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Review

Drinking for protection? Epidemiological and experimental evidence on the beneficial effects of coffee or major coffee compounds against gastrointestinal and liver carcinogenesis

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ABSTRACT

Recent meta-analyses indicate that coffee consumption reduces the risk for digestive tract (oral, esophageal, gastric and colorectal) and, especially, liver cancer. Coffee bean-derived beverages, as the widely-consumed espresso and "common" filtered brews, present remarkable historical, cultural and economic importance globally. These drinks have rich and variable chemical composition, depending on factors that vary from "seeding to serving". The alkaloids caffeine and trigonelline, as well as the polyphenol chlorogenic acid, are some of the most important bioactive organic compounds of these beverages, displaying high levels in both espresso and common brews and/or increased bioavailability after consumption. Thus, we performed a comprehensive literature overview of current knowledge on the effects of coffee beverages and their highly bioavailable compounds, describing: 1) recent epidemiological and experimental findings highlighting the beneficial effects against gastrointestinal/liver carcinogenesis, and 2) the main molecular mechanisms in these *in vitro* and *in vivo* bioassays. Findings predominantly address the protective effects of coffee beverages and their most common/bioavailable compounds individually on gastrointestinal and liver cancer development. Caffeine, trigonelline and chlorogenic acid modulate common molecular targets directly implicated in key cancer hallmarks, what could stimulate novel translational or population-based mechanistic investigations.

Abbreviations: 4-NQO, 4-Nitroquinoline-1-oxide; AC, Adenocarcinoma; ACF, Aberrant crypt foci; Ad-PTEN, Adenovirus-mediated transfer of phosphatase and tensin homolog; AhR, Aryl hydrocarbon receptor; Akt, Protein kinase B; AOM, Azoxymethane; ARE/XRE, Antioxidant/xenobiotic response elements; BSA, Body surface area; CCl_4, Carbon tetrachloride; COX-2, Cyclooxygenase 2; CQA, Caffeoylquinic acid; CRC, Colorectal cancer; CTGF, Connective tissue growth factor; CYP, Cytochrome P450; DEN, Diethylnitrosamine; DMBA, 7,12-Dimethylbenz[*a*]anthracene; DMH, 1,2-Dimethylhydrazine hydrochloride; DSS, Dextran sulphate sodium; EFSA, European Food Safety Authority; EGR1/mPGES-1, Early growth response protein-1/microsomal Prostaglandin E Synthase-1; EMT, Epithelial-mesenchymal transition; ERK, Extracellular signal-regulated kinase; G6Pase, Glucose 6-phosphatase; GCLC, Glutamate-cysteine ligase catalytic subunit; GR, Glutathione reductase; GSH, Reduced glutathione levels; GSH-Px, Glutathione peroxidase; GST-P, Placental glutathione-S-transferase; HCV/HBV, Hepatitis B/C virus; HCC, Hepatocellular carcinoma; HED, Human equivalent dose; HFD, High fat diet; HIF-1α, Hypoxia-inducible factor-1α; HSCs, Hepatic stellate cells; HWM, High molecular weight; IARC, International Agency for Research on Cancer; IBD, Inflammatory bowel disease; IFNγ, Interferon-γ; IRE1-α, Inositol-requiring enzyme 1 alpha; IL, Interleukin; MAM, Methylazoymethanol; miRs, microRNAs; MDA, Malondialdehyde; MEK1, Mitogen-activated protein kinase 1; MMP, Matrix metalloproteinase; MNNG, *N*-Methyl-N′-nitro-N-nitrosoguanidine; MNU, *N*-Methyl-N-nitrosourea; NAFLD, Non-alcoholic fatty liver disease; NF-κB, Nuclear fator κB; NMBA, *N*-Nitrosomethylbenzylamine; NQO1, NAD(P)H quinone dehydrogenase 1; Nrf2, Nuclear factor erythroid-related factor 2; OR, Odds ratio; PA, Palmitic acid; PCNA, Proliferating cell nuclear antigen; PGE_2, Prostaglandin E_2; PhIP, 2-Amino-1-methyl-6-phenylimidazo[4]=5-[b]pyridine; PKCα, Protein kinase Cα; PNL, Preneoplastic lesions; PPARγ, Peroxisome proliferator-activated receptor gamma; ROS, Reactive oxygen species; RR, Relative risk; SCC, Squamous cell carcinoma; SREBP1, Sterol regulatory element-binding protein 1; TAA, Thioacetamide; t-BOOH, Tert-butylhydroperoxide; TGF-β, Transforming growth factor β; TIMP, Tissue inhibitor of metalloproteinase; TNF-α, Tumor necrosis factor α; TOPK, T-LAK Cell-originated protein; UGT, UDP Glucuronosyltransferases; VEGF, Vascular endothelial growth factor.

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1. Introduction

It is estimated that upper, lower digestive tract (oropharyngeal, esophageal, gastric and colorectal) and liver malignant neoplasms accounted for 24% of new cases (~4.3 million) and 31% cancer-related deaths (~3 million) in 2018. Altogether, these cancers would reach the first position in terms of incidence and mortality globally, overcoming top-ranked prostate, breast and lung cancers (Bray et al., 2018). Nutritional habits and interventions, such as the high consumption of fruits, vegetables, and coffee, have been proposed to play important roles in reducing cancer risk (Wiseman, 2018). Indeed, recent studies showed that coffee consumption, a widespread habit usually incorporated into healthy eating patterns, may promote beneficial effects on a plethora of diseases, including a 13% reduction in overall cancer risk for regular consumers (Poole et al., 2017; Yu, Bao, Zou, & Dong, 2011).

In this paper, we provide a comprehensive literature overview of recent epidemiological and experimental findings highlighting the beneficial effects of coffee or bioactive coffee compounds consumption against digestive tract and liver carcinogenesis. Considering that caffeine, chlorogenic acid, and trigonelline are some of the most abundant bioactive compounds in popularly consumed coffee beverages ("common" brewed and espresso) and/or display high bioavailability after consumption, we focused on the effects and common molecular mechanisms linked to these compounds. This review should provide insights of clinical and translational significance for further mechanistic investigation.

2. Coffee origins and chemical composition

According to Ethiopia's popular legend, the discovery of coffee trees is attributed to a goatherd, Kaldi, who lived in the Kaffa highlands, a place which later named the plant. After eating coffee berries, his goats could not sleep. Kaldi reported his findings to a monk, who made a drink out of the berries (Tadesse, 2017). Despite this mythical origin, the production and consumption of coffee bean-derived beverages probably dated back from the 15th century, emerging in Africa and Asia, and rapidly spreading to Europe, along with the colonial voyages during the 17th century. Considered the "favorite drink of the civilized world", coffee beverages finally reached the "New World" in the 18th century, gaining popularity due to widespread production favored by the tropical climate in Latin America (Pendergrast, 2010). Brought from the French Guianas by Francisco de Melo Palheta and frequently portrayed in Candido Portinari's paintings, coffee production exponentially grew in Brazilian southeastern states, making this country by far the world's largest producer (~3.1 billion Kg/year, accounting for 34% of the worldwide production) and exporter (~1.8 billion Kg/year, representing 27% of all exports), and the sixth biggest consumer of coffee beans (6.25 Kg *per capita*) (Fig. 1) (International Coffee Organization, 2018). In terms of production, Brazil is followed by Central and South American, Asiatic and African countries that are part of an equatorial/tropical-climate privileged region called "bean belt" (Fig. 1) (International Coffee Organization, 2018).

Nowadays, because of the extensive production of *Coffea arabica* and *Coffea canephora* species, coffee beans, and their derived beverages are considered commodities of great economic importance (International Coffee Organization, 2018). Especially the "common" brewed and espresso brews are the most consumed drinks worldwide after water, estimated to reach around 2 billion cups consumed every day. When a cup of common (also known as "conventional" or "filtered") or espresso brew coffee is served, its composition reveals a multitude of substances, belonging to different chemical classes and thus, with many potential pharmacological properties (Caprioli et al., 2013; Caprioli et al., 2014; Derossi, Ricci, Caporizzi, Fiore, & Severini, 2018). There is an inherent fluctuation in the presence and levels of these compounds, depending on many factors that vary from "seeding to serving", such as plant species, growing conditions, time of harvesting, the roasting of the beans, types of preparations (common or espresso brews), among others. (Campa, Doulbeau, Dussert, Hamon, & Noirot, 2005; Caporaso, Genovese, Canela, Civitella, & Sacchi, 2014; Caprioli et al., 2013; Caprioli et al., 2014; Derossi et al., 2018; Fuller & Rao, 2017; Tolessa, D'heer, Duchateau, & Boeckx, 2017). The main bioactive compound present in coffee beverages is the caffeine (1,3,7-trimethylxanthine), a xanthine alkaloid derived from guanine (Fig. 2). This purine, which is also present in tea (*Camelia sinensis)* (Lin, Wu, & Lin, 2003) and cocoa (*Theobroma cacao*, L) (Risner, 2008), is the main compound responsible for the psychoactive activity of coffee beverages. Caffeine antagonizes adenosine receptors in the neuron cells in the brain, decreasing fatigue, increasing mental acuity and improving cognitive function (Kaster et al., 2015). Being the most prominent source of daily caffeine, "common" and espresso brews display varying concentrations of this compound: In common coffee, caffeine concentrations range from 0.55 to 1.55 mg/mL while in espresso, from 2.45 to 5.83 mg/mL (Fig. 2) (Caporaso et al., 2014; Caprioli et al., 2014; McCusker, Goldberger, & Cone, 2003). Decaffeinated coffee beverages are suggested in some caffeine-avoidance medical conditions, as treatments with bronchodilators and anti-anxiety drugs (European Food Safety Authority, 2015). Decaffeinated brews are compositionally similar to caffeinated beverages apart from having little (0.10 to 0.52 mg/mL in espresso and 0.02 mg/mL in common brew) or none caffeine (McCusker, Fuehrlein, Goldberger, Gold, & Cone, 2006). Although theobromine is less abundant than caffeine, this other common methylxanthine is present in coffee drinks as well (0.027 and 0.017 mg/mL in espresso and common brews, respectively) (Bispo et al., 2002; Gennaro & Abrigo, 1992).

In terms of abundance, caffeine is followed by a family of conjugated hydroxycinnamates collectively referred to as chlorogenic acids (Fig. 2), which are polyphenols that naturally occur in a wide variety of fruits and vegetables besides coffee beans (Ludwig et al., 2014; Upadhyay & Mohan Rao, 2013). These phenolic compounds are thermolabile and, hence prone to deterioration according to the roasting process of the beans (Ludwig et al., 2014). However, coffee beverages contain substantial amounts of total chlorogenic acid, ranging from 0.24 to 0.67 mg/mL in common coffee and from 1.52 to 3.37 mg/mL in espresso coffee (Fig. 2) (Caprioli et al., 2014; Crozier, Stalmach, Lean, & Crozier, 2012; Tfouni et al., 2014). In coffee beverages, the most common chlorogenic acid isomers are 5-caffeoylquinic acid (5-CQA), 3-CQA and 4-CQA (Caprioli et al., 2014; Crozier et al., 2012; Tfouni et al., 2014). Presenting similar concentration to chlorogenic acids, trigonelline (1-methylpyridinium-3-carboxylate) is another common alkaloid in coffee beverages. This pyrimidine is commonly found in fenugreek seeds (*Trigonella foenum-graecum*) and pumpkin (Cucurbitaceae family) (Panda, Biswas, & Kar, 2013; Yoshinari, Sato, & Igarashi, 2009), and displays 0.35–0.51 mg/mL and 1.69–2.70 mg/mL range variations in common and espresso brews, respectively (Fig. 2) (Caprioli et al., 2014; Furtado, Polletini, Dias, Rodrigues, & Barbisan, 2014; Kuhn, Lang, Bezold, Minceva, & Briesen, 2017; López-Galilea, De Peña, & Cid, 2007). Due to the roasting process, part of trigonelline is demethylated to nicotinic acid, which is mainly available in espresso (0.35 mg/mL) (Caprioli et al., 2014). Moreover, common and espresso brews contain lipids, mainly represented by cafestol and kahweol (Fig. 2). These diterpene alcohols remain abundant in unfiltered coffee preparations (as Turkish) but are almost completely removed when coffee is brewed with a paper filter (Gross, Jaccaud, & Huggett, 1997). Coffee is also a source of non-digestible fiber (6.5 and 4.7 mg/mL in espresso and common brews, respectively), mainly represented by mannose and galactose polysaccharide chains (Díaz-Rubio & Saura-Calixto, 2007).

The roasting process of beans contributes to the synthesis of another class of compounds named melanoidins (espresso: 1.75–3.65 mg/mL; common: 1.79 mg/mL) (Fogliano & Morales, 2011; Lopes et al., 2016). These heterogeneous, complex, and dark-colored molecules are end-products of Maillard reactions and have high molecular weights

Fig. 1. The top 6 coffee consumers and producers globally. Reference: International Coffee Organization, 2018.

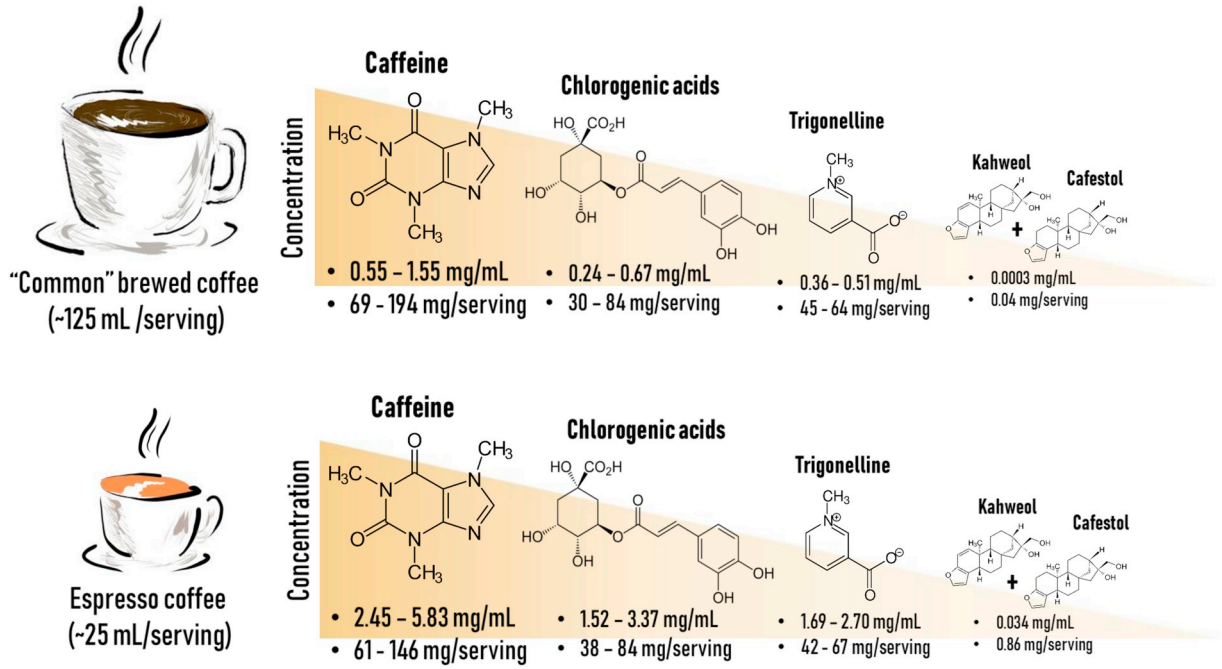

Fig. 2. Main compounds observed in common and espresso coffee brews according to their concentration and the total amount in usually applied servings.

