytochemicals against ethanol-induced oxidative stress in the SK-Hep-1 cells.                                                                                                                                                                                                                   | Cell proliferation: it was reduced to a range of 93-106% after co-treatment with Qu, Ct, Cf and Py; evaluation of cytotoxicity: it was decreased to 95-110% after co-treatment with Qu, Ct, Cf and Py; determination of reactive oxygen species: it was restored to a range of 98-123% after co-treatment with Qu, Ct, Cf and Py; lipid hydroperoxide: they decreased by 14-18% with the addition of Qu, Ct, Cf and Py; measurement of mitochondrial membrane potential: it was elevated to a range of 91-116% by the addition of Qu, Ct, Cf and Py; protein oxidation level: it was decreased by 10-14% with the addition of Qu, Ct, Cf and Py; determination of intracellular GSH content: it was increased to a range of 6.61-6.71 nmol/μg protein with the addition of Qu, Ct, Cf and Py; antioxidant enzyme activities: the addition of Qu, Ct, Cf and Py restored CAT and GPX activities to control levels and; caspase-3 activity: it shown an increase in non-oxidative ethanol metabolism. |
| Author/year   | Country | Population or sample | Inter details | Investigated outcomes | Study aims | Results before Inter | Results after Inter |
|--------------|---------|----------------------|---------------|-----------------------|------------|----------------------|---------------------|
| Xu et al. [29] | CN      | HepG2 cells.         | Addition of 100 mM EOH and/or 50 μM of Fe. | Cell viability, analysis of apoptosis, protein carbonylation, morphological analysis and integrated optical density, antioxidant activity. | Evaluate the CYP2E1-independent increase of ROS/RNS induced by alcohol and iron in HepG2 cells, and the effect of Ba. | Cell viability: lower normal cell ratio; analysis of apoptosis: higher apoptotic cell ratio; protein carbonylation: increased protein nitration and; morphological analysis: the expression of iNOS decreased in the nucleus and increased in the cytoplasm. | Cell viability: moderate cellular injury; analysis of apoptosis: pre-treatment with Ba markedly decreased apoptosis; protein carbonylation: reduced levels of protein nitration and; morphological analysis: Ba interfered with the distribution of iNOS. |

Wu et al. [30]  
N=146 men aged between 35–55 years, BMI 20–28 kg/m², alcohol consumption >30 g/day for at least five years, and ultrasound-defined fatty liver.  
CPs  
WP  
Anthropometric measurements, serum lipid profile, serum markers of liver injury, serum markers of oxidative stress and inflammation, liver ultrasound examination.  
Evaluated the effects of CPs supplementation on lipid profile, oxidative stress and alcoholic liver injury compared to WP and cornstarch (placebo) supplementation in men with chronic alcohol consumption.  
Anthropometric measurements: did not differ significantly between groups; serum lipid profile: TG: 2.4 mmol/L, TC: 5.23 mmol/L, LDL-C: 2.68 mmol/L, HDL-C: 1.26 mmol/L; serum markers of liver injury: TB: 13.58 μmol/L, ALP: 64.16 U/L, GGT: 64.75 U/L, ALT: 34.2 U/L, AST: 25.3 U/L, and; serum markers of oxidative stress and inflammation: GPx: 185.62 U/mL, SOD: 33.62 U/mL, MDA: 4.62 mmol/mL, TNF-α: 91.2 pg/mL.  
Anthropometric measurements: did not differ significantly between groups; serum lipid profile: TG: 2.03 mmol/L, TC: 5 mmol/L, LDL-C: 2.68 mmol/L, HDL-C: 1.12 mmol/L; serum markers of liver injury: TB: 12.23 μmol/L, ALP: 63.8 U/L, GGT: 64.58 U/L, ALT: 31.86 U/L, AST: 24.16 U/L, and; serum markers of oxidative stress and inflammation: GPx: 212.12 U/mL, SOD: 37.16 U/mL, MDA: 4.23 mmol/mL, TNF-α: 78.54 pg/mL.  

