Potential Mechanisms Underlying the Role of Coffee in Liver Health

Louise J.M. Alferink, Jessica C. Kiefte-de Jong, Sarwa Darwish Murad

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Abstract

Coffee, the most consumed hot beverage worldwide, is composed of many substances, of which polyphenols, caffeine, and diterpenoids are well studied. Evidence on potential effects of coffee on human health has been accumulating over the past decades. Specifically, coffee has been postulated to be hepatoprotective in several epidemiological and clinical studies. Several underlying molecular mechanisms as to why coffee influences liver health have been proposed. In this review, we summarized the evidence on potential mechanisms by which coffee affects liver steatosis, fibrosis, and hepatic carcinogenesis. The experimental models reviewed almost unanimously supported the theorem that coffee indeed may benefit the liver. Either whole coffee or its specific compounds appeared to decrease fatty acid synthesis (involved in steatogenesis), hepatic stellate activation (involved in fibrogenesis), and hepatic inflammation. Moreover, coffee was found to induce apoptosis and increased hepatic antioxidant capacity, which are involved in carcinogenesis.

Introduction

Coffee has never been such a timely topic as it is today. In earlier years, coffee was viewed negatively due to the cholesterol increasing capacity of unfiltered coffees,[123] and the positive confounding association between lifestyle factors, such as alcohol consumption and smoking, with caffeine consumption.[124,125] Today, there is a paradigm shift due to the emerging evidence favouring various health benefits of coffee. A recent umbrella review of Poole et al. explored the effect of coffee consumption on multiple health outcomes and found that coffee consumption seemed more likely to benefit than to harm human health.[125] This meta-analysis showed that coffee was associated with lower all-cause and cardiovascular mortality, lower incidence of cancer, and lower incidence of metabolic diseases. Interestingly, the authors of this umbrella review found a particular noticeable effect of coffee consumption on liver health outcomes. Indeed, coffee in relation to liver health has been well studied in both epidemiological and mechanistic research. In 1986, Arnesen and colleagues first described a beneficial association of coffee consumption with liver health, as assessed by gamma-glutamyltransferase (GGT) levels, in a large population-based study.[126] Subsequently, several other epidemiological studies confirmed the reduction in levels of transaminases and GGT in both whole[86,127] and decaffeinated coffee consumers.[128] Coffee was also found to be associated with less fibrosis, lower hospitalization rates, and lower mortality rate in alcoholic liver disease.[87,129] Moreover, our group[130] and others[131,132] have consistently shown that coffee was inversely associated with (severity of) fibrosis in population-based studies independent of important environmental and lifestyle traits. Coffee consumption has also been associated with improved response to antiviral therapy,[133] increased survival[134] and improved biochemistry[135] in patients with hepatitis C. On the other hand, although coffee was associated with less fibrosis in patients with steatosis,[130,136] clinical evidence on the protective effect of coffee on steatosis itself remains ambiguous.[91,137,138] And last but not least, there is evidence that coffee consumption is associated with a reduced incidence of hepatocellular carcinoma.[139,140] However, most of these epidemiological and clinical studies offer little pathological insights, inherent to the observational nature of such studies. In order to understand the mechanisms behind the effects of coffee on liver health, we aimed to summarize the existing experimental evidence on coffee associations with 1) hepatic steatosis, 2) hepatic fibrosis, and 3) hepatocellular carcinoma.

Methods

We performed an extensive search on Embase.com, Medline Ovid, Google Scholar, Cochrane Central and Web of Science on the 7th of March (see Supplementary Methods for

a full description). This search yielded 4568 articles, and 2498 articles after automatic de-duplication. One researcher selected on title/abstract, and excluded 2243 articles that did not had liver disease as study endpoint, remaining duplicates, non-English articles, articles on primarily caffeine metabolism, articles without full text, and articles on supplemental use of coffee substances for sport boosting purposes. All meta-analyses, reviews and non-experimental studies (n=178 studies) were excluded but used as background information for this review. The remaining 77 in vitro and in vivo studies were fully reviewed. An additional 10 articles were included based on cross-references of individual publications. Studies that focused merely on inflammation (without focus on either steatosis, fibrosis or carcinogenesis, n=7) or which were solely in vitro (n=16), were excluded. Hence, this review included 64 articles.

Coffee preparation and composition

Coffee is a complex chemical mixture that contains more than a thousand different compounds, such as lipids, vitamins, caffeine, melanoidins and more than 800 volatile compounds which are formed during the roasting process of coffee beans.[141] Coffee is derived from the coffee plant belonging to the genus Coffea. Amongst this genus, there are numerous species of which the Coffea Arabica and the Coffea Canephora (also known as Robusta), are the most important and well-known species. More than three quarters of the coffee bean production is that of Arabica which is superior in taste over the Robusta and contains less caffeine and polyphenols.[142] Today, Brazil is the leading country in cultivating coffee plants and exporting coffee beans, followed by Vietnam and Colombia. Meanwhile, the European continent is the largest consumer, followed by the United States and Brazil. From an economical perspective, coffee is the second largest traded commodity worldwide after petroleum (ICO 2018).

To create coffee, multiple preparations of the green Coffea beans are needed. This is important to take into account as the preparation method is highly responsible for the final composition of the coffee brew.[143,144] The roasting process, for example, incorporates the activation of the so-called Maillard reaction that causes an increase in antioxidant activity and degradation of polyphenols. The degradation of polyphenols itself causes a decrease in antioxidant activity, and it has therefore been postulated that the intensity of roasting (along with the intensity of the thermal process) are more important for the antioxidant activity of the brew than type of coffee bean alone.[142] Importantly, roasting at very high temperatures can cause the formation of acrylamide, a potential carcinogenic substance.[145] In addition, filtering can change coffee composition, e.g. paper-filtering can lead to a drastic decrease in lipid and vitamin content (USDA 2018). Lastly, the way of consumption can affect bioavailability of coffee compounds. For example, the addition of milk[146] or

non-dairy creamers,[147] have been shown to either impair or delay the bioavailability of polyphenols.

Although we often refer to polyphenols, the word 'polyphenol' is technically not a chemical term but merely a collective name for flavonoids, tannins, and phenolic acids.[148] The most common phenolic acid in coffee is 5-caffeoyl quinic acid, also known as chlorogenic acid (CGA). CGA is the ester of caffeic acid with quinic acid.[149] Polyphenols are regarded as healthy dietary components but despite extensive research, exact mechanism of action on human health remains an enigma.[148] Other well-known coffee constituents are the diterpenoids, i.e. cafestol and kahweol, which are naturally present in all oil-containing coffees, such as unfiltered or boiled coffees (i.e. Turkish and Scandinavian-style).[150] The diterpenoids have been blamed for the lipogenic effects of coffee,[151] while at the same time they have also been suggested to have anti-oxidant and anti-carcinogenic properties.[152] A group of less well-known substances of coffee are the melanoidins, defined as high-molecular weight nitrogenous and brown-coloured compounds. They have been proposed to exert beneficial effects on human health in terms of anti-oxidant and anti-inflammatory function, but as the exact structure of these components are largely unknown, exact health mechanisms remain to be elucidated.[153] Last but not least is caffeine (1,3,4-trimethylxanthine), the most well-studied coffee compound and probably the most widely-used stimulating drug in the world.[154] Caffeine is a methylxanthine, which contains three main metabolites, i.e. theobromine, paraxanthine, and theophylline. Caffeine remains stable during the roasting process. Some researchers ascribe the beneficial health effects of coffee solely to the caffeine.[155] Interestingly, twins studies have linked pharmacokinetic and pharmacodynamic responses to caffeine to certain single nuclear polymorphisms,[156] suggesting that response to and consumption patterns of caffeine may be influenced by genetic diversity. Moreover, it has been suggested that genes can modulate the effect of habitual caffeine consumption on health outcomes, and that tolerance to this psycho-active drug is different between individuals.[157] In addition, caffeine is almost exclusively metabolized in the liver, which has led to the question of reverse causality - perhaps patients with cirrhosis drink less coffee because of more pronounced side-effects.[158] However, it remains a topic of debate whether this fully explained the observed differences.

Mechanisms of coffee benefits

Liver steatosis

In contrast to human studies, many experimental studies found a beneficial effect of coffee on hepatic steatosis (*Table 1*). Building on previous evidence that CGA modulates the activity of glucose-6 phosphatase (G6Pase; a gluconeogenesis-inducing enzyme),[159]