(HMW). Coffee amino acids, polysaccharides, and phenolic compounds, especially the chlorogenic acids, contribute to the formation of coffee melanoidins (Perrone, Farah, & Donangelo, 2012). However, these HMW molecules may only reach high concentrations in over-extraction procedures during coffee beverage preparation (Bartel, Mesias, & Morales, 2015). In addition to the spectra of organic compounds, the mineral characterization of coffee beverages revealed great amounts of potassium in both espresso and common brews (3 and ~0.8 mg/mL, respectively) and phosphorus in espresso coffee (0.6 mg/mL) (Gillies & Birkbeck, 1983; Oliveira, Ramos, Delerue-Matos, & Morais, 2015).

With respect to the contribution of these molecules to the complex sensorial characteristics of coffee, the alkaloids (astringency) and

chlorogenic acids (acidity) are major compositional drivers of flavor, while chlorogenic acids and melanoidins are responsible for the brownish color (Sunarharum, Williams, & Smyth, 2014). Interestingly, high trigonelline and caffeine contents are positively associated with higher coffee cup quality (Farah, Monteiro, Calado, Franca, & Trugo, 2006). Therefore, based on the fluctuations on compound level data, it is clear that "a cup of coffee" measurement is not reliable and reproducible for epidemiological studies, yet commonly applied. Nonetheless, based on the same concentration data, caffeine, trigonelline, and chlorogenic acid seem to be some of the most important bioactive organic compounds of espresso and regular coffee beverages (Fig. 2).

3. Coffee consumption and metabolism

Considering the "coffee cup" term ambiguity, the most reliable coffee consumption data come from annual *per capita* consumption of ground coffee (in Kg). Thus, most countries presenting the higher consumption rates (6.29–9.12 Kg *per capita*) are in a "cup belt" in the north hemisphere, mainly in Europe (Fig. 1). It is worthy of note that many European Union countries (4.90 Kg *per capita*), as well as the US (4.84 Kg *per capita*), are also considered high coffee consumers (International Coffee Organization, 2018). On the other hand, Asian and African countries, such as China, Japan, South Korea, and Ethiopia, present mild to low coffee consumption (3.5 to 0.8 Kg *per capita*) (International Coffee Organization, 2016).

Epidemiological studies in Europe and the US indicate that coffee beverages are the predominant source of caffeine, contributing from 40% to 94% to total caffeine daily intake. In these regions, coffee/caffeine consumption increases in puberty and culminates in adult and elderly ages (European Food Safety Authority, 2015; Martyn, Lau, Richardson, & Roberts, 2018; Mitchell, Knight, Hockenberry, Teplansky, & Hartman, 2014). Findings from the European Food Safety Authority (European Food Safety Authority, 2015), including data from 22 different countries comprising high coffee consumers, showed that caffeine intake derived from coffee beverages reached maximum mean values of 280.7–382.6 mg/day in adults in some countries. In the US, 3 studies showed average consumption of 105.4–136.4 mg/day (all ages) and mean values of 109.4–271 mg/day in adults (Frary, Johnson, & Wang, 2005; Knight et al., 2004; Mitchell et al., 2014). In Asia, a recent Korean study that considered all sources of caffeine (cocoa, coffee, tea, and derivatives) showed average consumptions of 67.7 mg/day (all ages) and mean values of 81.9 mg/day in adults (Lim, Hwang, Choi, & Kim, 2015). In general, these findings are in keeping with *per capita* ground coffee consumption. Although the continents or countries present clear disparity in terms of coffee consumption, it is important to point that all the presented estimates are in accordance to European Food Safety Authority (2015) daily safe limit for caffeine intake for adults (400 mg/day).

Upon coffee beverage intake, caffeine is rapidly and almost completely absorbed (99%) in the gastrointestinal tract within 45 min, 20% in the stomach and the largest part in the small intestine, being hydrophilic and sufficiently lipophilic to cross the biological membranes (Liguori, Hughes, & Grass, 1997; Nehlig, 2018). Upon the consumption of 160 mg of caffeine, corresponding to approximately 1–2 cups of common coffee (Fig. 2), this xanthine is readily bioavailable, reaching a plasma peak of ~18 μM within 60–80 min (White Jr. et al., 2016). A higher coffee consumption, equivalent to 3 cups of common coffee (350 mL, Fig. 2), leads to a caffeine plasma peak of ~33 μM after 60 min and presents a half-life of ~5 h (Lang et al., 2013). In the liver, caffeine suffers demethylation by cytochrome P450 (CYP) subunit 1A2, which virtually accounts for total caffeine metabolism (Gu, Gonzalez, Kalow, & Tang, 1992). Caffeine biotransformation originates paraxanthine, dimethylxanthine, and theobromine (Gu et al., 1992; Lang et al., 2013). It is noteworthy that caffeine metabolism is relatively comparable in humans, rats, and mice, which facilitates the establishment of translational approaches (Walton, Dorne, & Renwick, 2001).

After the consumption of 350 mL of common coffee brew (~3 cups, Fig. 2), trigonelline is also mainly absorbed in the small intestine and presents a plasma peak of 5.6 μM within 3 h (Lang et al., 2013; Yuyama, 1999). In the liver, trigonelline is methylated to *N*-methylnicotinamide by nicotinamide *N*-methyltransferase and subsequently oxidized to *N*-methyl-2-pyridone-5-carboxamide and *N*-methyl-4-pyridone-5-carboxamide (Lang et al., 2013). Similar to caffeine, trigonelline has a long half-life of approximately ~5 h (Lang et al., 2013). Despite the few experiments available on trigonelline pharmacodynamics in rodents, findings indicate that a greater part of trigonelline is also absorbed in the small intestine (Yuyama, 1999). Around 30% of chlorogenic acid is absorbed in the small intestine after consumption, and most part reaches the colon (Olthof, Hollman, & Katan, 2001). After drinking 200 mL of common coffee containing a total of 96 mg of chlorogenic acid (~0.48 mg/mL, in keeping with Fig. 2), this polyphenol is almost undetectable in serum after 1 h (Nardini, Cirillo, Natella, & Scaccini, 2002). Higher common coffee consumption (350 mL) leads to a 0.035 μM plasma peak of chlorogenic acid in 45 min. In contrast, the concentration of (di)hydroxycinnamic acids, their sulfates, and glucuronides, that are well-known chlorogenic acid derivatives, appears to gradually increase in serum after coffee consumption (Lang et al., 2013; Nardini et al., 2002). In particular, catechol sulfate, one of the major metabolites, displays a 2.5 μM peak within 45 min (Lang et al., 2013). This is attributed to the fact that chlorogenic acid is heavily metabolized by colonic microbiota before absorption, considering that ileostomy-submitted patients present a 3-fold decrease in the excretion of some of these metabolites compared to healthy ones (Stalmach, Steiling, Williamson, & Crozier, 2010). Although part of chlorogenic acid is also metabolized by the gut microbiota in rats, about 16% of intact chlorogenic acid is absorbed in the stomach (Lafay et al., 2006).

Collectively, these data suggest that the pharmacokinetic profiling of coffee consumption indicates that caffeine, trigonelline and the metabolites of chlorogenic acid display high bioavailability in humans (Lang et al., 2013). Particularly caffeine and trigonelline accumulate in the plasma due to their long half-life times during habitual consumption of many cups of coffee distributed over the day (Lang et al., 2013). Indeed, both caffeine and trigonelline as well as some metabolites of chlorogenic acid, have been proposed as plasma/urinary biomarkers for coffee brew consumption in humans (Midttun, Ulvik, Nygård, & Ueland, 2018; Stalmach et al., 2009). The high levels and/or bioavailability of these compounds have direct implications on the epidemiological effects and, especially, on the experimental findings regarding coffee and gastrointestinal and liver carcinogenesis.

4. Epidemiological evidence

In the last decade, many meta-analyses of prospective, case-control and cohort studies have shed light onto the beneficial effects of coffee consumption on the digestive tract and liver cancers in different populations (Tables 1–3). As discussed, most of these studies usually apply the "cup of coffee" measurement, thus not considering the variations on the serving volume and type of beverage (Fig. 1). Moreover, depending on the population observed, high and low limits of consumption are variable. In general, the relative risk (RR) or odds ratio (OR) in these meta-analyses are usually calculated based on none or low consumption ($\leq$1 cup/day) *versus* high consumption ($\geq$3 cups/day). Despite these limitations and estimations, studies propose significant non-linear inverse associations between coffee consumption and the emerging oropharyngeal, gastric and colon tumors. In fact, the strongest data come from the hepatology field, which shows a linear inverse association between coffee beverage consumption and hepatocellular carcinoma (HCC) risk.

Although oropharyngeal cancers do not rank in the top ten most common malignant neoplasms, the annual estimated incidence is around half a million cases globally, mainly occurring in Asia, Europe, and South America. Tobacco and alcohol abuse cause > 80% of cases,

Table 1
Review of recent meta-analysis on the effects of coffee consumption against oropharyngeal and esophageal cancers.

Study	Countries/regions	Main findings	Sub group analysis
Oropharyngeal			
Miranda et al., 2017	Japan, Taiwan, Italy, Norway, Denmark, France, Switzerland, USA, Brazil	*vs.* low consumption • 31% reduction for high consumption (OR: 0.69)	• Reduction remains in adjustment for Asian (OR: 0.65) countries
Wang et al., 2016	Japan, Norway, USA	*vs.* low consumption • 31% reduction for high consumption (RR: 0.69)	• Reduction remains in adjustment for Asian (RR: 0.35) countries and smoking (RR: 0.68)
Li, Peng, & Li, 2016	Japan, Italy, Norway, Denmark, France, Switzerland, USA, Brazil	*vs.* low consumption • 37% reduction for high consumption (OR: 0.63)	• Reduction remains in adjustment for Asian (OR: 0.64) and European (OR: 0.62) countries
Turati, Galeone, La Vecchia, Garavello, & Tavani, 2011	Japan, India, Italy, Switzerland, Denmark, Brazil, USA	*vs.* low consumption • 36% reduction for high consumption (RR: 0.64) *vs.* ≤ one cup • 35% reduction for 3 cups/day (OR: 0.65)	• Reduction remains in adjustment for Asian (RR: 0.74), South American (RR:0.58) and European (RR: 0.61) countries
Esophagus			
Zhang, Zhou, & Hao, 2018	Japan, Taiwan, Sweden, Italy, Greece, Switzerland, Norway, Argentina	*vs.* low consumption • No association for high consumption in SCC (OR: 0.76) • No association for high consumption in AC (OR: 0.90)	• 36% reduction for high consumption (OR: 0.64) in Asian countries (SCC and AC)
Wang et al., 2016	Japan, Norway, Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden, United Kingdom, USA	*vs.* low consumption • No association for high consumption in SCC (RR: 0.89) • No association for high consumption in AC (RR: 0.91)	• No association in adjustments for European countries (RR: 0.89), alcohol (RR: 0.84) and smoking (OR: 0.85)
Zheng et al., 2013	Japan, China, Iran, Turkey, India, Italy, Greece, Switzerland, Norway, Sweden, USA, Argentina, Brazil, Uruguay, Paraguay	*vs.* low consumption • No association for high consumption in SCC (OR: 1.00) • No association for high consumption in AC (OR: 0.90)	• 33% reduction for high consumption (OR: 0.67) in Asian countries (SCC and AC) • No association in adjustments for European countries (OR: 0.95), men (OR: 0.82) and alcohol/smoking (OR: 0.89)
Turati et al., 2011	Japan, Taiwan, Italy, Switzerland, Greece, Sweden, USA, Argentina	*vs.* low consumption • No association for high consumption in SCC (RR: 0.87) • No association for high consumption in AC (RR: 1.18)	–

SCC = squamous cell carcinoma; AC = adenocarcinoma; RR = relative risk; OR = odds ratio.

and human papillomavirus infection may also be involved (Warnakulasuriya, 2009). Esophageal cancers, presenting ~572,000 new cases and ~508,000 related-deaths annually, are represented by 2 major subtypes, namely squamous cell carcinoma (SCC) and adenocarcinoma (AC). Both predominantly occur in men in Asian and European countries. Smoking and alcohol consumption are the main risk factors for esophageal SCC, while obesity and gastroesophageal reflux are also accounted for AC (Bray et al., 2018). Regarding coffee beverage consumption, many studies on oropharyngeal cancer present the same non-linear correlation describing that high consumption decreases the risk for this malignancy by 31% - 37% compared to low consumption. Moreover, this inverse association remains even if the data are adjusted for European, Asian countries or smoking (Table 1). For esophageal cancer data, meta-analysis results are inconsistent. Studies do not indicate any overall correlation between coffee consumption and the risk for esophageal SCC and AC development. Nonetheless, when data were subgrouped, high coffee consumption led to a 33%–36% risk reduction, considering both SCC and AC, in the highly affected Asian, but not in the European population (Table 1). Zheng et al. (2013) proposed that the genetic background difference between Europeans and Asians may account for distinct nutritional responses for coffee intake. In this case, more in-depth experimental *in vitro* and *in vivo* findings are needed in order to confirm the biological plausibility of this effect of coffee consumption in different populational backgrounds.