(Continued)
| Author/year | Country | Population or sample | Inter details | Investigated outcomes | Study aims | Results before Inter | Results after Inter |
|-------------|---------|----------------------|--------------|-----------------------|------------|----------------------|---------------------|
| Madushani Herath et al. [31]  
[31] | KR | HepG2 cells. | Addition of 800 mM EtOH and/or varying concentrations of various SQEs. | Cell viability, cytoprotective effects, RNS oxygen, catalase and GPX-1 activity and, apoptotic DNA formation. | To examine the protective capacity of *S. quelpaertensis* Nakai leaf extract against alcohol-induced fatty liver development. | Cell viability: Not reported; cytoprotective effects: viability of EtOH exposed HepG2 cells was significantly reduced at EtOH concentrations higher than 400 mM compared to untreated control; RNS oxygen activity: ROS production was increased by 35% in HepG2 cells stimulated by ethanol compared to untreated control; catalase and GPX-1 activity: in HepG2 cells stimulated by ethanol, catalase activity was decreased by 1.4 fold, and the activity of GPX-1 was not significantly affected in ethanol stimulated HepG2 cells compared to untreated control, and, apoptotic DNA formation: apoptotic DNA was significantly increased by 7-fold in ethanol stimulated cells compared to untreated control. | Cell viability: Viable cell percentage decreased when treated with SQEDW, SQEE20, and SQEE40 and it was increased in SQEE80 treated compared to untreated control; cytoprotective effects: none of SQEDW, SQEE20, and SQEE40 showed protective effect against alcohol-induced cytotoxicity in HepG2 cells. But, SQEE60 and SQEE80 treatment increased the viable cell population; RNS oxygen activity: ROS production was decreased in ethanol stimulated HepG2 cells treated with SQEE80; catalase and GPX-1 activity: SQEE80 treatment with 250 μg/mL significantly increased the activity of catalase by 1.7 fold in ethanol stimulated HepG2 cells and, treatment with 250 and 500 μg/mL of SQEE80 of ethanol in stimulated cells increased the hepatic GPX-1 activity significantly; and, apoptotic DNA formation: SQEE80 treatments (250 and 500 μg/mL) decreased the apoptotic DNA in ethanol stimulated cells by 62.2% and 55.5%. |
| Author/year | Country | Population or sample | Inter details | Other treatment | Investigated outcomes | Study aims | Results before Inter | Results after Inter |
|-------------|---------|----------------------|---------------|----------------|----------------------|------------|----------------------|----------------------|
| Madushani Herath et al. [32]^a | KR | HepG2 cells. | Addition of 400 mM EtOH and/or varying concentrations of different solvent fractions from 80% EtOH extract of S. quelpaertensis Nakoi leaf. | NA | Cell viability. | To evaluate different extracts of S. quelpaertensis for its cytoprotective effect in HepG2 cells and mice against alcohol-induced cytotoxicity. | Cell viability: EtOH exposure decreased (p<0.05) the cell viability compared to untreated control. | Cell viability: treatment with SQA did not have a significant effect on cell viability against EtOH induced toxicity in low to medium concentrations but significantly decreased the cell viability at higher concentrations. SQB and SQC did not show any significant change in cell viability across the whole concentrations tested. SQEA at 125, 250 and 500 μg/mL exhibited cytoprotective effect against EtOH induced toxicity and enhanced the cell viability by 25.3%, 16% and 17.2% respectively. |