Table 1: Experimental evidence on coffee effects in steatosis

Author	Coffee compound	Animal Model	Main study findings
Rodriguez de Sotillo, 2002[160]	CGA	(fa/fa) Sprague-Dawley Zucker rats treated with CGA or water 3 weeks	CGA ↓ total cholesterol and Tg CGA ↑ insulin sensitivity CGA 24% ↓ liver Tg
Ong, 2013[161]	CGA	In vivo Type II DM Leprdb/db mice +/– CGA, CGA + compound C or metformin treatment and lean control C57BL/6J mice 2 weeks In vitro HepG2 cells	In vivo CGA + metformin had ↑ adiponectin vs diabetic controls CGA + metformin ↓ G6Pase expression and activity vs diabetic controls CGA + metformin ↓ liver total cholesterol and Tg vs diabetic controls CGA + metformin ↑ expression + translocation of GLUT4 (↑ glucose transport in skeletal muscles) In vitro CGA ↓ glucose synthesis dose and time dependently CGA ↓ G6Pase expression and ↑ ACC and AMPK phosphorylation time and dose dependently CGA + metformin both ↓ FAS and formation of oil droplets and vacuole degeneration dose and time dependently AMPK knockdown mice (siRNA) completely blocked CGA-mediated ↓ in glucose production and FAS
Ma, 2015[162]	CGA	1. C57BL/6 mice on ND or HFD + CGA or placebo 15 weeks 2. 10 obese mice +/– CGA 6 weeks	HFD + CGA ↓ mRNA upregulation of macrophage specific marker genes (Cd68, Cd11b) in white adipocytes vs HFD HFD + CGA ↓ fat accumulation in liver vs HFD HFD + CGA ↓ genes hepatic fat accumulation: mRNA expression of PPAR-γ 1 and 2, Cd36, FABP4, MGAT1 genes vs HFD HFD + CGA ↑ fatty acid metabolism (mRNA of CPT 1a and 1b, FGF21) vs HFD HFD + CGA ↓ hepatic inflammation (mRNA Cd68, Cd11b and Cd11c) vs HFD CGA ↔ weight, ↓ liver weight and ↓ lipid accumulation liver vs obese CGA ↓ genes hepatic fat accumulation: mRNA expression of PPAR-γ 1 and 2, Cd36, FABP4, MGAT1 genes AND ↑ PPAR-α and its target genes ACOX1 and FGF21. ↔ mRNA CPT1 vs obese CGA ↓ expression of macrophage markers: CCR2, F4/80, Cd68, and TNF-α (also mRNA ↓ in adipose tissue) vs obese
Vitaglione, 2010[118]	DC Polyphenols Melanoidins	Wistar rats controls or HFD (3 months) treated with water, DC, Polyphenols or Melanoidin	HFD + DC or polyphenols or melanoidins ↓ lipid droplets, inflammatory infiltrate and fibrotic septa vs HFD + water HFD + DC or polyphenols ↑ IL6, IL10, IL4 and HFD + polyphenols ↓ IFNγ vs HFD + water HFD + polyphenols or melanoidins ↓ TNFα, and oxidized glutathione vs HFD + water HFD + DC or polyphenols ↓ TNFα and TGFβ, ↑ adipo-R2, PPARα expression in liver vs HFD + water
Shokouh, 2017[163]	Caffeic acid Trigonelline Cafestol	Sprague Dawley rats HFD +/– nutraceutical mix 12 weeks	HFD + Mix ↑ adiponectin vs HFD HFD + Mix ↓ semi quantitative scoring of severe steatosis and ↓ plasma ALT. Trend towards ↓ Liver Tg
Murase, 2011[164]	Coffee polyphenols	In vivo C57BL/6J mice on ND or HFD +/– Polyphenols 15 weeks In Vitro Hepa 1–6 cells	In vivo HFD + polyphenols 1% ↓ liver Tg and cholesterol vs HFD HFD + polyphenols 1% ↓ mRNA levels FAS, ACC1 and stearoyl-CoA desaturase HFD + polyphenols 1% ↓ mRNA level of SREBP-1c In vitro Polyphenols ↓ mRNA expression of ACC1-2, stearoyl-CoA desaturase, SREBP1 and FAS Polyphenols ↔ PPAR activation Polyphenols ↔ AMPKα and ACC phosphorylation

Table 1 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
Panchal, 2012[166]	Colombian Coffee Extract	Wistar rats on ND or HFD (8 weeks) + half of the littermates Coffee (8 weeks more)	Coffee + HFD ↓ liver fat deposition and no inflammatory infiltration nor portal fibrosis vs HFD Coffee + HFD ↓ ALT, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase vs HFD
Salomone, 2014[167]	DC	Wistar rat on HFD or ND +/−DC 3 months	HFD + DC mild steatosis but ↓ ballooning, inflammatory infiltrate and fibrosis vs HFD HFD + DC ↓ isoprostanes and 8-deoxyguanosine (=↓ oxidative stress) HFD + DC modulates expression of cell chaperones involved in protein folding, autophagy, and immune activation
Watanabe, 2017[168]	CC DC	15 Tsumura-Suzuki obese/ diabetic mice treated with water, DC or CC 11 months	DC and CC had ↓ ballooning after 6 months (control developed NASH) vs obese ↔ NAS score between groups CC had ↓ fibrosis and DC had NO fibrosis vs obese All groups ↔ liver tumour frequency, but atypical tumours occurred ↓ in CC and DC vs obese
Takahashi, 2014[169]	CC DC Unroasted CC	C57BL/6J mice on ND or HFD +/− treatment of water, CC, DC and unroasted CC 9 weeks	HFD + CC or DC or unroasted CC ↓ liver weight vs. ND HFD + CC or DC or unroasted CC ↓ lipid metabolism-related genes regulated by PPAR-γ (i.e. complement factor D, FABP2, ACC, CD36 and cell death-inducing DFFA like effector A and perilipin) vs HFD HFD + CC or unroasted coffee ↓ PPAR-γ and CD36 mRNA levels ↓ hepatic PPAR-γ levels (most in unroasted CC) vs HFD
Yun, 2008[170]	Caffeine	C57BL/6J mice on ND or HFD (12 weeks) or HFD + caffeine (4+8 weeks)	HFD + caffeine ↓ large microvacuolar steatosis vs HFD HFD + caffeine ↑ adiponectin and CPT1 activity vs HFD HFD + caffeine ↑ expression of phosphorylated ACC only at week 2 vs HFD
Sinha, 2014[171]	Caffeine	In vivo C57BL6 mice "short term" caffeine and CQ 3 days "long term" ND (4 weeks) or HFD + caffeine (4+4 weeks) In vitro HepG2 cells	In vivo Short-term caffeine ↑ flux through β-oxidation pathway in liver, ↑ hepatic lipolysis Short-term caffeine ↓ levels of p62 and ↑LC3-II (↑ autophagic flux), ↓ mTOR signalling Short-term caffeine ↑ β-oxidation, dependent of the autophagy-lysosomal pathway (after autophagy inhibitor CQ) Short-term caffeine↑ ACC and CPT-1 were independent of the autophagy-lysosomal pathway HFD + caffeine ↓ intrahepatic lipid accumulation HFD + caffeine ↑ autophagic, lipolytic and fatty acid oxidation pathways in liver In vitro Caffeine ↑ LC3 expression ↔ lipase levels Caffeine ↑ autophagosomes resulting in ↑ proautophagic proteins (ATL 5 and 7, Beclin) Caffeine ↓ mTOR signalling ↑ autophagic flux ATL5 knockdown (siRNA) blocks caffeine-mediated steatosis and hence is autophagy dependent
Helal, 2017[172]	Caffeine	Wistar rats ND (8 weeks) or HFD + caffeine (8+8 weeks)	HFD + caffeine 20 and 30 ↓ transaminases and bilirubin vs HFD (even lower than control) HFD + caffeine partially ↓ lipid peroxidation (i.e., MDA) vs HFD HFD + caffeine 20 and 30 partially ↑ glutathione vs HFD HFD + caffeine dose-dependently ↓ mRNA expression of ACC and FAS vs HFD

Table 1 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
			HFD + caffeine 20 and 30 ↑ hepatic CPT-1 and PPAR-α mRNA expression vs HFD HFD + caffeine ↓ histologically assessed steatosis, inflammation, ballooning and mild fibrosis vs HFD
Sugiura, 2012[173]	Caffeine Catechin Epigallocatechin gallate	ICR mice treated with water, caffeine, catechin + caffeine or epigallocatechin gallate + caffeine 4 weeks	↔ liver Tg and total cholesterol Catechin + caffeine ↓ FAS activity, mRNA expression and protein levels in liver Catechin + caffeine ↑ ACC and CPT-II enzymatic activity, but ↔ ACC and CPT-II mRNA expression ↔ in PPAR-α, SREBP-1c mRNA expression in liver
Zheng, 2015[174]	Caffeine	Wild-type zebrafish larvae overfed steatosis or ND + caffeine 20 days	Caffeine 5–8% ↓ hepatic steatosis rate and liver Tg content vs steatosis Caffeine 2.5–8% ↓ amount and size of lipid droplets in liver vs steatosis Caffeine 5% ↓ level of gene expression of fatty acid transport protein (incl. fatty acid translocase/Cd36) vs steatosis Caffeine ↓ mRNA levels of UCP2 and SREBP1 vs steatosis Caffeine ↓ gene expression of key lipogenic enzymes involved in FAS including ACC-1 vs steatosis Caffeine 5% ↓ mRNA level of genes involved in ER stress vs steatosis Caffeine 5% ↓ expression level of IL1β and TNFα vs NAFLD (incl. protein level of IL1β and TNFα vs steatosis Caffeine 5% ↓ mRNA level of ATG12 and Beclin1 (involved in autophagy) vs steatosis

Abbreviations ACC: acetyl-CoA carboxylase, ACOX: acyl-coenzyme A oxidase, ALT: alanine transferase, AMPK: adenosine monophosphate-activated protein kinase, ATG: Autophagy-related protein, ATL: adult T-cell leukaemia, CC: conventional coffee, CCR: C-C chemokine receptor, CD: cluster differentiation, CGA: chlorogenic acid, CPT: carnitine palmitoyltransferase, CQ: chloroquine, DC: decaffeinated coffee, ER: endoplasmatic reticulum, FABP: fatty acid binding protein, FAS: fatty acid synthesis, FGF: fibroblast growth factor, G6Pase: glucose-6-phosphatase, GLUT: glucose transporter, HFD: high fat diet, IL: interleukin, LC: lipidation of microtubule-associated protein light-chain, MDA: malondialdehyde, MGAT: monoacylglycerol O-acyltransferase, mTOR: mammalian target of rapamycin, NAS(H): non-alcoholic steatosis (hepatitis), PPAR: peroxisome proliferator-activated receptor, SREBP: sterol element binding transcription factor, Tg: triglyceride, TGF: tissue growth factor, TNF: tumour necrosis factor, UCP: uncoupling protein.