Concerning gastric cancer, which is responsible for a million new cases and 800,000 deaths *per* year, the age-standardized incidence and mortality rates are higher in Eastern Asia, Central, and Eastern Europe and South America and 2-fold higher in men than women. > 90% of gastric cancers are AC, which can be classified according to the anatomic site as cardia and noncardia subtypes. Cardia AC has similar risk factors to esophageal AC, while almost 90% of noncardia AC cases are attributed to *Helicobacter pylori* infection. For both, smoking, alcohol, high salt and low fruit diet are also established risk factors (Bray et al., 2018). A single recent meta-analysis showed that any daily coffee intake significantly reduces the risk of gastric cancer by 7% when compared to non-consumers. A stronger association was observed in high consumers (3–4 cups/day), which displayed a 12% risk decrease. Nonetheless, most meta-analyses demonstrate that coffee consumption does not modulate overall gastric cancer risk, considering pooled RR and adjustments to smoking, alcohol drinking, Europe, and Asia

Table 2
Review of recent meta-analysis on the effects of coffee consumption against gastric and colorectal cancers.

Study	Countries/regions	Main findings	Sub group analysis
Stomach			
Deng et al., 2016	Japan, Korea, Singapore, Norway, Netherlands, Sweden, Denmark, France, Germany, Greece, Italy, Spain, United Kingdom, USA	*vs.* low consumption • Increased risk for high consumption in cardia (RR: 1.50) • No association for high consumption in non-cardia (RR: 0.99)	• Increased risk in adjustment for USA (RR: 1.36) • No association in adjustments for Asia (RR: 0.96) and Europe (RR: 1.12)
Xie, Huang, He, & Su, 2016	Japan, Singapore, Taiwan, Turkey, India, Norway, Sweden, Finland, Poland, Italy, Spain, Uruguay, Venezuela	*vs.* non-consumption • 7% reduction for any consumption (RR: 0.93) • 12% reduction for 3–4 cups/day (RR: 0.88) • 8% reduction for 1–2 cups/day (RR: 0.92) • 5% reduction for < 1 cup day (RR: 0.95)	–
Zeng et al., 2015	Singapore, Norway, Sweden, Finland, USA	*vs.* low consumption • No linear association • No association for 6.5 cups/day (RR: 1.18) • No association for 3.5 cups/day (RR: 1.06) • No association for 1.5 cups/day (RR: 0.97)	• Increased risk in adjustment for USA for 6.5 cups/day (RR: 1.36) • No association in adjustments for Asia (RR: 0.96), Europe (RR: 1.07), smoking (RR: 0.95), and alcohol for 6.5 cups/day (RR: 1.24)
Xie et al., 2014	Japan, Singapore, Norway, Netherlands, Sweden, Finland, USA	*vs.* low consumption • No association for high consumption for both (RR: 1.12)	• Increased risk in adjustment for USA (RR: 1.35), • No association in adjustments for Europe (RR: 1.08), smoking (RR: 0.99), and alcohol (RR: 1.21)
Colorectal			
Gan et al., 2017	Japan, Singapore, Norway, Netherlands, Sweden, Finland, Denmark, France, Germany, Greece, Italy, Spain, United Kingdom, USA	*vs.* low consumption • No association for high consumption for colorectal (RR: 0.98) • No association for high consumption for colon (RR: 0.92) • No association for high consumption for rectum (RR: 1.06) • 7% reduction for 4 cups increment for colon (RR: 0.93)	• 11% reduction for colorectal in adjustment for high decaffeinated consumption (RR: 0.89) • No associations for colorectal in adjustments for Europe (RR: 1.03), Asia (RR: 0.97), USA (RR: 0.92), smoking (RR: 0.96), alcohol (RR: 0.95), red meat consumption (RR: 0.98), low fruit intake (RR: 1.00), no physical activity (RR: 0.99)
Akter et al., 2016	Japan	*vs.* low consumption • No association for high consumption for colorectal (RR: 0.95) • No association for high consumption for colon (RR: 0.98) • No association for high consumption for rectum (RR: 0.99)	• No associations for colorectal in adjustments for men (RR: 1.05) and women (RR: 0.82)
Wang et al., 2016	Japan, Singapore, Norway, Netherlands, Sweden, Finland, Denmark, France, Germany, Greece, Italy, Spain, United Kingdom, USA	*vs.* low consumption • No association for high consumption for colorectal (RR: 0.96) • 13% decrease for high consumption for colon (RR: 0.87) • No association for high consumption for rectum (RR: 0.94)	• No associations in adjustments for Europe (RR: 0.97), Asia (RR: 1.03), USA (RR: 0.89), smoking (RR: 0.97), alcohol (RR: 0.96), red meat consumption (RR: 0.95), no fiber intake (RR: 0.93), no physical activity (RR: 0.93)
Je, Liu, & Giovannucci, 2009	Japan, Norway, Sweden, Finland, USA	*vs.* low consumption • No association for high consumption for colorectal (RR: 0.91) • 10% decrease for high consumption for colon (RR: 0.90) • No association for high consumption for rectum (RR: 0.98)	• 38% reduction in adjustment for colon in Japanese women (0.62) • No associations in adjustments in colorectal cancer for Europe (RR: 0.91), USA (RR: 0.93) and Japan (RR: 0.83)

RR = relative risk.

(Table 2). Although epidemiological data on coffee intake are not usually subgrouped considering different types of coffee (caffeinated or decaffeinated), a cohort study revealed that decaffeinated coffee consumption does not present a significant correlation with gastric cancer risk (Sanikini et al., 2015). In contrast, one subgroup analysis revealed that coffee may increase the risk for cardia, but not noncardia, gastric cancer. In addition, high coffee consumption (6.5 cups/day) also showed enhanced risk for both cardia and noncardia gastric cancers in U.S. adjustment (Table 2). However, Xie, Wang, Huang, and Guo (2014) and Deng et al. (2016) propose that these positive associations should not be overinterpreted, because residual confounding effects of other nutritional factors could exist, considering that coffee consumption tends to be related to the unhealthy behaviors of "western lifestyle", such as smoking and high salt consumption. Furthermore, in comparison to HCC, there are few prospective studies available to establish a solid correlation for gastric cancer. Indeed, findings are inconsistent, and experimental *in vivo* and *in vitro* data point to the opposite direction, as will be further presented.

Colorectal cancer (CRC), considered the main type of digestive tract malignant neoplasm (accumulating 1.8 million cases and 880,000 deaths in each year), is usually linked to smoking, alcohol drinking, sedentary lifestyle and poor dietary habits (low fiber and vegetable and high red meat and fat intakes) (Bray et al., 2018). The occurrence of inflammatory bowel disease (IBD) (*i.e.* ulcerative colitis or Crohn's disease) is also suggested to increase CRC risk (Wang & Fang, 2014). Although CRC onset mainly involves environmental factors (~95%), hereditary risk factors, such as Familial Adenomatous Polyposis and Lynch syndrome (~5%), are also accounted for this malignancy (Bray et al., 2018). The highest age-standardized incidence/mortality rates for CRC are found in Europe, North America, and Eastern Asia, predominantly affecting men. Some of the recent meta-analyses on coffee consumption and CRC are focused on Asian populations (Table 2), considering that rates markedly increased in these regions over the last decades due to a "western lifestyle" turnover (Bray et al., 2018). Coffee drinking was not significantly associated with CRC risk in most studies (Table 2). Nonetheless, non-linear overall risk reductions of 7% to 13% were observed in high coffee consumers considering colon, but not rectal cancer. These marginal correlations appeared to be stronger only at higher ranges of intake, with 7% risk reductions for every 4 cups/day of coffee. Upon data adjustment for Japanese women, risk reduction is 38% (Table 2). On the other hand, high decaffeinated coffee consumption led to an 11% reduction for both colon and rectal cancer risks (Gan et al., 2017) (Table 2). Gan et al. (2017) suggested that this effect may be attributed to residual factors, since participants that drink decaffeinated coffee tend to have healthier lifestyles, with higher fruit/vegetable and low red meat intake. However, the direct contact of the colonic mucosa with common bioactive coffee compounds, rather than caffeine, should be addressed for this inverse association. As previously discussed, chlorogenic acid isomers are heavily metabolized by colonic microbiota, giving rise to many other bioactive compounds that have direct contact with the colonic mucosa. Although clinical findings on coffee consumption and CRC are missing, Kang et al. (2011) reported that any consumption (> 1 cup/day) of caffeinated or decaffeinated coffee showed similar responses on downregulating extracellular signal-regulated kinase (ERK) phosphorylation in CRC tissue compared to non-consumers. These parallel modulations of a key regulator on colon tumorigenesis underscore the importance of *in vivo* and *in vitro* approaches to unravel other mechanisms involved in caffeinated and decaffeinated coffee intake and decreased CRC risk, as will be discussed further on.

Liver cancers, mainly represented by HCC, accounted for about 840,000 incident cases and 780,000 deaths in 2018 (Bray et al., 2018). HCC, a poor prognosis malignancy that comprises 75%–85% of all liver cancer cases and deaths, usually occurs in a background of fibrosis or cirrhosis (> 90% of cases), which is considered the main risk factor. This cirrhotic context is mostly caused by chronic hepatitis B and/or C virus (HBV/HCV) infections, alcohol abuse and non-alcoholic fatty liver disease (NAFLD) (Bray et al., 2018; Yang et al., 2011). Incidence data for HCC presents clear gender and geographic disparities, usually occurring in men and in Asian countries (Bray et al., 2018). In the last decade, there is increasing and accumulating evidence proposing an inverse linear dose-response correlation between coffee consumption and HCC risk in different populations (Table 3). In general, coffee consumption at any level leads to remarkable 27%–39% and 34%–43% reductions in fibrosis/cirrhosis and HCC risks when compared to non-consumers, respectively (Table 3). These inverse associations are stronger for high coffee consumers. Moreover, 1 to 2 extra cups *per* day on top of any consumption may lead to an additional 15%–27% reduction for HCC risk. Interestingly, when HCC data are stratified to specific risk conditions such as the history of chronic hepatitis and HCV or HBV serologic evidence or highly incident areas as Asia, these significant reductions are still observed (Table 3). In contrast, decaffeinated coffee consumption failed in showing a significant reduction for HCC risk in a recent meta-analysis (Godos et al., 2017). In addition, the daily consumption of 2 extra cups of decaffeinated coffee on top of any intake decreased HCC risk, but to a lesser extent in comparison to caffeinated coffee (14% vs. 27%) (Kennedy et al., 2017). These data suggest that decaffeinated coffee consumption presents a weak or none inverse association to HCC risk compared to caffeinated coffee consumption (Table 3). Thus, based on the epidemiological data available, one may raise the question if these protective effects majorly attributed to caffeine or to its combination or association to other highly abundant and bioavailable coffee compounds, as trigonelline and chlorogenic acid. Considering that a recent prospective cohort study also failed in showing that caffeine alone reduces HCC risk (Tamura et al., 2018), the combination of highly bioavailable coffee components may be accounted for the protective effects of coffee consumption against HCC. This insight should be considered for further translational investigations using *in vivo* and *in vitro* HCC models, since the available studies predominantly investigate the effects of coffee compounds individually, not in combination.

5. *In vitro* findings

In vitro studies demonstrate antiproliferative, antioxidant, antifibrotic or proapoptotic effects of coffee brews or major bioavailable coffee compounds on bioassays using SCC, AC, CRC, HCC and hepatic stellate cells (HSC) (Figs. 3 and 4). In general, these described beneficial effects are in line with the epidemiological evidence of inverse associations between gastrointestinal and liver cancer risks and coffee consumption, as pointed before. Interestingly, despite being administered individually, major coffee compounds exhibit a common modulation of key pathways involved in many cancer hallmarks (Figs. 3 and 4). Nonetheless, some insights based on human consumption and bioavailability of coffee compounds are necessary in order to improve novel experimental approaches. Most *in vitro* assays are based on exposing tumoral cells to bioactive coffee compounds individually, lacking the complexity of compound combination as observed in whole coffee beverages. Even when comparing the effects of whole coffee *versus* coffee compounds, these studies only focus on individually selected coffee compounds, and not on the combination of the most common and/or highly bioavailable ones (Kalthoff, Ehmer, Freiberg, Manns, & Strassburg, 2010; Nakayama, Funakoshi-Tago, & Tamura, 2017). Some studies evaluated the effects of cafestol and kahweol combinations (Kalthoff et al., 2010), although these lipids are almost completely absent in coffee brews after filtering. In addition, these assays applied coffee compounds in supraphysiological concentrations ranging from high micromolar (μM) to millimolar (mM) levels, usually mimicking the concentration observed in brewed coffee preparations (Kalthoff et al., 2010) (Fig. 2). Nonetheless, a "metabolic approach" should be considered for *in vitro* studies, such as the physiologically applicable concentrations based on serum or plasma peaks after coffee

Table 3
Review of recent meta-analysis on the effects of coffee consumption against liver fibrosis/cirrhosis and hepatocellular carcinoma (HCC).