(Continued)
| Author/year | Country | Population or sample | Interventions | Other treatment | Hematological and biochemical parameters. | Study aims | Results before Inter | Results after Inter |
|-------------|---------|----------------------|---------------|----------------|-----------------------------------------|------------|---------------------|----------------------|
| Mammen et al. [33] |  | N=16 healthy adult males aged between 25 and 55 years. | Intake of 1 g/kg body weight/day of EtOH; and placebo or 250 mg of Clovinol | NA | To demonstrate the efficacy of Clovinol in ameliorating the oxidative stress and inflammation caused by the accumulation of acetaldehyde after binge drinking. | Blood alcohol and acetaldehyde concentration: Not reported; antioxidant status and lipid peroxidation: Both SOD and GSH showed a significant change after 4, 12 h of alcohol consumption; measurement of 8-isoprostane: alcohol consumption increase the oxidative stress as evident from the generation of 8-isoprostane in plasma; measurement of CRP and IL-6: a significant increase in the inflammatory markers, CRP and IL-6, was found after heavy consumption of alcohol, and; survey of hangover severity: Not reported. | Blood alcohol concentration: In the Clovinol group at various post-alcohol consumption time intervals were lower than those in the placebo group; blood acetaldehyde levels: a significant decrease (65.10%) for the Clovinol group as compared with the placebo; antioxidant status and lipid peroxidation: Clovinol administration was found to maintain the baseline level of SOD with significant inhibition (92.5% p<0.001) in SOD depletion and inhibit the GSH depletion with an average enhancement of 34.5% (p<0.001) as compared with placebo; measurement of 8-isoprostane: Clovinol administration was found to maintain the baseline 8-isoprostane levels; measurement of CRP and IL-6: Clovinol administration was found to inhibit the increase in the CRP and IL-6 levels significantly; and; survey of hangover severity: he mean overall hangover severity score for the Clovinol group was significantly lower than that for the placebo group with a reduction of 55.34%. |

\( a = \text{in vitro study; ADH: Alcohol dehydrogenase; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate transaminase; Ba: Balcaen; BMI: Body mass index; CAT: Catalase; CF: Caffeic acid; Clovinol: Standardized polyphenolic extract of Clove buds; CN: China; CPs: Corn peptides; CRP: C-reactive protein; Ct: Catechin; CYP2E1: Cytochrome P450 2E1; DNA: EtOH: Ethanol; FC: Ferric citrate; FSTJ: Fermented sea tangle juice; GGT: Gamma-glutamyl transferase; GPX-1: Glutathione peroxidase-1; GPX: Glutathione peroxidase; GSH: Glutathione; HDL-C: High-Density lipoprotein; ID: Indonesia; IL-6: Interleukin-6; iNOS: inducible Nitric oxide synthase; KR: Korea; LDL-C: Low-density lipoprotein; MDA: Malondialdehyde; MTT: Thiobenzyl blue tetrazolium bromide assay.; pNA: p-Nitroaniline; Py: Phytic acid; Qu: Quercetin; RNS: Reactive oxygen species; ROS: Reactive oxygen species; SOD: Superoxide dismutase; SQA: Sasa quelpaertensis Nakai leaf extract suspended by distilled water and partitioned into distilled water; SQB: Sasa quelpaertensis Nakai leaf extract suspended by distilled water and partitioned into n-butanol; SQC: Sasa quelpaertensis Nakai leaf extract suspended by distilled water and partitioned into chloroform; SQD: Sasa quelpaertensis Nakai leaf extract suspended by distilled water and partitioned into ethyl acetate; SQEDW: Sasa quelpaertensis leaf extract dissolved into distilled water; SQEE20: Sasa quelpaertensis leaf extract dissolved into 20% (v/v) EtOH; SQEE40: Sasa quelpaertensis leaf extract dissolved into 40% (v/v) EtOH; SQEE60: Sasa quelpaertensis leaf extract dissolved into 60% (v/v) EtOH; SQEE80: Sasa quelpaertensis leaf extract dissolved into 80% (v/v) EtOH; SQEs: Sasa quelpaertensis Nakai leaf extracts into different dissolvents; TB: Total bilirubin; TC: Total cholesterol; TG: Triglycerides; TNF-α: Tumor necrosis factor-alpha; WP: Whey protein; 4-MP: 4-Methylpyrazole.}
Table 2. Variables involved in effectiveness of antioxidant treatments on cytochrome P450 2E1 (CYP2E1) activity after alcohol consumption.