Rodriguez de Sotillo and Hadley examined the effects of three weeks intravenous CGA administration in nine week-old, metabolic unhealthy rats. The authors showed that CGA administration prevented weight gain, attenuated triglyceride (Tg) accumulation in the liver, and improved lipid profile and insulin sensitivity.[160] These findings were confirmed by the study of Ong and colleagues who showed similar results upon that two weeks of oral CGA administration in genetically diabetic mice.[161] Additionally, in vitro analysis on HepG2 cells (a human hepatoma cell line) confirmed the inhibitory effect of CGA on G6Pase expression. Moreover, CGA completely inhibited fatty acid synthesis (FAS), conceivably by phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and subsequent downregulation of acetyl-CoA carboxylase (ACC), as AMPK knock-out cells did not show CGA-mediated inhibition of glucose production.[161] Finally, Ma et al. administered

CGA intraperitoneally in mice fed a high fat diet (HFD) or in already obese mice.[162] Both preventive and therapeutic CGA administration lowered steatosis in the liver. The authors found that preventive CGA lowered the mRNA expression of genes involved in I) hepatic fat accumulation, mediated by peroxisome proliferator-activated receptor (PPAR) γ1 and γ2, cluster of differentiation (CD) 36, fatty acid binding protein 4, and monoacylglycerol O-acyltransferase 1; II) hepatic inflammation (CD68, CD11b and 11c); and III) increased mRNA expression of fatty acid metabolism (including carnitine palmitoyltransferase (CPT)-1a and 1b, and fibroblast growth factor (FGF) 21). In addition, therapeutic CGA lowered mRNA expression of encoding inflammatory enzymes; C-C chemokine receptor 2 and tumour necrosis factor (TNF) α.[162]

In addition, three studies examined polyphenols in rodents with diet-induced steatosis, and found that coffee polyphenols could prevent,[163,164] as well as treat,[118] hepatic steatosis. It was suggested that the preventive effect of polyphenols worked via downregulation of mRNA lipogenic genes (i.e. FAS, ACC1, and stearoyl-CoA desaturase1), and sterol element binding transcription factor (SREBP) 1c, the main regulator of FAS.[164] An elegant study by Vitaglione et al. found that polyphenols, but also decaffeinated coffee (DC) and melanoidins, affected steatosis via I) modulation of inflammatory genes, i.e. interleukin (IL) 4, 6 and 10 increment, interferon (IFN) γ and TNFα decrement, II) increased fatty acid β oxidation, and III) reduced liver oxidative stress mediated by glutathione.[118] In this study, only polyphenols increased the ferric reducing ability of plasma, an index of antioxidant capacity.[165] The components examined did not contain caffeine or diterpenoids, suggesting that neither caffeine nor diterpenoids were responsible for this observed anti-steatogenic effect.[118] In contrast, another study on caffeine and diterpenoid-containing Colombian coffee extract in rats on a high carbohydrate/HFD, found less steatosis and inflammation rats.[166]

Salomone et al. found that DC administration to rats on a HFD partially prevented steatosis by mediating stress proteins in the endoplasmic reticulum, and via mitochondrial chaperones in the liver.[167] Two additional studies examined both conventional coffee (CC) and DC,[168,169] and found similar effects of both beverages. Of note, Watanabe and colleagues found less ballooning but no preventative effect of CC or DC on steatosis alone in genetically diabetic mice.[168] Takahashi et al. did not examine lipid content of the liver, but found lower lipid metabolism-related genes regulated by PPARγ (i.e. complement factor D, fatty acid binding protein 2, ACC, and CD36), in both CC and DC, albeit more pronounced in CC, implying an additive effect of caffeine.[169]

Five in vivo studies on caffeine and steatosis identified caffeine as anti-steatogenic.[170-174] Four of the studies were conducted in a (diet-induced) steatosis model and found less steatosis in caffeine-treated animals.[170-173] In addition, one of these studies found an increased fatty acid flux upon acute caffeine administration via the β-oxidation pathway in the liver. They postulated that the lipolytic effects of caffeine are mediated through autophagy, as they demonstrated a decreased effect of caffeine upon blocking autophagy.[171] In addition,

Helal et al., nominated CPT1, ACC and FAS expression levels as mediators in caffeine effects on steatosis. In addition, they also found an upregulation of glutathione (normally depleted in presence of oxidative stress), upon caffeine administration.[172] Another study examining caffeine in addition to catechin (a tea polyphenol) detected a decreased mRNA expression and enzyme activity of FAS and increased enzymatic activity of acyl-CoA oxidase and CPT2.[173] Lastly, a study using zebrafish larvae (which bear great similarity with the human genome) showed that overfed larvae had an upregulation of lipid β-oxidation, a downregulation of lipogenesis-associated genes (i.e. ACC1, CD36, uncoupling protein 2 and SREBP1), endoplasmic stress associated genes, and inflammatory cytokines (IL-1β and TNFα) upon exposure to moderate caffeine doses.[174]

Fibrosis

Coffee has been studied comprehensively in relation to fibrosis (*Table 2*). Polyphenols are amongst the most well-studied coffee components in fibrosis.[175-180] Caffeic acid, a specific 5-lipoxygenase inhibitor, was used therapeutically in carbon tetrachloride (CCl4) poisoned rats and led to lower liver enzymes, liver collagen content, and lipid peroxidation as measured by malondialdehyde (MDA; a marker for oxidative stress).[175] Other studies confirmed the transaminase-lowering capacity of caffeic acid using different toxins, such as acetaminophen.[176,178,179] Amongst them, two subsequent studies from Pang et al. assessed caffeic acid in vivo and in vitro.[178,179] Caffeic acid appeared to attenuate liver damage on histology and lowered MDA and reactive oxygen species formation in vivo. The authors ascribed these findings to the decreased Kelch-like ECH-associated protein (KEAP) 1 and increased nuclear factor erythroid 2-related factor (Nrf) 2 expression in vitro.[179] KEAP1 is an inhibitor of Nrf2 activation, and Nrf2 activates downstream phase II antioxidative enzymes such as nicotinamide adenine dinucleotide (phosphate) quinone oxidoreductase 1 and heme oxygenase 1. Hence, the KEAP1-Nrf2-cascade is thought to play a critical role in liver oxidative injury, which is in line with previous in vitro studies.[181] Therapeutically, caffeic acid reduced phosphorylation of extracellular signal-regulated protein kinase (ERK)1 and 2, which led to I) reduced mRNA expression of early growth response 1; II) reduced inflammatory cytokines IL6, IL1β and tissue factor; and III) reduced growth arrest and DNA damage inducible gene 45α mRNA expression (involved in growth arrest and apoptosis). Henceforth, caffeic acid led to restored cell viability in L-02 and HepG2 cell lines upon acetaminophen-intoxication.[178] Two additional studies examined CGA in presence of either CCl4 or arsenic toxic and found a transaminase-lowering effect of CGA.[177,180] One additionally showed that CGA lowered fibrosis histologically. Also, CGA treated rats showed decreased expression of α-Smooth Muscle Antigen (SMA; an actin isoform that can be used to identify activated hepatic stellate cells (HSCs)), and connective tissue growth factor (CTGF; an inhibitor of extracellular matrix deposition).[177] In addition, CGA reduced liver,

Table 2: Experimental studies on coffee effects in fibrosis

Author	Coffee compound	Animal Model	Main study findings
Reyes, 1995[175]	Caffeic acid	Wistar rats +/– CCl4 intraperitoneally and +/– caffeic acid 8 weeks	CCl4 + caffeic acid 50 partially ↓ serum activities of alkaline phosphatase, and glutamic pyruvic transaminase and completely ↓ GGT CCl4 + caffeic acid 20–50 ↓ lipid peroxidase (i.e. MDA) CCl4 + caffeic acid 20–50 (dose-dependently) ↓ liver collagen content
Janbaz, 2004[176]	Caffeic acid	1. Swiss mice with acetaminophen (1g/kg) +/– caffeic acid 2. Albino Wistar rats +/– acetaminophen (640mg/kg) or CCl4 +/– caffeic acid	Caffeic acid pre-treatment ↓ mortality 1g/kg acetaminophen 80% Caffeic acid pre-treatment ↓ transaminases rise by 640mg/kg acetaminophen and CCl4
Shi, 2013[177]	CGA	Sprague-Dawley rats +/– CCl4 +/– CGA 8 weeks	CCl4 + CGA ↓ transaminase rise and ↑ albumin vs CCl4 CCl4 + CGA ↓ degree of liver fibrogenesis and inflammatory cell infiltration vs CCl4 CCl4 + CGA ↓ αSMA and CTGF collagen I vs CCl4 CCl4 + CGA ↓ mRNA and protein levels of TLR4, MyD88, inducible NOS, COX2 rise and ↑ Bambi vs CCl4 CCl4 + CGA ↓ inflammatory cytokines expression and serum (TNFα, IL6 and IL1β) rise vs CCl4
Pang, 2016[179]	Caffeic acid preventive	*In vivo* C57BL/6 Mice +/– caffeic acid prior to +/– acetaminophen *In Vitro* L-02 cells, HepG2 cells +/– acetaminophen and CA 5–50uM	*In Vivo* Acetaminophen + caffeic acid 30 ↓ transaminase rise, ROS level and myeloperoxidase vs acetaminophen Acetaminophen + caffeic acid 10 and 30 ↑ liver glutathione vs acetaminophen Acetaminophen + caffeic acid 10 and 30 ↓ histology severity vs acetaminophen *In Vitro* Acetaminophen + caffeic acid 50 ↑ the reduced cell viability Acetaminophen + caffeic acid 10–50 ↓ ROS rise Acetaminophen + caffeic acid 50 ↑ Nrf2 expression and transcriptional activation Acetaminophen + caffeic acid ↔ on control knockout Nrf2 cells and viability Acetaminophen + caffeic acid 50 partially ↑ downstream antioxidative enzymes of Nrf2 (i.e. NADQ1, heme oxygenase1) Acetaminophen + caffeic acid 50 ↓ expression of KEAP1 (=inhibitor of Nrf2 activation) Acetaminophen + caffeic acid ↔ CYP2E1, CYP1A2 and CYP3A4 (=acetaminophen converters)
Pang, 2016[178]	Caffeic acid therapeutic	*In vivo* ICR mice +/– acetaminophen +/– caffeic acid 6 hours *In vitro* L-02 cells, HepG2 cells +/– acetaminophen and CA 5–50uM	*In vivo* Acetaminophen + caffeic acid 10–30 partially ↓ transaminase and MDA rise vs acetaminophen Acetaminophen + caffeic acid 10–30 partially ↓ severity of liver histology vs acetaminophen Acetaminophen + caffeic acid 10–30↓ tissue factor, IL1β and 6 and ↓ Egr1 expression vs acetaminophen Acetaminophen + caffeic acid 10 ↓ serpine1 level vs acetaminophen