Study	Countries/regions	Main findings	Sub group analysis
HCC			
Bravi, Tavani, Bosetti, Boffetta, & La Vecchia, 2017	China, Japan, Finland, multicentre Europe, USA	*vs.* low/non-consumption • 34% reduction for any consumption (RR: 0.66) • 50% reduction for high consumption (RR: 0.50) • 15% reduction for 1 cup increment (RR: 0.85)	• Reduction remains in adjustment for Asian countries (RR: 0.68)
Godos et al., 2017	China, Japan, Finland, multicentre Europe, Italy, Greece, USA	*vs.* non-consumption • Linear association: - 32% reduction for 2 cups/day (RR: 0.68) - 43% reduction for 3 cups/day (RR: 0.57) - 53% reduction for 4 cups/day (RR: 0.47) • 15% reduction for 1 cup increment (RR: 0.85)	• Non-significant reduction for decaffeinated (RR: 0.85) • Significant reduction remains in adjustments for Asian countries (RR: 0.42) and chronic hepatitis (RR: 0.56)
Kennedy et al., 2017	China, Japan, Hong Kong, Singapore, Finland, multicentre Europe, Italy, Greece, France, USA	• 27% reduction for 2 cups increment (RR: 0.73)	• 14% reduction for 2 decaffeinated cups increment (RR: 0.86)
Yu et al., 2016	Japan, Singapore, Finland, multicentre Europe, USA	*vs.* non-consumption • Linear association: - 24% reduction for 2 cups/day (RR: 0.76) - 33% reduction for 3 cups/day (RR: 0.67) - 42% reduction for 4 cups/day (RR: 0.58)	• Reduction remains in adjustments for Asian countries (RR: 0.50), gender (male RR: 0.58, female RR: 0.57) and history of liver diseases (RR: 0.48)
Wang et al., 2016	Japan, Singapore, Finland, multicentre Europe, USA	*vs.* low consumption • 54% reduction for high consumption (RR: 0.46) • 27% reduction for 2 cups increment (RR: 0.73)	• Reduction remains in adjustments for Asian countries (RR: 0.51), men (RR: 0.29) and history of liver diseases (RR: 0.36)
Bravi, Bosetti, Tavani, Gallus, & La Vecchia, 2013	China, Japan, Finland, Italy, Greece, Serbia	*vs.* non-consumption • 40% reduction for any consumption (RR: 0.60) • 56% reduction for high consumption (RR: 0.44) • 20% reduction for 1 cup increment (RR: 0.85)	• Reduction remains in adjustments for gender (male RR: 0.58, female RR: 0.70), serologic evidence of HBV and/or HCV (RR: 0.52) and history of hepatitis (RR: 0.70)
Bravi et al., 2007	Japan, Italy, Greece	*vs.* non-consumption • 41% reduction for any consumption (RR: 0.59) • 55% reduction for high consumption (RR: 0.45) • 23% reduction for 1 cup increment (RR: 0.77)	• Reduction remains in adjustments for history of hepatitis (RR: 0.53)
Fibrosis/Cirrhosis			
Kennedy et al., 2016	Singapore, Norway, Finland, Italy, France, USA	• 44% reduction in all-cause cirrhosis for 2 cups increment (RR: 0.56) • 42% reduction in alcoholic cirrhosis for 2 cups increment (RR: 0.58)	–
Liu et al., 2015	Hong Kong, Italy, France, UK, Brazil, USA	*vs.* non-consumption • 39% reduction in cirrhosis for any consumption (OR: 0.61) • 47% reduction in cirrhosis for high consumption (OR: 0.53) • 27% reduction in fibrosis for any consumption (OR: 0.73)	• Reduction remains in adjustment for alcohol drinking in cirrhosis (OR: 0.49) and for HCV infection in fibrosis (OR: 0.65)

RR = relative risk; OR = odds ratio.

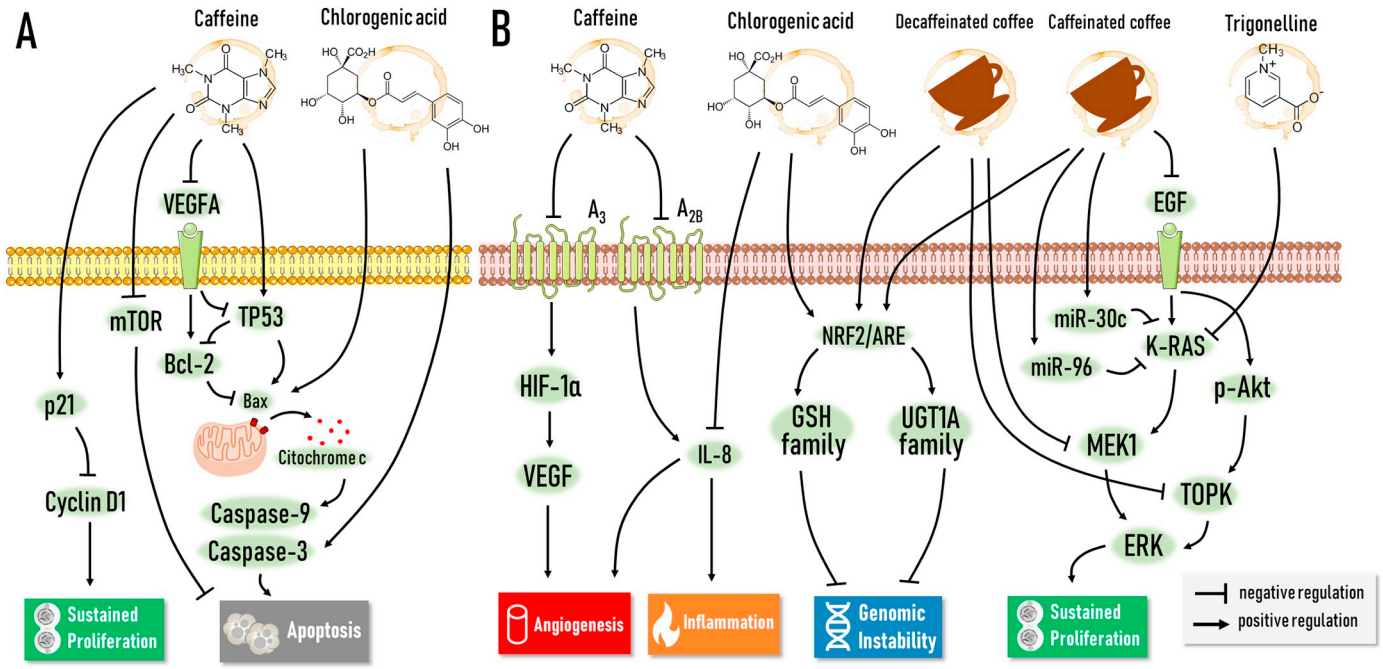

Fig. 3. Main molecular pathways modulated *in vitro* by whole caffeinated, decaffeinated coffee brews or highly bioavailable isolated coffee compounds in (A) gastric adenocarcinoma (AC) and (B) colon cancer cells. Caffeine and chlorogenic acid modulate common molecular targets in the proapoptotic axis in gastric AC cells. Caffeinated and decaffeinated coffee are suggested to share the regulation of pro-proliferative (K-RAS pathway) and antioxidant pathways (Nrf2 pathway), as well as both caffeine and chlorogenic acid, can commonly attenuate IL-8-mediated pro-inflammatory response.

compound biotransformation that range from mid to low micromolar (μM) concentrations. Furthermore, the time points selected for parameter evaluation in these bioassays should be based on the half-life of the compounds in the human body, hence still simulating a physiologically reliable context. Finally, despite exerting antiproliferative and/or proapoptotic effects on different tumor cell lines, only few studies have included normal cell line controls to show a potential selective effect or absence of toxic effects in cells isolated from normal tissues (Amigo-Benavent, Wang, Mateos, Sarriá, & Bravo, 2017; Liu, Zhou, & Tang, 2017; Saito et al., 2003).

5.1. Upper digestive tract cancer cells

There are few studies available on the effects of coffee brews and selected coffee compounds on upper digestive tract cancer *in vitro* models. With respect to whole brewed coffee exposure, both caffeinated

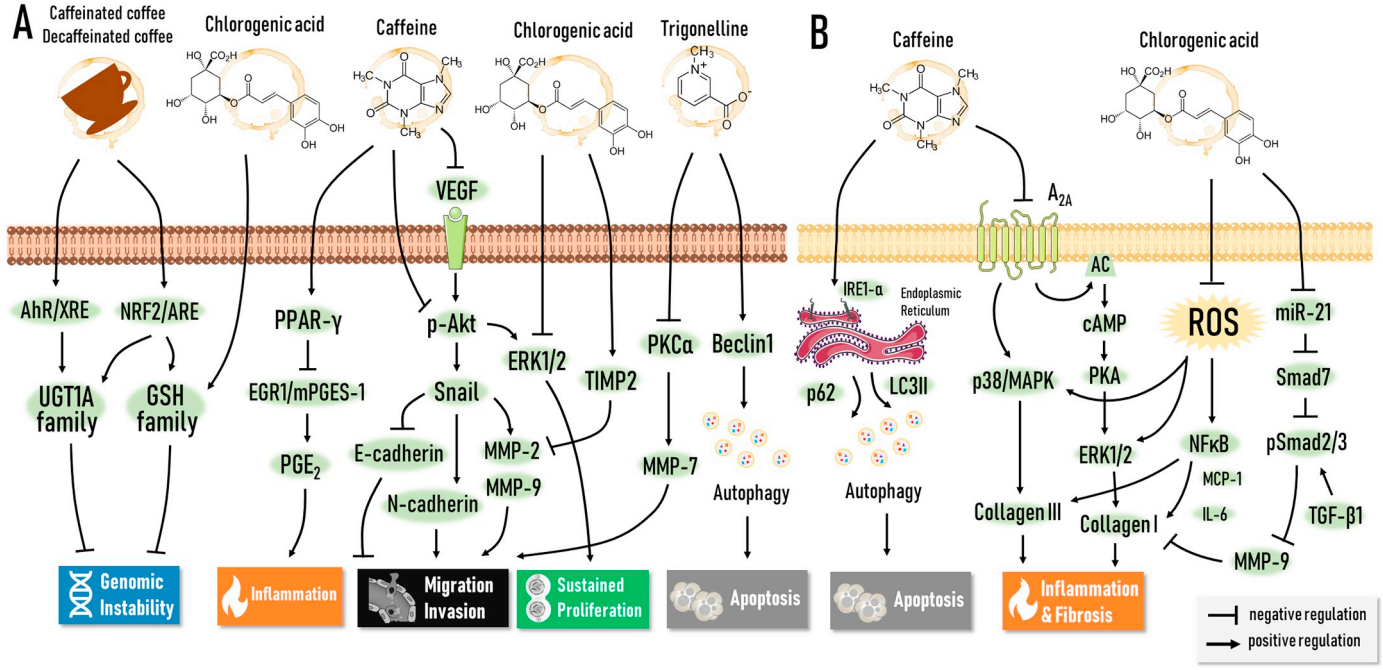

Fig. 4. Main molecular pathways modulated *in vitro* by whole caffeinated, decaffeinated coffee brews or highly bioavailable isolated coffee compounds in (A) hepatocellular carcinoma (HCC) cells and (B) hepatic stellate cells (HSC). Isolated coffee compounds share molecular targets implicated on important cancer hallmarks, such as migration/invasion in HCC cells (MMP modulation) and inflammation and fibrosis in HSC (p38/ERK1/2/collagen I/III pathway).

(12% in the medium, with ~400 μM of caffeine) and decaffeinated coffee increased the transcription of genes encoding antioxidant UDP glucuronosyltransferases 1A (UGT1A) family through the upregulation of aryl hydrocarbon receptor (AhR), nuclear factor erythroid-related factor 2 (Nrf2) and antioxidant or xenobiotic response elements (ARE or XRE) proteins in esophageal SCC cells (KYSE70) (Kalthoff et al., 2010). In the same study, this important antioxidant pathway was not modulated by xanthines (including caffeine, 3.4 mM) and lipids present in coffee beverages, considering that these compounds individually did not increase UGT1A family mRNA. The contribution of chlorogenic acid and trigonelline to this antioxidant effect is still to be investigated. Recently, Amigo-Benavent et al. (2017) showed that chlorogenic acid (10, 100 and 1000 μM) reduced cell viability and proliferation of esophageal SCC cells (OE-33) in a time- and concentration-dependent manner. However, chlorogenic acid also reduced the viability and proliferation of normal fibroblasts (CCD-18Co), mainly in the highest applied dose (1000 μM), considered supraphysiological. In non-toxic doses to normal cells (0.1–1 μM), chlorogenic acid also increased cytotoxicity in SCC cells (Amigo-Benavent et al., 2017). In the same study, caffeine (10, 100 and 1000 μM) showed no effect on both normal and SCC cells. In accordance with these findings, high chlorogenic acid doses (0.1 to 10 mM) decreased the viability of oral SCC cells (HSC-2) in a concentration-dependent manner. Chlorogenic acid-treated cells displayed clear DNA fragmentation and nuclear condensation, typical features of apoptosis that were correlated with increased levels of the caspase cleavage product of cytokeratin 18 (Jiang et al., 2000). Molecular insights into the antiproliferative or proapoptotic effects of coffee compounds on SCC cell lines are still missing.

Caffeine showed beneficial effects on both well-differentiated (SGC-7901) poorly differentiated (MGC-803) human gastric AC cell lines. High doses of caffeine (0.5, 1, 2, 4 and 8 mM) showed similar results on both cell lines, reducing cell viability, inhibiting cell cycle progression and increasing apoptosis on a concentration-dependent way (Liu et al., 2017). Since caffeine treatment above 2 mM also exerted proapoptotic effects on normal gastric mucosa cells, the 0–2 mM range was selected for further analysis. In keeping with the cell cycle arrest effects, caffeine increased p21 protein production in poorly differentiated AC cells whereas decreasing cyclin D1 protein levels in both types of cell lines (Fig. 3A). In addition, a caffeine-mediated positive modulation of caspase 3 and 9 axis was suggested, corroborating with the pro-apoptotic effect in AC cells. Indeed, caffeine reduced vascular endothelial growth factor A (*VEGFA*) and mammalian target of rapamycin kinase (*MTOR*) expression, while it increased *TP53* mRNA expression (Fig. 3A). In addition, caffeine decreased anti-apoptotic Bcl-2 protein quantities, but enhanced expression of pro-apoptotic Bax, cytochrome *c* and cleaved caspase-3 and 9 proteins (Fig. 3A). Notably, both protein and gene expression signatures in this proapoptotic axis were sustained even 24 h after caffeine withdrawal in the cell culture medium. Hence, it was suggested that caffeine may modulate the expression of microRNAs (miRs) involved in this pathway, yet further elucidation is warranted. Notably, chlorogenic acid exposure showed similar results as caffeine on increasing apoptosis and hindering cell cycle progression accompanied by the upregulation of Bax and caspase 3 gene expression in AC cells (Jafari, Zargar, Delnavazi, & Yassa, 2018) (Fig. 3A). These findings suggest that caffeine and chlorogenic acid share the induction of apoptosis as a mechanism for abrogating AC.