| Measurement                  | Cell or metabolic changes | References |
|------------------------------|----------------------------|------------|
| **Antioxidant activity**     | Clovinol administration was found to maintain the baseline level of SOD with significant inhibition (92.5%; p < .001) in SOD depletion as compared with placebo. Clovinol administration inhibits the decrease in GSH levels with an average improvement of 34.5% compared to placebo. The level of the antioxidant GSH was found to be 3-fold upregulated in VL-17A cells treated with ethanol, which may be a metabolic adaptation of the oxidative stress. The SQEA, rich in phenolic acids such as p-coumaric acid and flavonoids, particularly myristin, showed a hepatoprotective effect against EtOH (400 mM) in HepG2 cells. Baicalin inhibits oxidative stress induced by the combination of alcohol and iron, mainly by chelation of iron. Co-treatment of cells with ethanol and Qu, Ct, Cf and Py significantly inhibited oxidative metabolism-induced cytotoxicity by blocking ROS production. Ct was very effective at recovering levels of intracellular ROS and MMP altered by ethanol treatment. | Mammen et al. [33]a,c Mammen et al. [33]a,c Chandrasekaran et al. [26]a,b Madushani Herath et al. [31]a,b Xu et al. [29]a,b Lee et al. [28]a,b |
| **Cell viability**           | Untreated VL-17A cells exhibited apoptosis and oxidative stress when compared with untreated HepG2 cells. Chronic alcohol exposure, i.e., 100 mM ethanol treatment for 72 h caused a significant decrease in viability (47%) in VL-17A cells but not in HepG2 cells. SQEA at 125, 250 and 500 µg/mL exhibited cytoprotective effect against EtOH induced toxicity and enhanced the cell viability by 25.3%, 16% and 17.2% respectively (all p < .05). Increased in cells treated with SQEE80 (0–1000 µg/mL) after 24 hours of incubation at high concentrations of SQEE80 compared to untreated control: by 10% at 250 µg/mL, by 20% at 500 µg/mL and by 18% to 1000 µg/mL (p < .05). Co-treatment of cells with 100 mM ethanol and Qu, Ct, Cf or Py (1 or 5 µM) significantly increased proliferation of SK-HeP-1 cells to a range of 93–106%. Treatment with SQEA at 125 and 250 µg/mL reduced the apoptotic DNA fraction in the sub-G1 phase by 2.1 (p < .05) and 2.3 (p < .005) times. Pretreatment with 8a markedly decreased apoptosis (p < .05) in the EtOH-Fc-Ba group (25 µM) and the EtOH-Fc-Ba group (50 µM) compared to the EtOH-Fc group. The effect of 8a was concentration dependent. SQEE80 treatments (250 and 500 µg/mL) decreased the apoptotic DNA in sub-G1 groups in ethanol-stimulated cells by 62.2% and 55.5% compared to those without SQEE80 treatment. | Chandrasekaran et al. [26]a,b Chandrasekaran et al. [26]a,b Madushani Herath et al. [31]a,b Madushani Herath et al. [32]a,b Lee et al. [28]a,b |