Table 2 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
			In Vitro Acetaminophen + caffeic acid 10–50 ↑ cell viability cells vs acetaminophen Acetaminophen + caffeic acid 20–50 ↓ mRNA expression rise in Egr1 vs acetaminophen Caffeic acid 10 ↓ GADD45α mRNA expression (=regulated by Egr1 leading to apoptosis) Acetaminophen ↔ cell viability in siRNA knockdown expression of Egr1 and GADD45α Acetaminophen + caffeic acid 50 ↓ ERK1 and 2 phosphorylation and C-jun N-terminal kinase in L-02 cells vs acetaminophen Acetaminophen + caffeic acid ↓ c-Raf & MEK1/2 phosphorylation (=upstream ERK signals) vs acetaminophen ERK1 and 2 inhibitors ↓ nuclear translocation of Egr1 but C-Jun N-terminal kinase inhibitors ↔ nuclear translocation of Egr1
Ghahhari, 2017[180]	CGA	Mice +/– CGA prior to +/– arsenic trioxide	Arsenic + CGA 10–100 ↓ transaminase rise vs arsenic Arsenic + CGA 10–100 ↑ glutathione
Lee, 2007[183]	Kahweol Cafestol	ICR Mice +/– kahweol or cafestol prior to CCl4 3 days	CCl4 + kahweol 50–100 or cafestol 50–100 dose-dependently ↓ transaminases and MDA rise vs CCl4 CCl4 + kahweol 50–100 or cafestol 50–100 dose-dependently ↑ glutathione content and activity vs CCl4 CCl4 + kahweol 50–100 or cafestol 50–100 dose-dependently ↓ necrosis and hepatic lesions vs CCl4 Kahweol and cafestol dose-dependently ↓ CYP2E1 activity, but ↔ its expression vs controls Kahweol and cafestol dose-dependently ↓ lipid peroxidation (MDA) and superoxide scavenging activity vs controls
Poyrazoglu, 2008[184]	Unfiltered (Turkish) coffee	Sprague- Dawley rats +/– CCl4 +/– Turkish coffee 7 days	CCl4 + Turkish coffee ↑ transaminase and MDA and ↓ serum albumin vs CCl4 CCl4 + Turkish coffee ↑ inflammation and necrosis vs CCl4, Turkish coffee alone ↑ steatosis, inflammation and necrosis vs control
Seo, 2017[185]	Kahweol	*In vivo* C57BL/6 mice +/– TAA +/– kahweol 8 weeks *In vitro* AML-12 cell line, LX2-cells or primary hepatocytes	*In vivo* TAA + kahweol ↓ fibrosis and expression of type I collagen, αSMA, CTGF and TGFβ mRNA vs TAA TAA + kahweol ↓ transaminase rise vs TAA TAA + kahweol ↓ nuclear translocation of Smad2 and 3 (=signalling pathway TGFβ) in nucleus vs TAA TAA + kahweol ↓ STAT3 phosphorylation vs TAA Kahweol ↓ MAPK pathways leading to ERK phosphorylation and C-Jun N terminal protein kinase vs TAA + kahweol *In vitro* Kahweol ↓ TGFβ stimulated type I collagen and CTGF expression Kahweol ↓ TGFβ stimulated Smad3 protein and STAT3 phosphorylation expression Kahweol ↓ TGFβ stimulated Erk and C-Jun N terminal protein expression
He, 2001[186]	CC Caffeine Different tea types	Wistar rats treated CC, Caffeine, Tea or Water 10 days prior to LPS & D-galactosamine	All beverages ↓ LPS-induced enhancement of transaminases Caffeine dose-dependently ↓ LPS-induced enhancement of transaminases

Table 2 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
Ozercan, 2006[187]	Instant CC	Sprague Dawley rats +/– CCl4 +/– instant CC 7days	CCl4 + instant CC ↓ transaminase rise, necrosis and inflammation, but ↔ steatosis vs CCl4 CCl4 + instant CC ↓ serum MDA rise by CCl4, but ↔ MDA hepatic tissue vs CCl4 CCl4 + instant CC restored the lowered total antioxidant capacity vs CCl4
Shi, 2010[188]	Nescafe CC	Sprague Dawley +/– CCl4 +/– CC 8 weeks	CCl4 + CC 300 ↓ fibrosis scores and ↓ necrosis and infiltration of lymphocytes vs CCl4 CCl4 + CC 300 ↓ Collagen I and III, VEGF and TGFβ1 vs CCl4 CCl4 + CC 150–300 ↑ Bax mRNA expression vs CCl4 CCl4 + CC 150 ↓expression of αSMA, GRP 78, and GRP 94 vs CCl4
Shin, 2010[189]	Nestlé CC Nestlé DC Caffeine	*In vivo* Sprague Dawley rats +/– DMN +/– CC 4 weeks *In vitro* rat peritoneal macrophages from Sprague Dawley rats treated with LPS + DC or CC or caffeine	*In vivo* DMN + CC 100–300 dose-dependently ↑ body, liver and spleen weight vs DMN DMN + CC 100–300 ↓ transaminase rise and ↑ albumin decrease vs DMN DMN + CC 300 ↓ MDA vs DMN DMN + CC 100–300 dose-dependently ↓ hydroxyproline and αSMA expression, and ↑ tissue glutathione, SOD and catalase activity and SOD mRNA expression vs DMN DMN + CC 100–300 ↓ hepatocyte necrosis, atrophy, yellow pigment and inflammation vs DMN DMN + CC 100–300 ↓ mRNA expressions of PDGFβ, TGFβ, TNFα, IL1, inducible NOS vs DMN *In vitro* CC and DC ↓ nitrite production through macrophage LPS activation. Caffeine ↔
Abreu, 2011[190]	Coffee extract	Male littermates from pregnant rats given CC or control rats were treated +/– acetaminophen +/– CC	CC +/– acetaminophen ↓ TBARS (= lipid peroxidation) and ↑ glutathione vs controls CC +/– acetaminophen ↓ glutathione peroxidase activity and ↑ glutathione reductase vs controls CC +/– acetaminophen ↑ biotransformation phase II enzymes (GST, UGT1 and UGT2) vs controls
Moreno, 2011[191]	Instant CC Ground CC	Wistar rats +/– CCl4 +/– instant CC or ground CC 8 weeks	CCl4 + instant or ground CC ↓ ALP rise and ↓ TBARS, but ↔ ALT rise vs CCl4 CCl4 + ground CC ↑ glutathione vs CCl4 CCl4 + instant or ground CC partially ↓ hydroxyproline vs CCl4 CCl4 + instant < ground CC partially ↓ collagen, but ↔ necrosis vs CCl4 CCl4 + instant CC partially or ground CC completely ↓ TGFβ protein level, both ↓ TGFβ mRNA rise vs CCl4
Furtado, 2012[192]	CC DC Caffeine	Wistar rats +/– TAA +/– DC or caffeine or CC 8 weeks	TAA + DC or CC or caffeine ↓ ALT rise vs TAA TAA + DC or CC or caffeine ↓ liver injury score + collagen fibres fraction + TGFβ1 protein levels vs TAA TAA + DC or CC or caffeine ↑ reduced glutathione and ↓ oxidized glutathione vs TAA TAA + DC or CC ↓ proliferating cell nuclear antigen rise and ↓development of PNL vs TAA TAA + CC ↓ MMP-2 rise vs TAA, whereas TAA + DC ↑ MMP2 vs TAA