5.2. Colon cancer cells

In contrast to the upper digestive tract and liver cancer *in vitro* bioassays, literature is loaded with findings comparing the effects of coffee brews *versus* selected coffee compounds on CRC cell lines, as well as the effects of the most important compounds individually. Findings suggest that the combination of compounds in both caffeinated and decaffeinated coffee beverages may display the most pronounced antioxidant and antiproliferative effects on CRC cell lines (Fig. 3B). The exposure to a common caffeinated coffee brew (at 0.31, 0.63, 1.25, 2.5, 3.75 or 5.0%) decreased both mRNA and protein expression of K-RAS in Caco-2 cells in a concentration-dependent manner through the upregulation of miR-30c and miR-96 expression (Fig. 3B), which are direct negative regulators of this oncogene (Nakayama et al., 2017). *KRAS* activating mutations are commonly found in CRC cases, exerting essential roles in the sustained proliferation of tumor cells (Vaughn, ZoBell, Furtado, Baker, & Samowitz, 2011). Coffee also reduced epidermal growth factor (EGF)-induced activation of phosphorylated protein kinase B (Akt) and ERK in these cells, reinforcing the negative modulation of K-RAS signaling (Fig. 3B). Of note, in the same study, most of the isolated components, including caffeic and chlorogenic acids and caffeine (100 μM for 24 h), did not modulate K-RAS protein expression, except for a slight reduction observed in trigonelline treatment (100 μM for 24 h) (Nakayama et al., 2017) (Fig. 3B). The authors also observed that the reduction in K-RAS protein expression was inversely correlated with the roasting of the beans used in brew preparation. These findings suggest that K-RAS-mediated malignant growth of CRC cells may be modulated by a sort of interaction between coffee compounds, especially those emerging during the roasting of the beans (Nakayama et al., 2017). Moreover, decaffeinated coffee exposure (5, 10, 20 and 40 μg/mL) showed decreased mitogen-activated protein kinase (MEK1) and T-LAK cell-originated protein kinase (TOPK) activities, which are upstream activators of ERK (Fig. 3B), in a concentration dependent-manner, whereas chlorogenic acid displayed weak attenuation of TOPK in CT-26 cell line (Kang et al., 2011). In addition, caffeic acid showed stronger effects than chlorogenic acid, but the whole phenolic fraction of decaffeinated coffee, as well as interaction between these compounds, should be considered for explaining this effect. *In vitro* results of Nakayama et al. (2017) and Kang et al. (2011) are partially in line with clinical findings showing reduced ERK protein expression in colonic tissue of CRC patients that frequently consumed both caffeinated and decaffeinated coffee beverages (Kang et al., 2011).

Complex effects are also observed regarding the potential antioxidant activity of coffee brews. As addressed in SCC cells (KYSE70), Kalthoff et al. (2010) demonstrated that caffeinated and decaffeinated coffee exposures, (both 12% in the medium) similarly upregulated glucuronidation (UGT1A-related genes) via AhR/XRE and Nrf2/ARE induction in Caco-2 cells (Fig. 3B), an effect that was not accomplished by caffeine alone (~3.4 mM) and by cafestol combined with kahweol. Indeed, there is accumulating evidence regarding the positive modulation of AhR/Nrf2 pathways by different coffee brews on Caco-2 cells (Venkatasubramanian et al., 2017; Yazheng & Kitts, 2012). Findings from Bakuradze et al. (2010) suggest that the cellular antioxidant effectiveness of coffee beverages may be linked to the chlorogenic acid and *N*-methylpyridinium-derived (including trigonelline) fractions, present in both caffeinated and caffeinated coffee brews. According to these authors, tert-butyl hydroperoxide (t-BOOH)-induced reactive oxygen species (ROS) levels were reduced in HT-29 cells by chlorogenic acid isomer 5-CQA (30 μM), and mainly by chlorogenic acid- (1, 5, 10, 50 and 100 μg/mL) or *N*-methylpyridinium-rich (1, 5 and 100 μg/mL) fractions extracted from coffee. Interestingly, only the 5-CQA (most 1, 3, 10 and 30 μM) and chlorogenic acid-rich fraction (1, 10 and 100 μg/mL) of coffee increased the protein expression of ARE-dependent enzymes, such as NAD(P)H quinone dehydrogenase 1 (NQO1) and glutamate-cysteine ligase catalytic subunit (GCLC) (Fig. 3B). Recently, Liang and Kitts (2018) reinforced the antioxidant role of chlorogenic acid isomers (including the abundant 3-, 4- and 5-CQA) on phorbol-12-myristate-13-acetate or interferon-γ (IFNγ)-induced inflammation in Caco-2 cells, demonstrating that high concentrations (1 and 2 mM) of the most abundant isomers similarly increased Nrf2 protein expression (Fig. 3B), and 5-CQA upregulated gene expression of Nrf2 and its glutathione-related target genes (glutathione peroxidase 2, glutathione synthetase and glutathione-disulfide reductase). All isomers (0.2, 1.0 and 20 mM) similarly reduced interleukin-8 (IL-8) protein levels in a concentration-dependent manner. Shin et al. (2015) demonstrated that

chlorogenic acid not only decreased IL-8 secretion but also downregulated mRNA and transcriptional activity of this proinflammatory mediator in Caco-2 cells (Fig. 3B). In addition to these effects, chlorogenic acid treatment (100–1000 μM) decreased cell viability, increased cytotoxicity and induced S-phase cell-cycle arrest in a concentration-dependent manner, which was accompanied by the increased protein expression of the pro-apoptotic caspase-3 (Ekbatan, Li, Ghorbani, Azadi, & Kubow, 2018). In the same study, an equimolar mix of chlorogenic, caffeic acids and the selected microbial metabolites 3-phenylpropionic and benzoic acids in low concentrations showed more prominent results than isolated chlorogenic or caffeic acid individually in high concentrations (both at 500 and 1000 μM), indicating that these compounds may function together on abrogating CRC.

Although caffeine is not likely to be involved in the antioxidant effects of coffee brews in CRC cell lines, low concentrations of this xanthine (10 μM) modulated master regulators of tumor angiogenesis and migration in HT-29 cells under hypoxic conditions, a common feature of malignant tumors. This xanthine reduced the protein expression of (1) hypoxia-inducible factor-1α (HIF-1α) transcription factor and its downstream target VEGF via antagonism of adenosine A_3 receptor, and (2) IL-8 through blockade of adenosine A_{2B} receptor (Merighi et al., 2007) (Fig. 3B). Moreover, when combined with adenovirus-mediated transfer of phosphatase and tensin homolog (Ad-PTEN) treatment, caffeine administration attenuated growth and induced apoptosis through downregulation of Akt and modulation of p44/42 MAPK pathways in HCT116 cells in a synergistic manner, not exerting the same effects in normal CCD-18Co colon fibroblasts (Saito et al., 2003). Therefore, the authors proposed the combined treatment with Ad-PTEN and caffeine as a potential therapeutic alternative for CRC (Saito et al., 2003).

5.3. Liver cancer and hepatic stellate cells

There is a single report on the effects of whole brewed caffeinated (12% in the cell culture medium, with ~400 μM of caffeine) and decaffeinated coffee on HCC cells (HepG2) (Kalthoff et al., 2010). In a similar manner to SCC (KYSE70) and CRC (Caco-2) cell lines, both types of coffee upregulated UGT1A-induced glucuronidation by AhR signaling and Nrf2 binding to ARE and XRE (Fig. 4A). Again, this antioxidant effect was not related to the xanthine fraction of coffee, since the treatment with caffeine (3.4 mM) and other coffee xanthines individually did not increase UGT1A expression in HepG2 cells (Kalthoff et al., 2010). The study did not investigate whether other common coffee compounds, like chlorogenic acid and trigonelline, are implicated in this potential antioxidant effect of brewed coffee. Nonetheless, previous findings suggest that this effect in HCC cells is related to the hydroxycinnamic acid fraction of the beverage, including chlorogenic acid (Baeza et al., 2014). Low concentrations of chlorogenic acid (1, 10 and 20 μM) decreased t-BOOH-induced cytotoxicity, lipid peroxidation and protein oxidation in HepG2 cells by restoring reduced glutathione levels (GSH) and glutathione reductase (GR) and peroxidase (GSH-Px) activities (Fig. 4A), which are endogenous antioxidant agents modulated by the ARE/Nrf2 axis. In the same study, caffeine administration did not alter both oxidative stress and the antioxidant response. In addition, high concentrations of chlorogenic acid (0.5 and 1.0 mM) decreased cell viability and induced S-phase arrest in HepG2 cells in a dose-dependent manner (Yan, Liu, Hou, Dong, & Li, 2017). This antiproliferative effect was attributed to the downregulation of active ERK1/2 protein expression (Fig. 4A). Furthermore, chlorogenic acid decreased the matrix metalloproteinase (MMP)-2/tissue inhibitor of metalloproteinase (TIMP)-2 ratio, suggesting an attenuation in MMP-2 activity, which plays a relevant role in extracellular matrix (ECM) degradation and remodeling essential for tissue invasion and metastasis (Yan et al., 2017) (Fig. 4A).

Caffeine alone (200, 400 and 600 μM) reduced cell viability, invasion, and migration of two different HCC cell lines (HepG2 and Huh7) in a concentration-dependent manner. These effects were associated with the downregulation of VEGF and Akt and suggest an abrogation of downstream VEGF and Akt-mediated signaling. In this respect, caffeine-treated HCC cells presented a reduction in the expression of MMP-2 and -9 proteins, both related to ECM degradation and remodeling, and reduced protein expression of Snail and N-cadherin, but increased expression of E-cadherin, which are involved in epithelial-mesenchymal transition (EMT) (Dong et al., 2015) (Fig. 4A). Concerning Akt signaling, caffeine-mediated (1.0, 1.5 and 2.0 mM) reduction of Akt phosphorylation was implied in the decrease of cell proliferation of HCC cells (SK-Hep-1) as well (Edling, Selvaggi, Ghonaim, Maffucci, & Falasca, 2014) (Fig. 4A). Caffeine has shown beneficial effects on abrogating pro-inflammatory signaling mediated by Hepatitis B virus x protein (HBx) in HepG2 cells (Ma, Wang, & Tang, 2015). This xanthine significantly reduced prostaglandin E_2 (PGE_2) levels by stimulating the protein expression of peroxisome proliferator-activated receptor gamma (PPAR-γ), which is a negative regulator of PGE_2 synthesis (Fig. 4A). The caffeine-mediated increase in PPARγ is proposed to subsequently block protein expression and/or transcriptional activity of early growth response protein-1 (EGR1) and microsomal prostaglandin E synthase-1 (mPGES-1), ultimately leading to PGE_2 synthesis (Ma, Wang, & Tang, 2015). Interestingly, the treatment with trigonelline alone (50, 75 and 100 μM) also decreased migration of HCC cells (Hep3B) in a concentration-dependent manner without altering cell viability by reducing the protein levels of protein kinase Cα (PKCα) and mRNA levels of MMP-7 (Liao et al., 2015) (Fig. 4A). Recently, in a palmitic acid (PA)-induced model fatty liver disease in HepG2 cells, it was shown that trigonelline (50 μM) downregulated the protein expression of sterol regulatory element-binding protein 1 (SREBP1) and PPAR-γ, suggesting decreased PA-induced lipotoxicity. Furthermore, trigonelline was able to enhance the protein expression of Beclin-1, a positive regulator of autophagy and apoptosis (Sharma, Lone, Knott, Hassan, & Abdullah, 2018) (Fig. 4A).

In addition to HCC cells, activated HSC, which produce collagen type I and III, are also regarded as key therapeutic targets for fibrosis and cirrhosis, the major risk factor for HCC development. Individually, caffeine treatment decreased the viability in a time and concentration-dependent manner and reduced the activation of human (LX-2) and rat (HSC-T6) HSCs by exerting antifibrotic and proapoptotic activities (Li et al., 2017; Shim et al., 2013; Wang et al., 2014) (Fig. 4B). This xanthine (4 mM) appears to block 2 distinct adenosine A_{2A} receptor-mediated signaling pathways: (1) cAMP/PKA/SRC/ERK1/2 activation leading to procollagen type I synthesis, and (2) p38/MAPK activation leading to procollagen type III production (Wang et al., 2014). In addition, Li et al. (2017) recently showed that caffeine (20 mM) induces inositol-requiring enzyme 1 alpha (IRE1-α)-mediated endoplasmic reticulum stress, with subsequent increases in autophagy (p62 and LC3II accumulation) and apoptosis. Similar to caffeine, chlorogenic acid diminishes HSC activation. This antioxidant molecule (~25, 50 and 100 μM) attenuated oxidative stress in LX-2 and HSC-T6 cells, thus reducing the activation of the p38/ERK1/2/collagen I/III pathway and abrogating the redox-sensitive and profibrogenic nuclear factor κB (NF-κB) pathway as well (Shi et al., 2013, 2016). Moreover, chlorogenic acid exposure (~55, 110 and 220 μM) upregulated the antifibrogenic Smad7/MMP-9 pathway by decreasing miR-21 expression, which is a negative regulator of Smad7 (Yang et al., 2017) (Fig. 4B). No data are available on the effects of whole decaffeinated and caffeinated coffee and trigonelline in HSC cells.

Although coffee compounds are usually administered individually, these data shed light onto common molecular targets of these compounds in both HCC (MMP modulation in migration and invasion activities) and HSC (p38/ERK1/2/collagen I/III pathway modulation in inflammation) (Fig. 4). These shared molecular targets and pathways suggest that the combination of highly bioavailable coffee compounds may underlie the protective effects of coffee consumption against fibrosis/cirrhosis and/or HCC, as suggested in caffeinated coffee data

from epidemiological studies. Nonetheless, monolayer *in vitro* models lack the complexity of the fibrosis or cirrhosis-associated hepatocarcinogenesis microenvironment. The use of complex *in vitro* models, as 3D HCC and HSC spheroids (Abu-Absi, Hansen, & Hu, 2004), is needed to confirm these molecular beneficial effects of the combination of bioactive coffee compounds.