| **Apoptosis**                | Clovinol administration was found to maintain the baseline 8-isoprostane levels. Pretreatment with 8a reduced the level of ROS induced by EtOH-Fc in the EtOH-Fc-Ba group (25 µM) and the EtOH-Fc-Ba group (50 µM) after 1 h of EtOH treatment. ROS production was decreased in ethanol stimulated HepG2 cells treated with SQEE80 compared to those without SQEE80 treatment. The reduction of ROS production was 22.9% (p < .05) and 37.7% (p < .05) with 250 µg/mL and 500 µg/mL of SQEE80 treatments, respectively. Treatment with FSTJ at a concentration of 25 µg/mL increased GSH levels to 67.08%. In ethanol-treated cells, GSH content decreased to 44.35% of control (ethanol-untreated cell) value. Co-treatment of cells with ethanol and Qu, Ct, Cf or Cf (1 or 5 µM) decreased cytotoxicity to 95–110%. Lipid hydroperoxide levels were decreased by 14–18% with the addition of Qu, Ct, Cf and Py. The level of reduced glutathione increased to a range of 6.61–6.71 nmol/µg protein with the addition of Qu, Ct, Cf and Py. | Mammen et al. [33]a,c Xu et al. [29]a,b Madushani Herath et al. [32]a,b Kang et al. [27]a,b Kang et al. [27]a,b Lee et al. [28]a,b Lee et al. [28]a,b |
| **Cellular damage**          | Clovinol administration reduced the extent of lipid peroxidation by 25% and helped it to remain in the normal range at all times after alcohol ingestion. Clovinol administration was found to maintain the baseline 8-isoprostane levels. Pretreatment with 8a reduced the level of ROS induced by EtOH-Fc in the EtOH-Fc-Ba group (25 µM) and the EtOH-Fc-Ba group (50 µM) after 1 h of EtOH treatment. ROS production was decreased in ethanol stimulated HepG2 cells treated with SQEE80 compared to those without SQEE80 treatment. The reduction of ROS production was 22.9% (p < .05) and 37.7% (p < .05) with 250 µg/mL and 500 µg/mL of SQEE80 treatments, respectively. Treatment with FSTJ at a concentration of 25 µg/mL increased GSH levels to 67.08%. In ethanol-treated cells, GSH content decreased to 44.35% of control (ethanol-untreated cell) value. Co-treatment of cells with ethanol and Qu, Ct, Cf or Cf (1 or 5 µM) decreased cytotoxicity to 95–110%. Lipid hydroperoxide levels were decreased by 14–18% with the addition of Qu, Ct, Cf and Py. The level of reduced glutathione increased to a range of 6.61–6.71 nmol/µg protein with the addition of Qu, Ct, Cf and Py. | Mammen et al. [33]a,c Xu et al. [29]a,b Madushani Herath et al. [32]a,b Kang et al. [27]a,b Kang et al. [27]a,b Lee et al. [28]a,b |
| **Protein expression**       | Incubating VL-17A cells with 100 mM ethanol for 72 h resulted in a 2.6-fold increase in the level of CYP2E1 when compared to untreated VL-17A cells. The expression of CYP2E1 was completely inhibited in cells treated with FSTJ at a concentration of 25 µg/mL. SQEE80 treatment with 250 µg/mL (p < .05) significantly increased catalase activity by 1.7-fold in ethanol-stimulated HepG2 cells compared to those without SQEE80 treatment. In addition, treatment with both doses of SQEE80 in ethanol stimulated cells significantly increased liver GPX-1 activity (p < .05) compared to cells without SQEE80 treatment. Clovinol supplementation significantly inhibited the alcohol-induced elevation in IL-6. | Madushani Herath et al. [32]a,b Madushani Herath et al. [32]a,b Madushani Herath et al. [32]a,b Madushani Herath et al. [32]a,b |
| **Biochemical parameters**   | | Mammen et al. [33]a,c |