Table 2 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
Arauz, 2013[193]	CC DC	Wistar rats +/– TAA +/– CC or DC 8 weeks	TAA + DC or CC ↓ transaminase and MDA rise vs TAA TAA + DC or CC partially ↑ glutathione peroxidase activity vs TAA TAA + DC or CC partially ↓ hydroxyproline, necrosis, TGFβ, αSMA and CTGF expression rise vs TAA TAA + DC or CC partially ↓ IL10, MMP2, 9, and 13 expression rise vs TAA
Arauz, 2017[194]	CC DC Caffeine	Wistar rats +/– BDL +/– CC or DC or caffeine	BDL + CC or DC ↔ ALT rise, but BDL + caffeine ↓ ALT rise vs BDL BDL + CC or DC or caffeine ↓ alkaline phosphatase and GGT vs BDL BDL + CC or DC or caffeine ↑ glutathione (peroxidase activity) and ↓ glutathione disulphide vs BDL BDL + CC or DC partially ↓ and caffeine completely ↓ MDA vs BDL BDL + CC or DC or caffeine ↓ hydroxyproline and collagenα1 protein expression + mRNA levels vs BDL BDL + CC or caffeine ↓ TGFβ mRNA + protein expression and CTGF, αSMA and IL-10 expression vs BDL
Sugiyama, 2001[195]	Caffeine	Mice injected anti-Fas antibody/TNFα or placebo + force-fed caffeine	Caffeine ↔ transaminase rise by anti-Fas antibody, but ↓ transaminase rise by TNFα Caffeine ↓ DNA fragmentation (i.e. apoptosis)
Chan, 2006[196]	Caffeine	*In vivo* Adenosine A2a/ a3 receptor deficient mice and wildtype control + CCl4 or TAA for 9 weeks AND C57BL/6 mice + CCl4 or TAA for 7 weeks + adenosine receptor (ant) agonists *In vitro* LX-2 and HepG2 cells treated with methotrexate or ethanol to generate adenosine	*In vivo* TAA ↑ adenosine A2a receptor expression in wildtype mice Adenosine receptor deficient mice developed no fibrosis and mild transaminase rise upon CCl4 or TAA Caffeine mimicked the protective effect of Adenosine receptor deficient mice *In vitro* Adenosine A2a receptor agonists dose-dependently ↑ collagen production in rat HSC Adenosine A2a agonists ↓ activity and expression of MMP (western blotting) which lead to ↓ collagen degradation and ↑ collagen production by HSC
Klemmer, 2011[198]	Paraxanthine	Sprague Dawley rats underwent sham or BDL +/– paraxanthine 30 days	BDL + paraxanthine ↔ transaminase rise vs BDL BDL + paraxanthine ↓ bile acid toxicity and bridging fibrosis on histology and ↓ MDA vs BDL BDL + paraxanthine ↓ intrahepatic levels CTGF, total SMAD2 and 3 vs BDL, but ↔ serum CTGF level
Gordillo-Bastidas, 2013[199]	Caffeine	Wistar rats +/– TAA or BDL +/– caffeine 7 weeks	BDL + caffeine ↓ transaminase rise vs BDL BDL + caffeine & TAA + caffeine ↓ extracellular matrix, CTGF Collagen I & TGFβ1 vs BDL & TAA BDL + caffeine & TAA + caffeine ↓ fibrosis and necro-inflammation vs BDL & TAA BDL + caffeine & TAA + caffeine ↓ CD11b, IL1β, IL6 and TNFα expression rise vs BDL & TAA BDL + caffeine & TAA + caffeine ↑ SOD expression and catalase activity vs BDL & TAA BDL + caffeine & TAA + caffeine ↑ protein level of Nrf2 vs BDL & TAA BDL + caffeine & TAA + caffeine ↓ Snail-1 (pro-fibrogenic transcription factor) vs BDL & TAA

Table 2 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
Shim, 2013[116]	Caffeine	*In vivo* Sprague Dawley rats +/– TAA +/– caffeine 8 weeks In vitro LX-2 cells	*In vivo* TAA + caffeine ↓ fibrosis and peri-portal inflammation histologically vs TAA TAA + caffeine ↓ αSMA and TGFβ level rise vs TAA *In vitro* Caffeine ↓ cell proliferation, wound healing and wound size and >50% ↑ apoptotic cells Caffeine ↓ F-actin expression and stellate cell adhesion (=involved cell migration & adhesion) Caffeine ↑ intracellular cAMP levels, modulating TGFβ/SMAD-induced expression of ECM Caffeine ↓ αSMA & procollagen I production, time and dose-dependently
Arauz, 2014[200]	Caffeine	Wistar rats +/– TAA +/– caffeine 8 weeks	TAA + caffeine ↓ transaminase and MDA rise vs TAA TAA + caffeine ↑ glutathione peroxidase vs TAA TAA + caffeine ↓ hydroxyproline, TGFβ protein level, αSMA expression and necrosis vs TAA TAA + caffeine ↓ IL10, MMPs, ↔ TIMP and ↓ TGFβ, αSMA and collagen α1 mRNA expression vs TAA
Hsu, 2015[197]	Caffeine	Sprague Dawley rats +/– BDL or TAA +/– caffeine prophylactically or therapeutically 4 weeks	BDL + therapeutic & prophylactic caffeine ↓ portal pressure vs BDL BDL + therapeutic caffeine & prophylactic caffeine ↓ portosystemic shunting and ↓ fibrosis vs BDL BDL + prophylactic caffeine ↓ intrahepatic angiogenesis, endothelial NOS, VEGF vs BDL BDL + prophylactic caffeine ↔ inducible NOS, COX1 and 2 and, p-ERK vs BDL BDL + prophylactic caffeine + adenosine A2a and A1a receptor agonists ↔ on portal pressure and fibrosis TAA + therapeutic caffeine & prophylactic caffeine ↓ portal pressure and ↓ fibrosis vs TAA
Wang, 2015[201]	Caffeine	Sprague-Dawley rats +/– ethanol +/– caffeine 8 or 12 weeks	Ethanol + caffeine ↓ transaminase and serum level fibrosis markers (hyaluronic acid, N-terminal procollagen type III, laminin, and type IV collagen) vs ethanol Ethanol + caffeine ↓ steatosis and inflammatory necrosis and ↓mRNA expression of procollagen I and III vs ethanol Ethanol + caffeine 20 ↓ collagen fiber and caffeine 10 and 20 ↓ expression of αSMA vs ethanol Ethanol + caffeine dose-dependently ↓ cAMP vs ethanol
Amer, 2017[202]	Caffeine	Albino rats +/– TAA +/– caffeine 8 weeks	TAA + caffeine ↓ transaminase and MDA rise vs TAA TAA + caffeine ↓ TNFα, IL-1β and IL-6 and TBARS vs TAA TAA + caffeine ↑ glutathione and glutathione peroxidase activity and SOD of cytoplasm vs TAA TAA + caffeine ↓ collagen deposition, MMP-9 and collagen type IV histologically vs TAA
Cachón, 2017[203]	Caffeine	Wistar rats +/– CCl4 +/– caffeine 10 weeks	CCl4 + caffeine 5–10 or N-acetyl cysteine ↓ relative liver weight vs CCl4 CCl4 + caffeine 5–10 or N-acetyl cysteine ↓ transaminase rise, (un) conjugated bilirubin vs CCl4 CCl4 + caffeine 5–10 or N-acetyl cysteine ↑ glutathione vs CCl4 CCl4 + caffeine 5–10 or N-acetyl cysteine ↓ fibrosis, but ↔ steatosis, inflammation and collagen level

Table 2 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
Mohamed, 2017[204]	Caffeine	Wistar rats +/– CCl4 +/– caffeine 5 weeks	CCl4 + caffeine or N-acetyl cysteine ↓ fibrosis markers (i.e. TGFβ1 + hydroxyproline) vs CCl4 CCl4 + caffeine or N-acetyl cysteine ↓ inflammatory markers (TNFα + myeloperoxidase) vs CCl4 CCl4 + caffeine or N-acetyl cysteine ↓ relative liver weight vs CCl4 CCl4 + caffeine or N-acetyl cysteine transaminase, bilirubin, and MDA, and ↑ albumin vs CCl4 CCl4 + caffeine or N-acetyl cysteine ↑ glutathione + catalase activity vs CCl4 CCl4 + caffeine partly ↓ dilated congested blood vessel and activated Von Kuppfer cells
Eraky, 2018[205]	Caffeine	Sprague-Dawley rats +/– TAA +/– caffeine and/or silymarin 8 weeks	TAA + caffeine or silymarin or both ↑ albumin and ↓ transaminase and bilirubin rise vs TAA TAA + caffeine or silymarin but most in both ↓ inflammation and necrosis vs TAA TAA + caffeine or silymarin or both ↓ fibrosis% vs TAA TAA + caffeine or silymarin or both ↓ TGFβ1, CTGF and αSMA mRNA expressions vs TAA

Abbreviations ALT: alanine transferase, BDL: bile-duct ligation, CC: conventional coffee, CCl4: carbon tetrachloride, CCR: C-C chemokine receptor, CD: cluster differentiation, CGA: chlorogenic acid, COX: Cyclo-oxygenase, CTGF: connective tissue growth factor, CYP: cytochrome P, DC: decaffeinated coffee, DMN: dimethylnitrosamine, ECM: extracellular matrix, EGR: early growth response, ERK: extracellular signal-regulated protein kinase, GADD: growth arrest and DNA damage inducible gene, GGT: γ-glutamyl transferase, GST: glutathione S-transferase, IL: interleukin, KEAP: Kelch-like ECH-associated protein, LPS: lipopolysaccharide, MDA: malondialdehyde, MMP: matrix metalloproteinase, MyD: Myeloid differentiation primary response, NOS: nitric oxidase synthase, Nrf: Nuclear factor (erythroid-derived)-like, PDGF: platelet derived growth factor, ROS: reactive oxygen species, SMA: smooth muscle antigen, SOD: superoxidase dismutase, STAT: signal transducer activator of transcription, TAA: thioacetamide, TBARS: thiobarbituric acid reactive substances, TGF: tissue growth factor, TIMP: tissue inhibitor matrix metalloproteinase, TLR: toll-like receptor, TNF: tumour necrosis factor, UCT: uncoupling protein glucuronosyl transferase, VEGF: vascular endothelial growth factor.