6. *In vivo* findings

Evidence stemming from most *in vivo* studies predominantly demonstrate anti-carcinogenic effects of coffee brews or major bioavailable coffee compounds on tongue, esophagus, stomach, colon, and liver carcinogenesis preclinical bioassays, in keeping with the epidemiological and *in vitro* findings. Based on the available data, some critical points can be raised that might be helpful in designing future *in vivo* studies. Firstly, the dosages used in most studies do not usually mimic human coffee consumption and bioavailability of coffee compounds, and supraphysiological approaches are frequent. Allometric calculation approaches should be applied to suitably translate the dosage from one species to the another. The well-accepted Human Equivalent Dose (HED) calculation considers the body surface area (BSA) normalization for dose translation. BSA correlates well with important parameters of both rodent (rats and mice) and humans, such as basal metabolism, oxygen utilization and renal function (Reagan-Shaw, Nihal, & Ahmad, 2008). Thus, considering the data on average caffeine consumption from coffee in the USA, the 109.4–271 mg/day [1.82–4.52 mg/Kg body weight (b.wt.)/day in 60 Kg adults] range would correspond to 22.5–55.8 and 11.4–28.3 mg/Kg b.wt. doses in mice and rats, respectively. Secondly, several studies evaluated the anti-carcinogenic effects of the paper-filtered cafestol and kahweol lipids (Fig. 2), and these findings will not be included in this section. The main effects of coffee and most bioavailable coffee compounds (caffeine, chlorogenic acid, and trigonelline) on experimental tongue/esophagus, stomach, colon, and liver carcinogenesis are discussed in this section and summarized in Tables 4–6. Details on the experimental procedures (*i.e.*, carcinogen, dose, concentration, regimen of treatment, etc.) and the main effects on the endpoint lesions or cellular processes are also presented. In general, these studies focus on changes in incidence and/or multiplicity of preneoplastic and neoplastic lesions, with few data on potential related mechanisms. Indeed, the most prominent mechanistic findings come from hepatocarcinogenesis models or bioassays mimicking HCC main risk conditions, such as fibrosis and NAFLD. Therefore, the main pathways and biological processes commonly modulated by whole caffeinated coffee, decaffeinated coffee, and isolated compounds during these liver rodent bioassays are depicted in Fig. 5.

6.1. Upper digestive tract carcinogenesis bioassays

7,12-dimethylbenz[*a*]anthracene (DMBA), 4-nitroquinoline-1-oxide (4-NQO), diethylnitrosamine (DEN) and *N*-nitrosomethylbenzylamine (NMBA) are known carcinogens used for the induction of SCC in the oral cavity (tongue and buccal pouch) and esophagus (Nagini & Kowshik, 2016; Sallet, Zilberstein, Andreollo, Eshkenazy, & Pajecki, 2002; Tang, Knudsen, Bemis, Tickoo, & Gudas, 2004) while *N*-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and *N*-methyl-N-nitrosourea (MNU) are used for the induction of SCC in forestomach and AC in glandular stomach of rodents (Tsukamoto, Mizoshita, & Tatematsu, 2007; Yu, Yang, & Nam, 2014). Due to their extensive similarities to human cancer, these animal models are usually applied to investigate multistage carcinogenesis and to assess the efficacy of chemopreventive agents (Nagini & Kowshik, 2016; Tsukamoto et al., 2007). A few studies on the effects of coffee, caffeine or chlorogenic acid on oral, esophageal and gastric carcinogenesis have been published (Table 4). The literature lacks animal studies investigating the effects of trigonelline on upper digestive tract carcinogenesis.

Miller, Formby, Rivera-Hidalgo, and Wright (1988) showed that dietary supplementation with coffee bean powder is implicated in the attenuation of oral SCC development in a DMBA-induced buccal pouch painting hamster model, displaying a marked reduction (11-fold) in SCC mass (Table 4). Using a similar model, Saroja, Balasenthil, Ramachandran, and Nagini (2001) described that common coffee administration exerted no preventive effect on SCC development, resulting in increased mean tumor volume (Table 4). In the same study, common coffee treatment reduced lipid peroxidation and increased GSH levels and GSH-Px activity in oral pouch mucosa. The exact molecular mechanisms of these contrasting effects need further clarification. In a 4-NQO-induced tongue carcinogenesis model, Tanaka et al. (1993) demonstrated that the consumption of chlorogenic acid during the initiation step significantly reduces the incidence of squamous cell hyperplasia, moderate and severe dysplasia and total incidence of papillomas and SCC. In addition, chlorogenic acid reduced cell replication in non-neoplastic surrounding squamous epithelium. In a DEN-induced esophageal carcinogenesis model, Balansky, Blagoeva, Mircheva, and De Flora (1994) observed that caffeine intake does not interfere with the development of esophagus squamous cell papillomas in female BD6 rats. As in animal studies on oral and esophageal carcinogenesis, there are few animal studies on the effects of caffeine or chlorogenic acid on gastric carcinogenesis (Table 4). Nishikawa et al. (1995) observed that caffeine treatment reduced the incidence of pyloric AC and lipid hydroperoxide levels in the gastric mucosa of male Wistar rats submitted to MNNG and NaCl-induced carcinogenesis. Ultimately, the chlorogenic acid treatment led to a reduction of the incidence of MNU-induced adenomatous hyperplasia and AC, as well as to a decrease in proliferating cell nuclear antigen (PCNA) labeling indexes in non-neoplastic glandular areas (Shimizu et al., 1999). As could be observed in epidemiological studies, results from animal models also lack sufficiently strong evidence of beneficial effects of coffee and its main components on upper digestive tract carcinogenesis. Thus, these few findings indicate the need for additional animal studies to convincingly prove the beneficial effects and potential interactions of coffee bioactive compounds on oral and esophageal SCC and gastric AC development.

6.2. Colon carcinogenesis models

1,2-dimethylhydrazine hydrochloride (DMH) and its metabolites azoxymethane (AOM), methylazoymethanol (MAM) or MNNG and heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo[4,5]-[b]pyridine (PhIP), are widely applied chemicals that promote the development of both preneoplastic (non-dysplastic and dysplastic aberrant crypt foci [ACF]) and neoplastic (adenomas and adenocarcinomas) lesions in the colon of rodents (Ward & Treuting, 2014). These lesions are mainly detected in the middle and distal colon and used as putative endpoints in short and medium-term rodent bioassays, enabling the evaluation of potential environmental and dietary factors or preventive compounds on different stages of colon carcinogenesis (Ward & Treuting, 2014).

Although epidemiological data show protective effects for both caffeinated and decaffeinated coffee consumption at higher levels, experimental animal data comparing these different types of coffee or isolated compounds are limited. Recently, Soares, Kannen, Jordão Junior, and Garcia (2018) showed that caffeinated coffee intake, but not decaffeinated coffee or caffeine, counteracted the development of dysplastic ACF during the initial stages of MNNG-induced colon carcinogenesis (Table 4). Rather than decaffeinated coffee, caffeinated coffee-treated rats also displayed fewer ACF positive for metallothionein, which is proposed as a stem cell mutation marker (Soares et al., 2018). In the same study, all treatments reduced DNA damage (phosphorylate H2A histone family/member X, cH2AX) and only caffeinated coffee and caffeine diminished proinflammatory cyclooxygenase 2 (COX-2) expression in colonic mucosa. The authors suggested that the anti-inflammatory and antigenotoxic effects exerted by caffeinated

Table 4
Review of the main studies on the effects of whole coffee or highly bioavailable isolated coffee compounds on oral, esophageal, gastric and colon carcinogenesis/colitis rodent models.

Carcinogen/Procedure (dose, exposure)	Animal	Coffee or specific compound	Dose/Concentration/Regimen	Before (1), during (2) or after (3) carcinogen exposure/model establishment	Main findings	Reference
Oral cavity or esophagus						
DMBA (0.5%, buccal pouch painting, 3×/week for 16.5 weeks)	Female Syrian golden hamsters	Coffee powder	200 g/Kg in diet for 16.5 weeks	1	• Reduced SCC mass	Miller et al., 1988
DMBA (0.5%, buccal pouch painting, 3×/week for 14 weeks)	Male Syrian golden hamsters	Common coffee	3×/week, on alternate days to DMBA, i.g., for 14 weeks	1	• Increased SCC volume	Saroja et al., 2001
4-NQO, (20 mg/L in drinking water for 5 weeks)	Male F344 rats	Chlorogenic acid	500 mg/Kg in diet for 7 weeks	1, 2 and 3	• Reduced incidence of tongue hyperplasia and moderate/severe dysplasia • Reduced incidence of tongue tumors (papillomas plus SCC)	Tanaka et al., 1993
DEN (80 mg/kg b.wt., i.p., 1×/week for 7 or 9 weeks)	Female BD6 rats	Caffeine	0.3 or 0.6 mg/mL in drinking water during 8–12 or 12–24 weeks	1 and 2 or 3	• No effects on esophageal papilloma development	Balansky et al., 1994
Stomach						
MNNG (100 mg/L in drinking water for 8 weeks) and NaCI (50 g/Kg diet for 8 or 40 weeks)	Male Wistar rats	Caffeine	2.5 mg/mL in drinking water for 32 weeks	3	• Reduced incidence of pyloric AC	Nishikawa et al., 1995
MNU (400 mg/L) in drinking water for 12 weeks	Male F344 rats	Chlorogenic acid	250 or 500 mg/Kg in diet for 22 weeks	3	• Reduced incidence of adenomatous hyperplasia (500 mg/Kg) • Reduced incidence of gastric AC (250 mg/Kg)	Shimizu et al., 1999
Colon						
MNNG, (2.5 mg/exposure, i.r., 2×/week for 2 weeks)	Male Wistar rats	Caffeinated or decaffeinated coffee (instant powder) and caffeine	Coffee and decaffeinated at 200 mg/kg b.wt./day; caffeine at 5.4 mg/kg b.wt./day. All i.g. for 4 weeks	3	• Only coffee reduced dysplastic ACF number/mm^2	Soares et al., 2018
DMH (40 mg/Kg b.wt., s.c., 2×/week for 2 weeks)	Male Wistar rats	Common coffee (commercial or organic)	Infusions: 5, 10 or 20 g of powder in 100 mL water/Kg diet for 12 weeks; Powder: 40 g /Kg diet for 12 weeks	1, 2 and 3	• No effects on conventional or mucin-depleted ACF development	Carvalho et al., 2011
–	*Apc*$^{Min/-}$ mice	Common coffee	Filtered or unfiltered coffee at 10 mg/Kg diet for 14 weeks	–	• No effects on colonic tumor development	Oikarinen et al., 2007
CT-26 xenograft model (i.v., single dose)	Male Balb/C mice	Decaffeinated coffee and chlorogenic acid	Decaffeinated coffee at 0.5, 1.0 and 2.0 g/Kg b.wt./day; chlorogenic acid at 0.1, 0.5 and 1.0 g/Kg b.wt./day. All i.v. for 2 weeks.	2 and 3	• Both reduced the number of CRC xenograft metastatic tumors in the lung	Kang et al., 2011
PhIP (100 mg/Kg b.wt., i.g., every other day for 2 weeks)	Male F344 rats	Caffeine	0.50 mg/mL in drinking water for 11 weeks	3	• Reduced number of total ACF • Reduced number of small ACF (< 4 crypts)	Carter et al., 2007
PhIP (400 mg/Kg diet for 10 weeks)	Male F344 rats	Caffeine	0.01, 0.1 or 1.0 mg/mL in drinking water for 10 weeks	2	• Increased number of total ACF (1 mg/mL)	Tsuda et al., 1999
PhIP (200 mg/Kg in diet for 54 weeks)	Female F344/DuCrj rats	Caffeine	1.0 mg/mL in drinking water for 54 weeks	2	• Increased incidence of adenomas and adenocarcinomas; • Increased multiplicity of tumors (all)	Hagiwara et al., 1999
DSS (35 mg/mL in drinking water for 5 days)	Male C57BL/6 mice	Caffeine	~0.49 mg/mL in drinking water for 18 days	1, 2 and 3	• Decreased colitis histological score	Lee et al., 2014
PhIP (three cycles of 50 mg/Kg b.wt./day, i.g. for 2 weeks) and high fat diet (three cycles for 4 weeks), in alternated cycles.	Male F344 rats	Caffeine	0.65 mg/mL in drinking water for 34 weeks	3	• Increased tumor incidence and volume	Wang et al., 2008
AOM (15 mg/Kg, b.wt., s.c. 1×/week for 3 weeks)	Male F344 rats	Chlorogenic acid	250 mg/Kg diet for 5 or 32 weeks	2 (5-week-long) or 3 (32-week-long)	• Reduced tumor multiplicity (5-week-long)	Matsunaga et al., 2002
AOM (15 mg/Kg b.wt., s.c., 1×/week, for 3 weeks)	Male F344 rats	Chlorogenic acid	250 mg/Kg diet for 6 or 12 weeks	3	• Reduced number of total ACF; • Reduced number of small ACF (< 3 crypts)	Morishita et al., 1997

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Table 4 (*continued*)

Carcinogen/Procedure (dose, exposure)	Animal	Coffee or specific compound	Dose/Concentration/Regimen	Before (1), during (2) or after (3) carcinogen exposure/model establishment	Main findings	Reference
AOM (10 mg/Kg, b.wt., i.p. 1 ×/week, for 6 weeks)	Male A/J mice	Chlorogenic acid	100 or 1000 mg/kG in diet for 20 weeks	1, 2 and 3	● No effects on ACF and tumor development	Park et al., 2010
DSS (25 mg/mL in drinking water for 8 days)	Female C57BL/6 mice	Chlorogenic acid	~0.35 mg/mL in drinking water for 15 days	2 and 3	● Decreased colitis activity index	Zhang et al., 2017
DSS (30 mg/mL in drinking water for 8 days)	Female C57BL/6 mice	Chlorogenic acid	~0.35 mg/mL in drinking water for 15 days	2 and 3	● Decreased colitis histological score	Shin et al., 2015

"-" = not applicable; DMBA = 7,12-dimethylbenz[*a*]anthracene; 4-NQO = 4-nitroquinoline-1-oxide; DEN = diethylnitrosamine; MNNG = *N*-methyl-N '-nitro-N-nitrosoguanidine; MNU = *N*-methyl-N-nitrosourea; DMH = 1,2-dimethylhidrazine; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5]-[b]pyridine; DSS = dextran sulphate sodium; i.g. = intragastrical route; i.r. = intrarectal route; s.c. = subcutaneous route; i.p. = intraperitoneal route. SCC = squamous cell carcinoma; AC = adenocarcinoma; ACF = aberrant crypt foci; AC = aberrant crypt.

coffee may be implicated in reducing ACF development. In keeping with these findings, Silva et al. (2014) demonstrated that the administration of common organic or commercial caffeinated coffee brews diminished DMH-induced mutagenicity (micronuclei) and toxicity (apoptotic cells) in colonocytes of male Swiss mice. Interestingly, the most pronounced results were observed in infusions prepared with organic coffee powder, despite presenting similar caffeine, chlorogenic acid, and trigonelline levels compared to the commercial preparation. The same research group reported that although similar organic and commercial common coffee brews reduced malondialdehyde (MDA) levels in the liver, the major organ responsible for DMH metabolism (Ward & Treuting, 2014), these treatments did not modulate the development of conventional or high-risk mucin-depleted ACF (Carvalho et al., 2011) (Table 4). Besides chemically-induced models, the dietary exposure to common caffeinated coffee did not exert antitumoral effects in multiple intestinal neoplasia ($Apc^{Min/+}$) mice and did not alter β-catenin and cyclin D1 protein levels in colonic adenomas either (Oikarinen, Erlund, & Mutanen, 2007) (Table 4). In a xenograft mice model using CT-26 CRC cell line, both decaffeinated coffee and chlorogenic acid treatments showed similar results on reducing the number of metastatic CT-26 tumors in the lung (Table 4), with stronger reductions at higher doses (Kang et al., 2011). This effect was possibly accomplished by reductions in COX-2 protein expression, corroborating with findings from an AOM-induced model (Soares et al., 2018). Additionally, reduced activity of matrix MMP-2 and MMP-9 and ERK phosphorylation was also observed, which are directly involved in the metastatic activity of CRC cells. Results were slightly stronger in decaffeinated coffee than chlorogenic acid treatment and the negative regulation of the ERK pathway corroborates with clinical and *in vitro* findings (Fig. 3B).