(Continued)
Table 2. (Continued).

| Measurement | Cell or metabolic changes | References |
|-------------|---------------------------|------------|
| Supplementation with CPs decreased ALT and AST, whereas participants who received WP or placebo showed no significant changes. Decrease in the blood acetaldehyde concentrations upon Clovinol treatment, as compared with control. | Wu et al.\textsuperscript{[30]a,b} | Mammen et al.\textsuperscript{[33]a,c} |

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Clovinol: Standardized polyphenolic extract of Clove buds; Cf: Caffeic acid; CPs: Corn peptides; Ct: Catechin; CYP2E1: Cytochrome P450 2E1; EtOH: Ethanol; FSTJ: Fermented marine tangle juice; GPX-1: Glutathione peroxidase-1; GSH: Reduced glutathione; MMP: Mitochondrial membrane potential; Py: Phytic acid; Qu: Quercetin; SOD: Superoxide dismutase; SQEA: Ethyl acetate fraction of 80% ethanol extract of Sasa quelpaertensis Nakai leaves extract; SQEE80: 80% ethanol extract of Sasa quelpaertensis; ROS: Reactive oxygen species.

Figure 1. PRISMA flow diagram.

**Relation between antioxidants and alcohol exposure**

Processes that protect against alcohol-induced deleterious effects include antioxidant enzymes,\textsuperscript{[19]} such as superoxide dismutase, catalase, glutathione, and glutathione reductase\textsuperscript{[18,19]}, the antioxidant GSH, metal-binding proteins, vitamins C and E; the carotenoids that deactivate the free radicals\textsuperscript{[34,35]}. 
and other antioxidants such as cinnamic acid and syringic acid that improving the inflammatory and oxidative damage produced by ethanol.\textsuperscript{[36]}

Few studies in humans have described the role of antioxidants on alcohol intake.\textsuperscript{[30,33]} In effect, some variables involved during alcohol exposure, such as time of exposure, age, dose, ethnicity, or type of alcohol consumed could hinder the process. Meanwhile, in vitro assays allow controlling the studied substance, without subjecting humans or animals to the possible side effects or toxicity of a new drug. The possible antioxidant strategies to use on CYP2E1 activity after alcohol exposure are shown in Figure 2.

**Antioxidant activity**

The antioxidant effects were indicated in almost all articles (Table 2). However, if the antioxidant activity was not measured; others measurements such as cell viability, cellular damage, protein expression, or enzymatic analysis were analyzed to evaluate it.\textsuperscript{[27,30,32]} The changes on GSH enzyme activity to evaluate antioxidant effects were often used.\textsuperscript{[26,31,32]} Although one study used to measure more than one enzyme involved in the antioxidant activity to evaluate antioxidant effects such as catalase and glutathione peroxidase,\textsuperscript{[28]} just one study used the ABTS radical cation to measure antioxidant activity.\textsuperscript{[29]}

**Cell viability**

Viability levels and/or proliferation rates of cells are good indicators of cell health. In effect, physical and chemical agents can affect cell health and metabolism. Thus, some in vitro studies have shown that cells treated with Ethyl acetate fraction of 80% ethanol extract of Sasa quelpaertensis Nakai leaves extract (SQEA) or 80% ethanol extract of Sasa quelpaertensis (SQEE80) have enhanced cell viability due to attenuated oxidative stress by decreased expression of CYP2E1.\textsuperscript{[31,32]} In the same way, the co-treatment of cells with ethanol and phytochemicals shown an increased proliferation of SK-Hep-1 cells due to suppression ROS and lipid hydroperoxide (LPO) production, protein oxidation,\textsuperscript{[28]} and loss of
mitochondrial membrane potential (MMP) and increased CAT and glutathione peroxidase-1 (GPX-1) activities during 4-methylpyrazole (4-MP) induced non-oxidative metabolism of ethanol.

**Apoptosis**

Flow cytometry is one of the most popular and versatile applications for studying apoptosis. In effect, antioxidants’ effects on ethanol-induced free radicals and hepatic DNA strand breaks have been studied. In this sense, the treatment with SQEA or SQEE80 decreased the apoptotic DNA fraction confirming their hepatoprotective effect against ethanol-induced cell death.\(^{31,32}\) Likewise, pre-treatment with Baicalin has shown decreased apoptosis levels on groups treated with ethanol and 25 µM and 50 µM of Baicalin, due to a decreased accumulation of ROS and prevention on HepG2 cells from protein oxidation damage and apoptosis.\(^{29}\)

**Cellular damage**

Reactive oxygen species plays an important role in alcohol-induced cell injury and disease; and it can cause various cellular injuries, such as DNA damage, lipid peroxidation, and protein modification. However, antioxidant exposure could decrease levels of lipid peroxidation and ROS. In this sense, Clovinol -standardized polyphenolic extract of clove buds- administration reduced lipid peroxidation and 8-isoprostane, a marker of oxidative stress, after alcohol ingestion.\(^{33}\) While Baicalin and SQEE80 reduced the levels of ROS after ethanol treatment.\(^{29,32}\)

Likewise, there are non-enzymatic defense mechanisms that include: GSH, which is almost exclusively in its reduced form and detoxifies the ROS produced on the mitochondrial electron transport chain. Thus, compounds as Fermented Sea Tangle Juice (FSTJ) and phytochemicals, such as quercetin, catechin, caffeic acid, and phytic acid; have shown increased GSH levels after ethanol exposure,\(^{27,28}\) promoting increased protection of this organelle to oxidative damage.