IL-1β, IL-6, and TNFα. The authors hypothesized that the protective effect of CGA could be mediated via the inhibition of the toll-like receptor 4/ myeloid differentiation factor 88/nuclear factor κ of activated B-cells (TLR4-MyD88-NFκB) signalling pathway, as TLR4 activates pro-inflammatory producing Kupffer cells, HSCs, and liver endothelial cells.[177] NFκB additionally induces cyclo-oxygenase (COX)2 expression, which is involved in HCC development.[182]

Diterpenoids are fairly well studied in fibrosis.[183-185] Lee and colleagues first studied diterpenoids in CCl4-induced liver damage. Both kahweol and cafestol attenuated the CCl4-induced rise in transaminases and MDA. In addition, the diterpenoids-treated mice appeared protected from glutathione depletion, showed less necrosis and less hepatic lesions. The authors believed these effects were mediated via the reduction of CYP2E1 activity, an enzyme that induces liver injury upon CCl4 administration.[183] The elegant in vivo/in vitro study of Seo et al. examined kahweol administration in thioacetamide (TAA)-poisoned

mice.[185] They ascribed the transaminase and fibrosis lowering capacity of kahweol to the downregulation of TGFβ, the master pro-fibrogenic cytokine, and thereby downregulation of HSC activation and stimulating proteins involved in CTGF expression in vitro (i.e. Smad 2 and 3, signal transducer activator of transcription 3, ERK, and C-Jun N terminal protein).[185] Paradoxically there was one study that showed a deleterious effect of Turkish (unfiltered, diterpenoid-rich) coffee on fibrosis, inflammation, and lipid peroxidation even without exposure to an exogenous toxin, suggesting a potential toxic effect of this type of coffeep.[184] This study is in contrast to all other studies on whole CC or DC and fibrosis that did not find a direct toxic effect.[186-194]

Many studies found comparable effects of CC and DC on fibrosis in presence of various exogenous toxins.[189,192,193] This hepatoprotective effect included increased glutathione levels, reduced fibrosis on histology, decreased hydroxyproline levels (a proxy of total collagen content), reduced protein expression of αSMA, and lowered mRNA expression of TGFβ. In addition, these studies showed that both CC and DC reduced lipid peroxidation (as assessed by MDA), and inflammation (i.e. reduced IL 1 and 10, inducible nitric oxidase synthase, and TNFα). Only two studies found a more pronounced effect of CC than DC[192,194] on fibrosis, specifically they found attenuated matrix metalloproteinases 2 (MMP; involved in degradation of extracellular matrix), upon TAA administration in the CC-treated arm.[192] In addition, four studies on instant CC, ground CC or coffee extract, demonstrated similar results via similar pathways, i.e. I) increased glutathione, II) decreased collagen content assessed by hydroxyproline, histology, or collagen and αSMA expression, and III) decreased lipid peroxidation assessed by MDA or thiobarbituric acid reactive substances.[187,188,190,191] One of these studies suggested that apoptosis played a prominent anti-fibrogenic role, while pro-apoptotic Bax mRNA was upregulated, and anti-apoptotic Bcl-2 mRNA was downregulated after whole coffee administration in CCl4 poisoned rats.[188]

Sugiyama et al. first studied the effect of caffeine-only on TNFα-intoxicated mice. The authors found lowered transaminases and less DNA fragmentation upon caffeine treatment and hence concluded that caffeine had hepatoprotective characteristics, possibly mediated via induction apoptosis.[195] The in vivo/in vitro study of Chan and colleagues, nicely established a plausible mechanistic explanation as to how caffeine could prevent fibrosis.[196] The authors showed that adenosine A-deficient mice did not develop fibrosis upon TAA or CCl4 administration, and that caffeine mimicked these effects. Indeed, caffeine was found to act as non-selective adenosine A(2A) receptor antagonist, suppressing collagen production. Hsu et al. examined caffeine in bile duct ligated rats and found attenuated fibrosis, lowered portal pressure and less portosystemic shunting.[197] Caffeine here acted again as an antagonist of adenosine receptor A2a and A1a, as the presence of a competing adenosine agonist attenuated the results. Later studies all confirmed the anti-fibrotic properties of caffeine as assessed by histology or liver biochemistry.[116,197-205] Most studies explained this by the inhibition of TGFβ and the subsequent decreased αSMA and CTGF

expression upon caffeine treatment.[116,199,200,204,205] Also, caffeine decreased MDA (i.e. lipid peroxidation), increased superoxidase dismutase and glutathione concentration.[199,200,202-204] Gordillo-Bastidas and colleagues treated rats with either bile duct ligation or TAA, and discovered that caffeine decreased transcriptional factor Snail-1 (important in the activation of HSCs) and increased Nrf2.[199] Another elegant in vivo/in vitro study suggested that the anti-fibrogenic activity of caffeine was due to its apoptosis-inducing capacity[116] as caffeine decreased cell viability, reduced cell adhesion and proliferation, decreased the wound healing capacity, and increased the apoptotic cell count by over 50%. Lastly, Klemmer et al. examined the specific effect of paraxanthine, the most effective and least toxic metabolic methylxanthine of caffeine[206] in bile duct ligated rats and found lower transaminases and less bridging fibrosis.[198]

Hepatocellular Carcinoma

Stich and colleagues first described coffee as anti-mutagenic substance and ascribed this effect to the polyphenolic compounds and their ability to reduce the formation of mutagenic units.[207] Subsequently, Mori et al. assessed the effect of CGA on methylazoxymethanol-induced carcinogenesis in hamsters and found significantly less liver cell foci in CGA-treated hamsters (*Table 3*).[208] Decades later, the anti-mutagenic capacity of CGA was examined again in an in vivo/in vitro study of Yan et al.[209] In this study, intraperitoneal CGA administration reduced liver tumour volume and weight in HepG2 xenografts in vivo. The authors found a decreased phosphorylation of ERK1/2, possibly due to reduced mitogen activated protein kinase activation in vitro. In addition, hepatic MMP-2 was lowered, but as tissue inhibitors of MMP (TIMP) were not affected, MMP-2/TIMP ratio was decreased, leading to prevention of extracellular matrix degradation and tumour inhibition.

Diterpenoids are well-studied coffee compounds in carcinogenesis.[210-214] Schilter et al. studied the effect of diterpenoids in rats without administration of an exogenous toxin.[210] The authors found a dose-dependent increase of glutathione S-transferases (GST)-placental form. GSTs are glutathione catalyzing enzymes and induction of GST has been associated with reduced carcinogenesis.[215] The GST-inducing capacity of kahweol and cafestol, with[211] or without[212-214] presence of an exogenous toxin, was confirmed by later studies. In addition, Cavin et al. found a 50% decrease in DNA adduct formation after diterpenoid administration, potentially leading to fewer mutations in proto-oncogenes and tumour suppressor genes.[211] Lastly, mRNA expression of carcinogen-detoxifying phase II mechanisms (CYP450[211] and sulfotransferase 1A1)[214] was decreased upon diterpenoid treatment but not after unfiltered coffee administration. Moreover, CYP2B2 and CYP1A1 activities were increased upon both filtered and unfiltered CC in this study.[213]

Ten studies on whole coffee consumption in relation to hepatic carcinogenesis were conducted,[216-219] of which six specifically looked into tumour morphology.[220-225] Only Hasegawa

Table 3: Experimental evidence on coffee effects on carcinogenesis

Author	Coffee compound	Animal Model	Main study findings
Mori, 1986[208]	CGA	Syrian golden hamsters +/– methylazoxymethanol +/– CGA 24 weeks	Methylazoxymethanol + CGA ↓ adenocarcinoma in intestine vs methylazoxymethanol ↔ liver tumours Methylazoxymethanol + CGA ↓ liver cell foci vs methylazoxymethanol
Yan, 2017[209]	CGA	*In vivo* Nude mice with HepG2 xenograft + placebo or CGA 6 weeks *In vitro* hepG2 cells	*In vivo* CGA 30 and 60 ↓ tumour volume and tumour weight vs placebo CGA ↓ phosphorylation of ERK 1/2 vs placebo CGA ↓ MMP2 and 9, but ↔ TIMP2: hence, ↓ MMP-2/TIMP2 ratio vs placebo *In vitro* CGA 250uM ↔ cell viability, but ↓ CGA 500uM cell viability (50%)à inhibiting in vitro proliferation CGA ↓ MAPK activation of ERK1/2 CGA ↓ MMP2, but ↔ MMP-9 and TIMP2. Hence ↓ MMP-2/TIMP2 ratio
Schilter, 1996[210]	Kahweol Cafestol	Sprague-Dawley rats + kahweol and cafestol 90 days	Kahweol + cafestol ↔ GSTα and GSTμ activity, but doses 2300 and 6200 ↑ GST-P mRNA expression, protein and enzymatic level Results were reversible after 1 month withdrawal
Cavin, 1998[211]	Kahweol Cafestol	Sprague Dawley rats + aflatoxin B1 +/– placebo or kahweol and cafestol 90 days	Aflatoxin B1 + kahweol + cafestol dose-dependently ↓ aflatoxin B1 DNA-binding Aflatoxin B1 + kahweol + cafestol 2300 and 6200 ↓ DNA adduct formation (50%) vs aflatoxin B1 Aflatoxin B1 + kahweol + cafestol ↓ CYP450 (i.e. CYP2C11, CYP3A2), but ↔ CYP1A1 & CYP1A2 expression vs aflatoxin B1 Aflatoxin B1 + kahweol + cafestol ↑ GST subunit Yc2 vs aflatoxin B1
Huber, 2002[212]	Kahweol Cafestol	F344 rats + control or kahweol and cafestol 10 days	Kahweol + cafestol ↑ UGT 9-fold Kahweol + cafestol ↑ GST 3-fold (all subclasses, including GST-θ for the first time)
Huber, 2004[213]	Kahweol Cafestol	F344 rats + kahweol and cafestol 10 days	Kahweol + cafestol dose-dependently ↓ N-acetyltransferase activation in the liver, but reversible upon withdrawal Kahweol + cafestol dose-dependently ↑ GST-μ, GST-α, and GST-4VP in the liver, but reversible upon withdrawal
Huber, 2008[214]	Kahweol Cafestol Turkish coffee Paper-filtered CC	F344 rats + control or kahweol and cafestol or filtered CC or Turkish coffee 10 or 20 days	Kahweol + cafestol ↓ hepatic CYP450 metabolism/mRNA (CYP1A1/2 and CYP2B1/2), but ↔ CYP2E1 vs controls Kahweol + cafestol ↓ sulfotransferase 1A1, Turkish coffee ↔ sulfotransferase 1A1 vs controls
Higgins, 2008[216]	CC	*In vivo* C57BL/6 mice and DBA/20 mice, wild type (Nrf2+/+ and Nrf2-/-) + CC 5 days *In vitro* Mouse embryonic fibroblasts Nrf2 -/- and Nrf2+/+	*In vivo* Nrf2 -/- ↓ NQO1 protein and ↓ CYP1A2 mRNA vs Nrf2+/+ Nrf2+/+ & coffee 3% or 6% dose-dependently ↑ hepatic NQO1 protein vs Nrf2+/+ Nrf2 -/- & coffee 6% ↑ hepatic NQO1 protein vs Nrf2-/- Nrf2+/+ & coffee 3% or 6% ↑ GSTα1/2 and GSTα4 vs Nrf2+/+ Nrf2 -/- & coffee ↔ GST α1/2 or α4 vs Nrf2-/- Nrf2+/+ ↓ UGT1A6 expression vs Nrf2-/- Coffee ↔ effect UGT1A6 Nrf2 +/+ & coffee 6% ↑ CYP1A2 mRNA vs Nrf2+/+