Concerning the most abundant coffee compounds, caffeine treatment right after PhIP exposure resulted in a significant reduction in the number of small ACF (< 4 aberrant crypts) and the total number of ACF (Carter et al., 2007) (Table 4). Despite not altering cleaved caspase-3 protein expression in colonocytes, this protective effect was accompanied by a significant reduction in cell proliferation index in the colonic crypts. In contrast, the co-administration of caffeine and PhIP resulted in a significant increase in colonic ACF development associated with a 2-fold enhancement in hepatic CYP1A2 protein expression in male rats (Tsuda et al., 1999) (Table 4). Similar findings were observed in a short-term study wherein Takeshita, Ogawa, Asamoto, and Shirai (2003) showed that caffeine intake simultaneously to PhIP exposure increased PhIP-induced DNA adduct formation as well as hepatic CYP1A2 mRNA levels in female rats. However, no alteration in cell proliferation, apoptosis or DNA repair enzymes were detected in the colon of PhIP- and caffeine-treated group. In addition to ACF, Hagiwara et al. (1999) demonstrated that caffeine intake during PhIP exposure significantly increases the incidence and multiplicity of colonic neoplastic lesions in female rats (Table 4). In addition to the increase in tumor incidence and volume, caffeine intake after cycles of PhIP exposure and HF diet intake increased the frequency of β-catenin mutations in colon tumors (79%, codon 34) compared to PhIP- and HF-exposed counterparts (36%, mainly in codons 32 and 34). Taken together, these findings suggest that caffeine exposure may modulate carcinogen metabolizing enzymes (as the universal CYP1A2) and, thus, the complex protective or promotional effects may depend on the time of administration of this xanthine (i.e., after, during and/or before carcinogen exposure) (Table 4). Other effects and mechanisms of caffeine on colon carcinogenesis, not considering the modification of carcinogen metabolism, should be evaluated in further studies using well-established transgenic and/or xenograft rodent models. Different from caffeine, most rodent bioassays point to a protective effect of chlorogenic acid on different stages of colon carcinogenesis. The administration of this polyphenol after AOM initiation resulted in a marked 43% -51% reduction in the total number of preneoplastic ACF in male rats (Morishita et al., 1997). In contrast, Park, Davis, Liang, Rosenberg, and

Table 5
Review of the main studies on the effects of whole caffeinated/decaffeinated coffee or highly bioavailable isolated coffee compounds on hepatocarcinogenesis, fibrosis or NAFLD rodent models.

Carcinogen/Procedure (dose, time)	Animal/ Liver Disease	Coffee or coffee compound	Dose/Concentration/Regimen	Before (1), during (2) or after (3) model establishment	Main findings	Reference
DEN (single, 200 mg/Kg b.wt., i.p.) and CCl_4 (1 ×/week, 0.5–1.0 mL/Kg b.wt., gavage, for 21 weeks)	Male Wistar rats, Fibrosis-associated Hepatocarcinogenesis	Common coffee or caffeine	Common coffee with 1.00, 0.51 and 0.41 mg/mL of caffeine, trigonelline and chlorogenic acid, respectively. Caffeine at 1 mg/mL. All in drinking water for 23 weeks.	2	• Only caffeine reduced the size and area of GST-P+ preneoplastic lesions and the number of neoplastic lesions; • Both reduced collagen area and collagen I mRNA; only common coffee reduced collagen III mRNA	Furtado et al., 2014
TAA (2×/week, 200 mg/Kg, b.wt., i.p., for 8 weeks)	Male Wistar rats, Fibrosis-associated Hepatocarcinogenesis	Common coffee, decaffeinated coffee or caffeine	Common coffee containing with 1.00 and 0.41 mg/mL of caffeine and chlorogenic acid, respectively. Decaffeinated coffee with 0.09 and 0.30 mg/mL of caffeine and 5-CQA, respectively. Caffeine 1 mg/mL. All in drinking water for 8 weeks.	2	• Only common coffee reduced the number of GST-P+ preneoplastic lesions • All treatments reduced collagen area	Furtado et al., 2012
DEN (single, 200 mg/Kg b.wt., i.p.) followed by 2-AAF (1 ×/day, 20 mg/Kg b.wt., gavage, for 6 days) and PH	Male Wistar rats, Hepatocarcinogenesis	Common coffee	Lyophilized, 15 mg/Kg diet for ~13 weeks	1, 2 and 3	• Reduced size, area and number of total, persistent and remodeling G6Pase- preneoplastic lesions	Silva-Oliveira et al., 2010
–	Long Evans Cinnamon rats, Inflammation associated Hepatocarcinogenesis	Common coffee	Common coffee with ~0.5 and ~0.09 mg/mL of caffeine and chlorogenic acid, respectively in drinking water for 27 weeks	–	• Reduced number of small GST-P+ preneoplastic lesions	Katayama et al., 2014
AFB1 (single, 0.75 mg/Kg b.wt., i.p.)	Male Wistar rats, Hepatocarcinogenesis	Common coffee or decaffeinated coffee	Common coffee with 0.045–0.065 mg/mL of caffeine. Decaffeinated coffee with 0.003 mg/mL of caffeine. Both in 25% and 50% solutions in drinking water for 8 days	1	• Both common and decaffeinated coffee reduced the number of GST-P+ preneoplastic lesions; • Only common coffee reduced the area of GST-P+ preneoplastic lesions	Ferk et al., 2014
BDL procedure	Male Wistar rats, Fibrosis	Common coffee, decaffeinated coffee or caffeine	Common coffee and decaffeinated coffee at 200 mg/Kg b.wt., caffeine at 50 mg/Kg b.wt. i.g., or 4 weeks.	2 and 3	• Only Common coffee and caffeine reduced collagen I protein and mRNA	Arauz et al., 2017
TAA (2×/week, 200 mg/Kg, b.wt., i.p., for 8 weeks)	Male Wistar rats, Fibrosis	Common coffee or decaffeinated coffee	Common coffee and decaffeinated coffee at 200 mg/Kg b.wt., by gavage for 8 weeks.	2 and 3	• Both common and decaffeinated coffee reduced collagen area	Arauz et al., 2013
HFD (for 3 months)	Male Wistar rats, NAFLD	Decaffeinated coffee, polyphenol combination, or melanoidin combination	Decaffeinated coffee with 2.8 and 1.5 mg/mL of polyphenols and melanoidins, respectively. Polyphenol combination with 2.8 mg/mL, Melanoidin combination with 1.5 mg/mL. All in drinking water for 2 months	2	• All treatments reduced both lipid droplets, inflammatory infiltrate and fibrosis	Vitaglione et al., 2010
Fat-, fructose- and cholesterol-rich diet for 6 weeks	Female C57BL/6 J mice, NAFLD	Decaffeinated coffee	~Freeze-dried decaffeinated coffee, 6 g/Kg diet for 6 weeks	2	• Reduced NAFLD score	Brandt et al., 2019
HFD (for 3 months)	Male Wistar rats, NAFLD	Decaffeinated coffee	1.5 mL /day in drinking water for 2 months	2	• Reduced steatosis, ballooning, inflammatory infiltration, fibrosis and liver triglycerides	Salomone et al., 2014
HFD (for 9 weeks)	Male C57BL/6 mice, NAFLD	Common coffee and decaffeinated coffee	Lyophilized common coffee, 20 g/Kg diet, with 0.50, 0.16 and 0.14 g/Kg diet of caffeine, trigonelline and chlorogenic acid. Lyophilized decaffeinated coffee, 20 g/Kg diet, with 0.02, 0.18 and 0.18 g/Kg diet of caffeine, trigonelline and chlorogenic acid. Both for 9 weeks.	2	• Both treatments decreased liver triglycerides	Takahashi et al., 2014
HFD and fructose in drinking water (for 14 weeks)	Male Sprague-Dawley rats, NAFLD	Common coffee or chlorogenic acid, caffeic acid and trigonelline combination	Common coffee i.g. for 14 weeks, 24, 12 and 7 mg/rat/day of chlorogenic acid, caffeic acid and trigonelline, respectively, i.g. for 14 weeks.	2	• Only common coffee reduced liver steatosis grades and triglyceride levels	

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Table 5 (*continued*)

Carcinogen/Procedure (dose, time)	Animal/ Liver Disease	Coffee or coffee compound	Dose/Concentration/Regimen	Before (1), during (2) or after (3) model establishment	Main findings	Reference
						Shokouh et al., 2018

"-"= not applicable; AFB1 = Aflatoxin B1; BDL = bile duct ligation; GST-P = placental glutathione-S-transferase; G6Pase = glucose 6-phosphatase; DEN = diethylnitrosamine; CCl_4 = carbon tetrachloride; TAA = thioacetamide; 2-AAF = 2-acetylaminofluorene; pH = partial hepatectomy; HFD = high fat diet; NAFLD = non-alcoholic fatty liver disease; i.g. = intragastric route; b.wt. = body weight; i.p. = intraperitoneal route; s.c. = subcutaneous route.

Bruno (2010) observed that chlorogenic acid intake before, during and after AOM exposure did not influence ACF and tumor development in male mice. Matsunaga et al. (2002) have also shown that chlorogenic acid treatment during AOM initiation phase reduced colon tumor multiplicity, while both exposures during initiation and post-initiation phases diminished PCNA labeling index in colonocytes in non-tumorous mucosa areas (Table 4).

Apart from the chemically-induced colon carcinogenesis models, some studies have shown similar protective effects of caffeine and chlorogenic acid on dextran sulfate sodium (DSS)-induced colitis in mice models (Lee, Low, Kamba, Llado, & Mizoguchi, 2014;Shin et al., 2015 ; Zhang et al., 2017) (Table 4). In patients with IBD, chronic mucosal inflammation is a key factor for carcinogenesis onset (Wang & Fang, 2014). Caffeine attenuated colitis by reducing bacterial and inflammatory cell infiltration, modulating cytokine production, including decreasing tumor necrosis factor α (TNF-α), IFNγ and IL-17F while increasing IL-10 and downregulating chitinase 3-like 1-associated Akt signaling pathway activation (Lee et al., 2014). Similarly, chlorogenic acid administration also attenuated colitis by reducing colonic infiltration of macrophages, neutrophils and CD3+ T cells, decreasing pro-inflammatory NF-κB, IFNγ, TNF-α, IL-1b, and IL-6 signaling, and modulating colonic microbiota (i.e. decreased *Firmicutes* and *Bacteroidetes* whereas increased mucin-degrading *Akkermansia* spp.) (Shin et al., 2015; Zhang et al., 2017).

6.3. Hepatocarcinogenesis, fibrosis,cirrhosis, and NAFLD models

In the past decade, increasing evidence from rodent models of hepatocarcinogenesis or from bioassays mimicking its main risk conditions (fibrosis, cirrhosis and NASH) allowed insight into the molecular pathways modulated by common filtered caffeinated, decaffeinated coffee and the most common and bioavailable compounds (Fig. 5). In general, data from these preclinical bioassays are in line with the findings of epidemiological studies showing beneficial effects of coffee consumption, especially caffeinated coffee intake (Tables 5 and 6). Different whole common coffee treatments reduced the mean number, size and/or relative area of placental glutathione-S-transferase (GST-P)-positive or glucose 6-phosphatase (G6Pase)-negative hepatocyte foci in several rodent studies (Table 5). These foci are well-established preneoplastic lesions (PNL) observed in models of chemically-induced hepatocarcinogenesis, featuring the absence (as some DEN-induced models) or presence of a fibrotic or cirrhotic [as in thioacetamide (TAA) or carbon tetrachloride (CCl_4)-induced ones] background (Silva-Oliveira, Fernandes, & Moraes-Santos, 2010; Furtado et al., 2012; 2014). These classical biomarkers, especially GST-P-positive foci, are prone to neoplastic transformation or regression under adequate stimuli, enabling the *in vivo* short-term screening of modulators of chemical hepatocarcinogenesis (Tatematsu, Tsuda, Shirai, Masui, & Ito, 1987), such as coffee beverages and its isolated compounds.

Only a few studies compared the effects of different types of coffee and/or isolated compounds on the fate of these lesions. Some findings suggested that caffeinated coffee displays more pronounced attenuation of PNL development compared to decaffeinated coffee and caffeine alone (Ferk et al., 2014; Furtado et al., 2012) (Table 5), but the precise mechanisms involved in these different responses are still unclear. Nonetheless, during hepatocarcinogenesis, whole coffee and caffeine increased GSH levels without altering GST-P-positive PNL and whole coffee reduced DNA strand breaks in the liver, while decaffeinated coffee exerted none of these effects (Ferk et al., 2014; Furtado et al., 2012) (Fig. 5). Both caffeinated and decaffeinated coffee, (without altering GST-P positive PNL) coffee also increased the activity of the antioxidant UGT enzyme (Fig. 5), an effect equally addressed in HCC cells (Kalthoff et al., 2010) (Fig. 4A). In the absence of fibrosis/cirrhosis, while caffeinated coffee diminished the levels of IL-6 and TNF-α (Katayama et al., 2014), which are considered potent hepatomitogenic cytokines, and reduced the number of PCNA-positive hepatocytes

Table 6
Review of the main studies on the effects of on highly bioavailable isolated coffee compounds on hepatocarcinogenesis, fibrosis or NAFLD rodent models.