**Protein expression**

Induction of CYP2E1 is one of the central pathways by which ethanol generates oxidative stress, where incubating VL-17A cells with ethanol results in an increase in CYP2E1 levels.\(^{26}\) Likewise, studies have described the expression of CYP2E1 was completely inhibited when cells were treated with FSTJ.\(^{27}\)

**Biochemical parameters**

Antioxidants promote hepatoprotective effects and improve the activities of the enzymes that are associated with alcoholic liver disease. In this sense, SQEE80 has been shown to increase CAT and GPX-1 activity in ethanol-stimulated HepG2 cells compared to those without SQEE80 treatment.\(^{32}\) Clove buds (Syzygium aromaticum L.), a popular kitchen spice, have been identified as one of the richest sources of antioxidants polyphenols among the various fruits, vegetables, and edible herbs. Thus, Clovinol has been shown to decrease acetaldehyde levels and inhibited the alcohol-induced elevation in IL-6 through its antioxidant and anti-inflammatory potential.\(^{33}\) Likewise, Corn Peptides (CPs) -a novel food prepared from corn gluten meal- have shown beneficial effects on early alcoholic liver disease and acute alcoholic injury in mice. In effect, CPs decreased serum activities of ALT and AST.\(^{30}\)

**Discussion**

The systematic review collates and synthesizes evidence from eight quantitative studies relating different kinds of antioxidant treatments and CYP2E1 activity after alcohol exposure in humans and *in vitro* models.
**Summary of key findings and interpretation**

It is important to note that the effects produced by alcohol can be counteracted by antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase as well as non-enzymatic mechanisms.\(^{[18,19]}\) These non-enzymatic defense mechanisms include: GSH; metal-binding proteins; vitamins C and E; the carotenoids that deactivate the free radicals\(^{[34,35]}\) and other antioxidants such cinnamic acid and syringic acid that suppress liver activity and/or protein expression of CYP2E1, ADH, NADPH oxidase, nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB), improving the inflammatory and oxidative damage produced by ethanol.\(^{[36]}\) In addition, baikalin can alleviate ethanol-induced liver damage in rats, probably due to its antioxidant, anti-inflammatory properties, and the activation of Shh, which plays an important role in the morphogenesis of tissues and liver repair.\(^{[15,37]}\)

In this sense, *in vitro* studies on HepG2 cells have shown that SQEA and SQEE80 enhance cellular growth rates.\(^{[31,32]}\) While, phytochemicals treatment has shown an increased proliferation on SK-Hep-1 cells.\(^{[28]}\) Thus, cell viability has been enhanced through a decreased oxidative stress and CYP2E1 expression, suppression of ROS and LPO production, protein oxidation, loss of MMP, and increased CAT and GPX-1 activities; suggesting that SQEA, SQEE80, and phytochemicals, such as quercetin, catechin, caffeic acid, and phytic acid; have a direct action on oxidative and non-oxidative metabolism of ethanol.

It has been reported that ethanol promotes the formation of ROS within the mitochondria and reduces the concentration of GSH, promoting increased susceptibility of this organelle to oxidative damage.\(^{[12,38]}\) Also, ROS can interact with lipids, proteins, and DNA in a process called peroxidation, leading to the production of malondialdehyde (MDA) and 4-hydroxynonenal (HNE).\(^{[39]}\) However, previous studies have described that exposure to antioxidants as Clovinol, Baicalin, SQEE80, FSTJ, and phytochemicals as quercetin, catechin, caffeic acid, and phytic acid; could decrease levels of lipid peroxidation and ROS.\(^{[27–29,32,33]}\) In effect, Clovinol reduces lipid peroxidation and 8-isoprostane in adult males after binge drinking.\(^{[33]}\) whereas Baicalin and SQEE80 reduced the levels of ROS after ethanol treatment on HepG2 cells.\(^{[29,32]}\) Likewise, compounds as FSTJ and phytochemicals increase GSH levels after ethanol exposure on HepG2 and SK-Hep-1 cells, respectively.\(^{[27,28]}\) These findings support previous hypotheses, suggesting a decreased oxidative stress and increased protection to oxidative damage after antioxidant exposure.