Table 3 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
			In vitro Kahweol + cafestol ↑ NQO1 induction on antioxidant response elements Nrf2+/+ & kahweol + cafestol ↓ acrolein toxicity Nrf2+/+ & CGA or Nrf2-/- ↔ acrolein toxicity
Morii, 2009[217]	Instant CC	ICR Mice +/– low vitamin diet +/– CC 2, 4, and 8 months	↔ hydrogen peroxide deleting capacity, SOD activities and MDA Low vitamin + CC ↓ 8-hydroxydeoxyguanosine activity but ↔ gene expression vs low vitamin + water Low vitamin + CC ↓ glutathione peroxidase expression vs low vitamin + water
Kalthoff, 2010[218]	Filtered CC Undiluted 12% CC Metal-filtered Paper-filtered CC DC Instant CC Boiled CC Cocoa Teas	*In vivo* humanized transgenic mice (human UGT1A-expression) +/– undiluted CC 3 days *In vitro* HepG2 cells	*In vivo* Undiluted CC ↑ UGT1A1 liver vs controls *In vitro* filtered, instant, boiled CC/DC ↑ UGT1A1, UGT1A7, UGT1A10 activity & protein expression vs controls (caffeine-independent) CCs and DC ↓ effect on UGT1A1 and UGT1A7 in genetic variants vs wild-type constructs UGT1A siRNA knockdown ↔ on proliferation Coffee compounds caffeine, paraxanthine, theobromine, theophylline, kahweol and cafestol ↔ UGT1A1, 1A10 or 1A7 CC and DC ↔ UGT1A1, UGT1A7 and UGT1A10 induction upon siRNA aryl-hydrocarbon receptor and Nrf2 knockdown CC and DC ↑ UGT1A genes by aryl-hydrocarbon receptor and Nrf2 signaling (transcriptional level) vs controls
Pietrocola, 2014[219]	Instant CC, Instant DC	C57BL/6 Mice and transgenic C57BL/6 mice expression fusion protein GFP-LC3 "long-term" design CC for 2 weeks "short-term" design CC or DC max 6h	CC and DC ↑ autophagic flux (lipidation of LC3) à ↓ overall abundance of the autophagic substrate sequestome-1 CC and DC ↑ phosphorylation cAMPK in short-term, but ↓ phosphorylation cAMPK long-term CC and DC ↓ phosphorylation of mTORC1 substrates both short and long-term
Hasegawa, 1995[220]	Instant CC Instant DC Brewed CCCaffeine Theophylline Theobromine	F3444 rats + DEN or saline injection +/– Instant CC or brewed CC or caffeine or theophylline or theobromine 6 weeks	Theophylline was the only component causing toxic effects DEN + instant CC or DC or Brewed CC ↔ liver foci frequency and area
Miura, 2004[221]	Instant CC	*In vivo* Donryu rats subcutaneously implanted with cells of AH109A in the back +/– instant CC 14 days *In vitro* AH109A cells or rat ascites hepatoma cell line + instant CC	*In vivo* Instant CC ↓ tumour growth speed and size vs controls Instant CC ↓ hepatoma weight vs controls and ↓ metastasis (0/9 vs 3/11) in controls Instant CC ↓ serum TBARS (indicating anti-oxidant capacity) vs controls *In vitro* Instant CC 0.3 mg/mL ↑ cells in S-phase à ↓ cells in the G1 phase and G2/M phase, and ↑ DNA fragmentation Instant CC 0.3 mg/mL ↓ intracellular peroxide level
Silva-Oliveira, 2010[222]	Roasted CC	Rats of which 50% was lactated from a coffee drinking mum treated +/– 2AAF and DEN and partial hepatectomy +/– CC	Toxins + CC ↔ hepatic tissue morphologically vs toxins + water Toxins + CC ↓ number of premalignant, persistent and remodelling lesions vs toxins + water

Table 3 (*continued*)

Author	Coffee compound	Animal Model	Main study findings
Ferk, 2014[223]	Metal-filtered CC Paper-filtered CC Metal-filtered DC	Sprague Dawley rats +/– aflatoxin B1 +/– metal-filtered CC or DC, or paper-filtered CC	Aflatoxin + metal/paper filtered CC dose-dependently ↓ DNA migration, aflatoxin + DC ↔ DNA migration vs aflatoxin Aflatoxin + caffeine pure dose-dependently ↓ DNA migration vs aflatoxin Aflatoxin + metal/paper filtered CC ↓82% or Aflatoxin + DC 57% ↓ number of liver foci vs aflatoxin Aflatoxin + metal/paper filtered CC or DC ↑ UGTs, but ↔ glutathione
Furtado, 2014[224]	Brewed CC Instant CC Caffeine	Wistar rats DEN + CCl4 +/– instant or brewed CC or caffeine 0.1% 23 weeks	Toxin + instant CC or caffeine ↓ number of neoplastic lesions per liver area vs toxin Toxin + instant CC or caffeine ↑ number of small preneoplastic lesions and ↓ number of large preneoplastic lesions vs toxin Toxin + instant/brewed CC or caffeine ↓ PCNA and ↑ GST-P vs toxin Toxin + brewed CC or caffeine ↓ liver collagen fibres, collagen I and III mRNA expression vs toxin Toxin + instant CC or caffeine ↑ Bax, but ↔ Bcl-2 and TGFβ protein levels vs toxins
Katayama, 2014[225]	Nestlé CC	Long-Evans Cinnamon rats +/– CC 27 weeks	CC ↓ glutamic pyruvic transaminase activity up to week 19, then ↔ vs controls ↔ copper and iron accumulation in liver and ↔ CTGF, TGFβ and Smad2 mRNA expression CC ↓ expression of inflammatory cytokines IL6 and TNFα and ↓ small GST-positive premalignant foci vs controls
Fujise, 2012[226]	Caffeine	Wistar rats +/– DEN +/– caffeine 14 weeks	DEN + caffeine ↓ HCC and number/size of lesions and ↓ PCNA positive cells vs DEN. All rats survived DEN + caffeine ↓ GST vs DEN
Hosaka, 2001[227]	Caffeine	ACI Male rats +/– 2AAF +/– caffeine12 weeks	2-AAF + caffeine 0.1%: 2 deaths, 2-AAF: 1 death 2-AAF + caffeine dose-dependently ↓ HCC-type, incident, and number of tumours vs 2-AAF

Abbreviations 2-AAF: 2-acetylaminofluorene, AMPK; adenosine monophosphate-activated protein kinase, CC: conventional coffee, CGA: chlorogenic acid, CTGF: connective tissue growth factor, CYP: cytochrome P, DC: decaffeinated coffee, DEN: diethylnitrosamine, ERK: extracellular signal-regulated protein kinase, GST: glutathione S-transferase, HCC: hepatocellular carcinoma, LC: lipidation of microtubule-associated protein light-chain, MDA: malondialdehyde, MAPK: mitogen activated protein kinase, MMP: matrix metalloproteinase, mTOR: mammalian target of rapamycin, NQO: NAD(P)H:quinone oxidoreductase 1, Nrf: Nuclear factor (erythroid-derived)-like, PCNA: proliferation cell nuclear antigen, SOD: superoxidase dismutase, TBARS: thiobarbituric acid reactive substances, TGF: tissue growth factor, TIMP: tissue inhibitor matrix metalloproteinase, TNF: tumour necrosis factor.

et al. found no difference in the frequency and location of diethylnitrosamine-induced tumours in coffee-treated rats.[220] All other studies found either reduced tumour frequency or size, upon coffee consumption in rats with endogenously or exogenously induced carcinogenesis.[221-225] Furtado et al. identified higher expression of pro-apoptotic Bax proteins in rats treated with instant coffee or caffeine, suggesting apoptosis-mediated protection against liver carcinogenesis.[224] This apoptosis-mediated hypothesis was supported by a preceding

in vivo/in vitro study on rats implanted with a hepatoma cell line treated with instant CC. In this study, instant CC had the ability to induce cell cycle arrest and lower DNA fragmentation, and therefore, apoptosis.[221] Another in vivo/in vitro study showed a coffee-mediated upregulation of genes involved in chemoprevention, i.e. nicotinamide adenine dinucleotide (phosphate) quinone oxidoreductase 1 and GSTα1-2 and α4, particularly in Nrf2-possessing mice.[216] In addition, CYP1A2 mRNA was upregulated upon high coffee administration in these mice. Nrf2-deficient mice exhibited lower UDP glucuronosyltransferase (UGT)-1A6 expression (proteins with indirect antioxidant and chemo-protective properties), and this was not affected by coffee treatment. These UGTs were studied in more detail by Kalthoff and colleagues, who found upregulation of these proteins regardless of coffee type in vitro.[218] The antiproliferative effect of coffee was attributed to this protein, as supported by UGT1A siRNA knockdown experiments. The authors hypothesized a synergistic effect of the combined components, as the compounds caffeine, paraxanthine, and diterpenoids alone had no effect on UGT upregulation. Most studies comparing CC with DC found similar anti-carcinogenic effects,[218-220] only Ferk et al. found a dose-dependent decrease of DNA migration in CC and caffeine, but not in DC. In addition, the number of liver foci decreased with 82% in CC-treated mice as compared to only 57% in DC-treated mice.[223] Two studies specifically examined the effect of caffeine on liver carcinogenesis and found a protective effect in terms of hepatocellular carcinoma incidence and number of lesions.[226,227] Of note, mortality rate was slightly higher in the caffeine-treated group as compared to the control group, suggesting a possible toxic effect of caffeine itself.[227]