Peroxidation increases the release of cytochrome c, as a result of the increase in mitochondrial permeability, promoting apoptosis.\(^{[40–42]}\) However, the treatment with SQEA or SQEE80 decreased the apoptotic DNA fraction.\(^{[31,32]}\) Likewise, Baicalin has shown decreased apoptosis levels.\(^{[29]}\) These results confirming the protective effects of SQEA, SQEE80, and Baicalin against ethanol-induced cell death, probably due to a decreased accumulation of ROS and prevention from protein oxidation damage and apoptosis.

Biochemical tests can be useful in determining the toxic effects and health wellness.\(^{[43]}\) It has been described that hepatic damage could be observed after 4 and 12 weeks of ethanol treatment, which is evidenced by higher levels of hepatic enzyme markers and malondialdehyde.\(^{[44]}\) For alcoholics, abnormal values for two or more of the five parameters Alanine Aminotransferase (ALAT), Aspartate Aminotransferase (ASAT), gamma-glutamyl transferase (GGT), and creatinine gave a diagnostics sensitivity of 85% and a diagnostic specificity of 64%. Likewise, elevated ALAT and ASAT levels have been described as a general indicator of tissue and organ damage caused by alcohol, viruses, infections, drugs, or toxins.\(^{[45]}\) In this sense, CPs have decreased serum activities of ALAT and ASAT promoting beneficial effects on early alcoholic liver disease and acute alcoholic injury.\(^{[50]}\) In addition, antioxidant enzymes such CAT and GPX-1 increase their activities after SQEE80 exposure, enhanced alcohol oxidation.\(^{[32]}\)

Ethanol-induced oxidative stress is the result of the combined impairment of antioxidant defenses and the production of reactive oxygen species by the mitochondrial electron transport chain, the alcohol-inducible CYP2E1, and activated phagocytes. Currently, it is known that antioxidants can be used as a therapeutic strategy in various pathologies, including ethanol consumption. It has been
found the activation of stellate pancreatic cells is inhibited when they are incubated with antioxidants.\cite{46} Therefore, antioxidants described previously may represent a potential therapeutic strategy for damage caused by alcohol.

**Scope and limitations**

The goal of this study was to seek an explanation that relates antioxidant treatments and CYP2E1 activity after alcohol exposure. However, the lack of data linking CYP2E1 and alcohol dehydrogenase activities/expressions with antioxidants therapies remains a limitation of this study and needs to be addressed in future studies. Unfortunately, the results provide little support for this notion. At best, we could only discern a trend toward enhanced enzymatic activities/expressions in most of studies with significant improvement in those who had antioxidants exposure. Whereas in light of these data, it appears that antioxidants exposure, did significantly improve oxidative stress, cell viability, DNA damage, and biochemical parameters after alcohol exposure in humans, HepG2, and SK-Hep-1 cells.

Our systematic review excluded animal studies, due to different variables involves such as nutritional status; type of food ingested, genetic factors, environmental factors such as animal facility; and different amounts of daily ethanol intake or antioxidants, which could influence or interfere with the interpretation of the data related to the physiological response of ethanol consumption and antioxidant treatment.

**Conclusion**

Our results suggest that antioxidant treatments could be beneficial and effective during ethanol exposure in human or *in vitro* models due to decreasing CYP2E1 activity and apoptosis levels and; an increase in GSH levels and cell proliferation. However, despite all the advances that have been made in clarifying effects caused by antioxidants supplementation, future studies should clarify more efficiently the relationship between ethanol consumption, antioxidant treatments, and defense mechanisms against oxidative stress; including enzymatic and non-enzymatic mechanisms using specific cell lines or clinical trials.

**Disclosure statement**

No potential competing interest was reported by the authors.

**Funding**

This work was supported by the Universidad de La Frontera under Grant DI20-0003.

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CS and VS designed this study; DC and CC supervised the study; CS, DC, CC, and VS conducted the literature searches, data extraction, and independent search and reviewing; CS and VS prepared a first draft of the manuscript; and CS, DC, CC, and VS finalized it.
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