Human experimental studies

To the best of our knowledge, only five experimental studies on coffee consumption and liver health have been conducted in humans so far. Boekschoten et al. conducted two trials in which healthy volunteers were given unfiltered coffee or coffee oils, high in cafestol. Both trials showed an increase in serum transaminases (with a large inter-individual variance) and a slight decrease in GGT and alkaline phosphataseoek.[228,229] Bichler et al. performed an experimental study with coffee (2/3 paper-filtered, 1/3 metal-filtered) on healthy volunteers. DNA-damage caused by reactive oxygen radical treatment in these individuals was found to be strongly reduced after coffee consumption but not after consumption of diterpenoids alone. Also, there was an upregulation of antioxidant enzymes, in particular superoxide dismutase.[230] Shaposhnikov and colleagues completed a placebo controlled intervention trial in 160 healthy volunteers administering water, three, or five cups of coffee per day during eight weeks. The authors did not find an effect of coffee on liver biochemistry (except for a small increase in GGT) nor on biomarker assays for oxidative stress or inflammation.[231] Finally, a recent randomized clinical trial was conducted among 44 patients with steatosis who were given green coffee bean extracts or placebo for eight

weeks. The coffee extracts significantly improved serum transaminases, lipid profile, insulin sensitivity and total antioxidant capacity as compared to placebo. However, steatosis was not affected by the coffee extract.[232]

Summary, conclusion and future perspectives

Over the past decades, both epidemiological and experimental evidence has accumulated on the hepatoprotective effect of coffee. In vivo studies using different experimental models to induce liver disease found beneficial effects of the various individual compounds of coffee as well as of whole coffee.

Several molecular pathways on the anti-steatotic, anti-fibrotic and anti-carcinogenic effects of coffee have been postulated. In *Figure 1* we graphically summarized the most important pathways. Briefly, the steatosis-lowering effect of coffee mainly resulted in decreased FAS

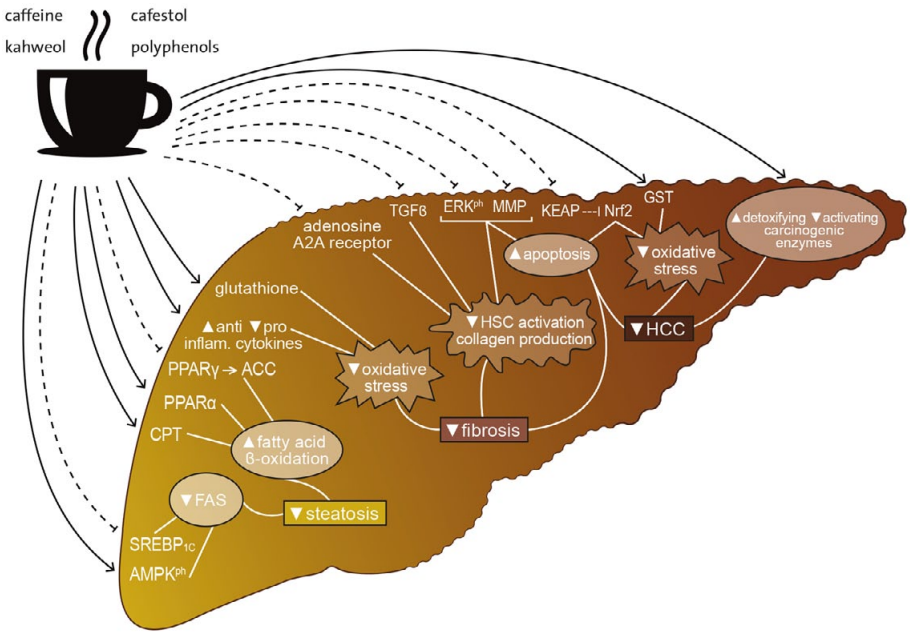

Figure 1: Graphical summary of potential mechanisms underlying the role of coffee in liver health

Simplified illustration of potential mechanisms of action underlying the role of coffee in liver health, specifically in steatosis, fibrosis and hepatocellular carcinoma.

Symbols ▲increasing; ▼decreasing; ➔stimulating; --I blocking.

Abbreviations ACC: acetyl-CoA carboxylase, AMPK: adenosine monophosphate-activated protein kinase, CPT: carnitine palmitoyltransferase, ERK: extracellular signal-regulated protein kinase, FAS: fatty acid synthesis, GST: glutathione S-transferase, HCC: hepatocellular carcinoma, HSC: hepatic stellate cell, KEAP: Kelch-like ECH-associated protein, MMP: matrix metalloproteinase, Nrf: Nuclear factor (erythroid-derived)-like, Ph: phosphorylated PPAR: peroxisome proliferator-activated receptor, SREBP: sterol element binding transcription factor, TGF: transcription growth factor

and increased fatty acid β-oxidation. Also, hepatic glutathione content was increased upon coffee administration, leading to reduced oxidative stress, and hence less steatosis and fibrosis. Coffee-induced inhibition of liver fibrosis mainly included the lowering of TGFβ and the antagonizing effect of caffeine specifically, on adenosine A2A receptors, inhibiting hepatic stellate activation. Both fibrosis and carcinogenesis were inhibited through upregulation of Nrf2, decreased ERK1/2 phosphorylation and increased apoptosis. In addition the balance between detoxifying and activating carcinogenic enzymes was altered by coffee consumption, resulting in reduced tumour growth. The mechanisms of action per coffee compound are depicted more comprehensively in *Table 4*.

Beyond the mechanisms described above, there is some evidence that coffee consumption may influence liver health via the gut microbiome, by modulation of gut microbiota[233-235] or by maintaining intestinal balance.[234]

That all said, it is important to realize that the observed hepatoprotective effects in experimental models cannot be directly translated to the human situation. Studies are largely heterogeneous in using many differences in doses, ways of administration, and coffee constitutes.[236] Similarly, there are the obvious differences between man and mice, and

Table 4: Proposed mechanisms of action per coffee compound

Compound	Mechanism of Action	Effect
Polyphenols	↑ glutathione	
	↑ fatty acid β oxidation	
	↓ C-C chemokine Receptor 2	anti-steatogenic
	↑ anti-inflammatory, ↓ pro-inflammatory cytokines	
	↓ mRNA expression PPARγ and fatty-acid binding protein	
	↑ mRNA expression of FGF21 and CPT1	
	↓ ROS formation	
	↓ lipid peroxidation	
	↓ collagen content	anti-fibrotic
	↓ KEAP -> ↑ Nrf2 -> ↑ phase II antioxidative enzymes	
	↓ TLR4-MyD88-NFkB -> ↓ pro-inflammatory cytokines	
	↓ MAPK activation -> ↓ ERK phosphorylation -> ↓ pro-inflammatory cytokines & ↑ cell viability	anti-fibrotic & anti-carcinogenic
	↓ MMP2/TIMP ratio	anti-carcinogenic
Diterpenoids	↑ glutathione	
	↓ TGFβ	anti-fibrotic
	↓ CYP2E1 activity	
	↑ GST	anti-fibrotic & anti-carcinogenic
	↓ carcinogen-activating enzymes	anti-carcinogenic
	↓ DNA adduct formation	
Whole coffee	↓ PPARγ -> ↓ ACC & ↓ fatty-acid binding protein	anti-steatogenic

Table 4 (*continued*)

Compound	Mechanism of Action	Effect
	↓ pro-inflammatory cytokines	
	↑ glutathione	
	↓ TGFβ	anti-fibrotic
	↓ collagen content	
	↓ MMP2	
	↓ anti-apoptotic Bcl-2, ↑ pro-apoptotic Bax	anti-fibrotic & anti-carcinogenic
	↑ Nrf2	anti-carcinogenic
	↑ phase II carcinogen-detoxifying enzymes	
Caffeine	↓ SREBP-1c	
	↓ ACC	
	↓ mRNA expression CPT2	anti-steatogenic
	↓ pro-inflammatory cytokines	
	↑ fatty acid β oxidation	
	↑ apoptosis	anti-steatogenic & anti-fibrotic & anti-carcinogenic
	↓ adenosine A2A receptor	
	↓ lipid peroxidation	
	↓ collagen content	
	↑ glutathione	anti-fibrotic
	↓ Snail -> ↓ TGFβ	
	↓ pro-inflammatory cytokines	
	↑ Nrf2	
	↑ SOD expression	
	↑ GST	anti-carcinogenic

Abbreviations ACC: acetyl-CoA carboxylase, CPT: carnitine palmitoyltransferase, CYP: cytochroom P, ERK: extracellular signal-regulated protein kinase, FGF: fibroblast growth factor, GST: glutathione S-transferase, KEAP: Kelch-like ECH-associated protein, MAPK: mitogen activated protein kinase, MMP: matrix metalloproteinase, Nrf: Nuclear factor (erythroid-derived)-like, PPAR: peroxisome proliferator-activated receptor, ROS: reactive oxygen species, SOD: superoxidase dismutase, SREBP: sterol element binding transcription factor, TGF: tissue growth factor, TIMP: tissue inhibitor matrix metalloproteinase; TLR4-MyD88-NFκB: toll-like receptor 4/ myeloid differentiation factor 88/nuclear factor κ of activated B-cells

between real life circumstances and laboratory conditions. Likewise, although different coffee compounds seem to all contribute to the observed effects on liver health, specific recommendations on using whole coffee or these compounds separately to treat human diseases cannot be made as of yet. Moving forward however, data from these experimental animal models can be used to design human phase I studies, examining bioavailability and dose-response relation of the various compounds, and eventually well-conducted clinical trials using standardized amounts and preparation methods of coffee. Ultimately this may then lead to clinical recommendations on the optimal preparation method of coffee, and on the optimal amount of coffee to benefit the liver.