Carcinogen/Procedure (dose, time)	Animal/ Liver Disease	Coffee compound	Dose/Concentration/Regimen	Before (1), during (2) or after (3) model establishment	Main findings	Reference
DEN (40 mg/Kg b.wt./day, i.p., for 10 or 14 weeks)	Male Wistar rats, Hepatocarcinogenesis	Caffeine	0.2 mg/mL in drinking water, for 10 and 14 weeks	2	• Reduced number/size of GST-P + preneoplastic lesions	Fujise et al., 2012
Alcohol (2×/day, 5–8 g/Kg b.wt., gavage, for 8 or 12 weeks)	Male Sprague–Dawley rats, Alcoholic liver fibrosis	Caffeine	5, 10 and 20 mg/Kg b.wt./day, i.g., for 8 or 12 weeks	2	• All treatments reduced collagen area, protein and mRNA (time- and dose-dependent manner)	Wang et al., 2015
TAA (2×/week, 200 mg/Kg, b.wt., i.p., for 8 weeks)	Male Wistar rats, Cirrhosis	Caffeine	50 mg/Kg b.wt./day, i.g., for 8 weeks.	2	• Reduced collagen levels	Arauz et al., 2014
TAA (2×/week, 200 mL/Kg, b.wt., i.p., for 8 weeks)	Male Sprague-Dawley rats, Cirrhosis	Caffeine	50 mg/Kg b.wt./day, i.g., for 4 weeks.	2	• Reduced fibrosis and inflammation scores	Shim et al., 2013
HFD (for 4 weeks)	Male C57BL/6 J mice, NAFLD	Caffeine	0.5 mg/mL in drinking water, for 4 weeks	3	• Reduced lipid accumulation and liver triglyceride levels	Sinha et al., 2014
HFD (for 16 weeks)	Male Wistar rats, NAFLD	Caffeine	20 or 30 mg/Kg/day, i.g., for 8 weeks	2	• Reduced liver inflammation, lipid accumulation, serum cholesterol and triglyceride levels	Helal et al., 2018
HepG2 xenograft model (single, s.c.)	Male nude mice, Hepatocarcinogenesis	Chlorogenic acid	30, 60 or 120 mg/Kg b.wt., i.p./day for 6 weeks	3	• Reduced xenograft tumor volume and weight	Yan et al., 2017
CCl_4 (2×/week, 4–2 mL/Kg b.wt., 40% solution, i.p., for 8 weeks)	Male Sprague-Dawley rats, Fibrosis	Chlorogenic acid	15, 30 and 60 mg/Kg b.wt./day, i.g., for 4 weeks.	2	• Reduced collagen I area (dose-dependent manner)	Yang et al., 2017
CCl_4 (2×/week, 3 mL/Kg b.wt., 40% solution, i.p., for 8 weeks)	Male Sprague-Dawley rats, Fibrosis	Chlorogenic acid	60 mg/Kg b.wt./day, i.g., for 8 weeks.	2	• Reduced collagen area and hydroxyproline levels	Shi et al., 2016
HFD (for 15 weeks)	Male C57BL/6 J mice, NAFLD	Chlorogenic acid	100 mg/Kg b.wt., i.p., 2×/week for 15 weeks	2	• Reduced lipid accumulation, serum cholesterol and triglyceride levels	Ma, Wang, & Tang, 2015
High cholesterol and HFD (for 16 weeks)	Male C57BL/6 J mice, NAFLD	Trigonelline	50 mg/Kg b.wt., 3×/week, i.g., for 16 weeks	2	• Decreased liver triglycerides and steatosis	Sharma et al., 2018
HFD (for 8 weeks)	Male Sprague–Dawley rats, NAFLD	Trigonelline	40 mg/Kg b.wt./day, i.g., for 8 weeks.	3	• Decreased cholesterol, triglycerides and steatosis	Zhang, Zhang, Zhang, Zhang, & Li, 2015

GST-P = placental glutathione-S-transferase; DEN = diethylnitrosamine; CCl_4 = carbon tetrachloride; TAA = thioacetamide; HFD = high fat diet; NAFLD = non-alcoholic fatty liver disease; b.wt. = body weight; i.g. = intragastric route; i.p. = intraperitoneal route; s.c. = subcutaneous route.

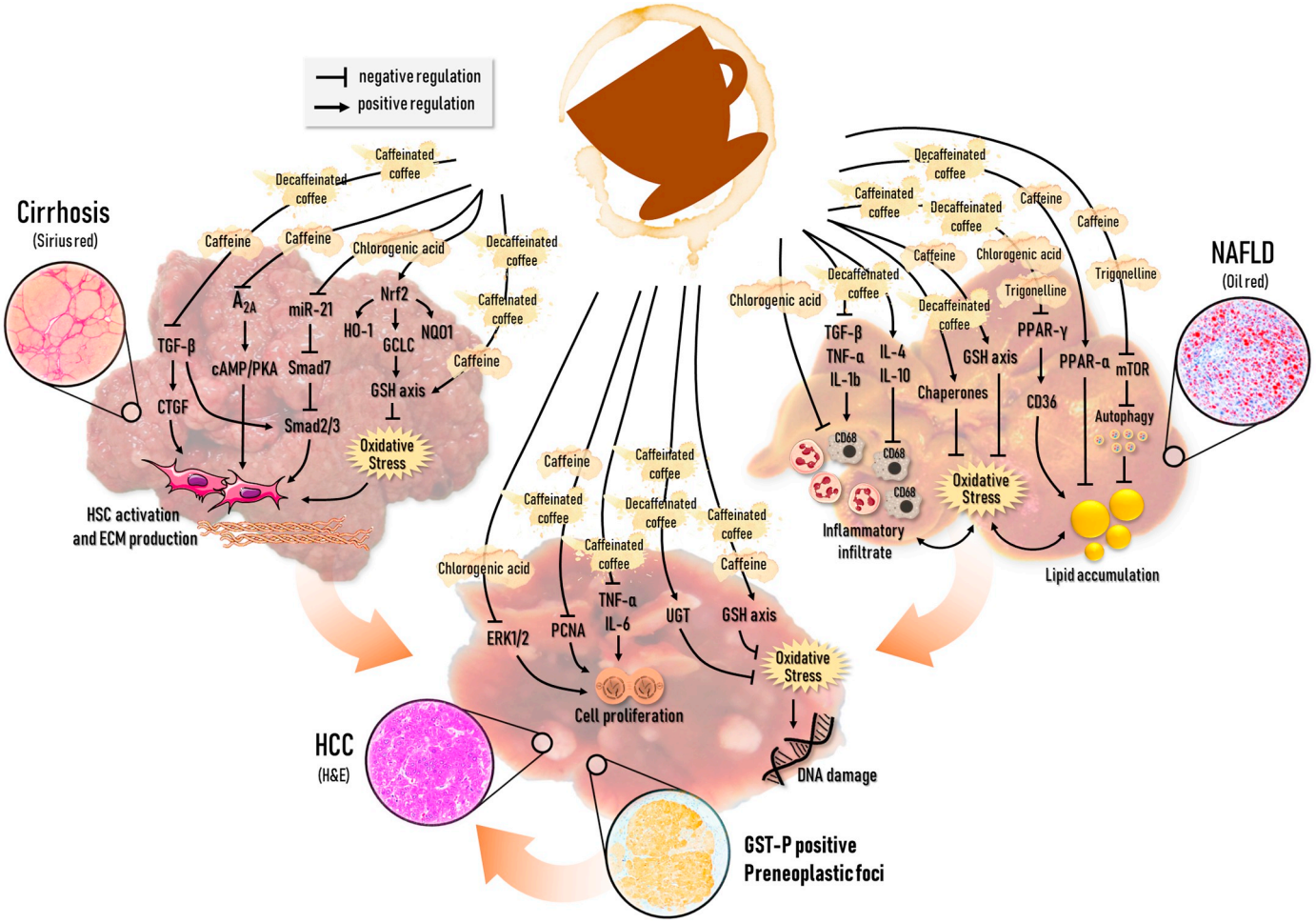

Fig. 5. Main molecular pathways and biological processes modulated by whole caffeinated, decaffeinated coffee brews or highly bioavailable isolated coffee compounds in fibrosis/cirrhosis (left), non-alcoholic fatty liver disease (NAFLD) (right) and hepatocarcinogenesis (down) *in vivo* models. Caffeinated/decaffeinated coffee and highly bioavailable isolated coffee compounds share many molecular targets. Notably, some modulated pathways *in vivo* are also addressed *in vitro*, as summarized in Fig. 4.

(Fig. 5), decaffeinated coffee did not (Furtado et al., 2012). These antioxidant, antigenotoxic and antiproliferative effects, especially exerted by whole coffee, may contribute to lessening GST-P-positive PNL development. However, other underlying mechanisms should be considered as well. Indeed, a single study comparing whole coffee *versus* caffeine reported that only caffeine reduced the size and area of GST-P-positive PNL and the number of neoplastic lesions, (including adenomas and HCC) (Furtado et al., 2014) (Table 5). In the same study, despite not altering antiapoptotic Bcl-2 protein expression, caffeine administration increased protein levels of proapoptotic Bax, whereas common caffeinated coffee did not. In line with these findings, caffeine showed similar results when administered alone, reducing GST-P-positive PNL and PCNA protein levels in the liver (Fujise et al., 2012) (Table 6, Fig. 5). Chlorogenic acid administration alone attenuated the progression of HepG2 xenograft tumors in mice, reducing tumor volume and weight in a dose-dependent manner (Yan et al., 2017) (Table 6). In xenograft tumors, chlorogenic acid reduced ERK1/2 phosphorylation (Fig. 5) and MMP-2/TIMP2 and MMP-9/TIMP2 ratios, which are implicated in sustained cell proliferation and invasion of tumor cells, respectively (Yan et al., 2017). Interestingly, the same effects on the ERK1/2 and MMP-2/TIMP2 pathways were observed when chlorogenic acid was added to the cell culture medium of HepG2 cells (Yan et al., 2017) (Fig. 4A). Regrettably, no studies on the effects of trigonelline treatment in hepatocarcinogenesis in *in vivo* models are available. Altogether, these studies underscore the complexity of understanding the effects of coffee beverages on hepatocarcinogenesis, since beneficial effects are not restricted to whole coffee and/or caffeine alone. Thus, additive or synergistic effects of the most common or highly bioavailable compounds may be considered. As it follows, complex similar responses were also observed in models recapitulating HCC risk conditions.

Featured in 75–80% of human HCC cases (Bray et al., 2018), the fibrotic/cirrhotic background associated to chronic liver disease is recapitulated by well-established experimental rodent models, especially in those induced by chemicals, (e.g. TAA/CCl_4) (Tables 5 and 6). Considering that the profibrotic microenvironment is essential to preneoplastic and neoplastic lesion development, alleviating this feature may indirectly slow down hepatocarcinogenesis. Some studies report that whole coffee and caffeine have stronger effects than decaffeinated coffee on diminishing profibrogenic signaling mediated by transforming growth factor β (TGF-β) and downstream connective tissue growth factor (CTGF), thus reducing HSC activation, fibrotic areas and collagen I mRNA production (Arauz et al., 2017). Furtado et al. (2012, 2014) demonstrated that only whole coffee administration diminished collagen III mRNA expression and total and active MMP-2 levels compared to decaffeinated and/or caffeine alone. In contrast, other authors addressed similar effects of whole coffee, decaffeinated coffee and caffeine on reducing oxidative stress (reducing lipid peroxidation and increasing GSH axis) (Fig. 5), profibrogenic signaling (TGF-β and CTGF) (Fig. 5) and ECM remodeling (MMP-2, 9 and −13) (Arauz et al., 2014;

Arauz, Moreno, Cortés-Reynosa, Salazar, & Muriel, 2013; Furtado et al., 2012). In line with *in vitro* studies with HSC (Fig. 4B), caffeine treatment alone attenuated fibrosis/cirrhosis presumably via interaction with adenosine A_{2A} receptors and reduction of the downstream cAMP/PKA/CREB signaling pathway (Chan et al., 2006; Wang et al., 2015) (Fig. 5). In fact, adenosine A_{2A} receptor plays a central role in the pathogenesis of hepatic fibrosis, since knockout mice ($A_{2A}^{-/-}$) showed a 10-fold decrease in collagen areas during TAA-induced fibrosis (Chan et al., 2006). Due to these effects, some authors pointed caffeine as the main antifibrotic agent in coffee, supporting the "caffeine hypothesis" (Dranoff, 2018). Nonetheless, the effects of chlorogenic acid on the antifibrotic miR-21-regulated Smad7 signaling pathway and antioxidant Nrf2 axis should also be considered for explaining the effects of coffee beverages (caffeinated coffee and, especially, decaffeinated coffee) on liver fibrosis. When administered to rats individually, this polyphenol reduced HSC activation, fibrosis areas, collagen I and III mRNA production and hydroxyproline levels (Shi et al., 2016; Yang et al., 2017). Chlorogenic acid may suppress fibrosis through the attenuation of oxidative stress (MDA levels) in liver tissue, increasing the protein expression of Nrf2 transcription factor, and gene expression of its downstream targets, heme oxygenase-1 (HO-1), NQO1 and GCLC, resulting in augmented GSH levels (Fig. 5), superoxide dismutase and catalase activities (Shi et al., 2016). Furthermore, chlorogenic acid treatment downregulates miR-21 expression in liver, subsequently increasing the mRNA and protein expression of Smad7, a direct miR-21 target (Yang et al., 2017) (Fig. 5). Smad7 upregulation was implicated in decreasing profibrotic Smad2 and Smad3 signaling in rat liver (Yang et al., 2017). Of note, the modulation of the miR-21/Smad7 axis by chlorogenic acid was also observed in HSC *in vitro* (Yang et al., 2017) (Fig. 4B).

Being a growing risk factor for human HCC in high-income countries, experimentally-induced NAFLD was found to be alleviated by whole and decaffeinated coffee interventions, as well as caffeine, chlorogenic acid and trigonelline individually (Tables 5 and 6). A limited number of studies compared the effects of different types of coffee in dietary-induced NAFLD models (Table 5). Shokouh et al. (2018) showed that common whole coffee treatment reduced both plasma and liver triglycerides levels as well as steatosis scores in the liver, while a combination of chlorogenic acid, trigonelline, and caffeic acid did not (Table 5). The molecular events underlying this pronounced response in common coffee treatment are still to be unveiled. On the other hand, another study showed that both caffeinated and decaffeinated coffee regimens triggered strikingly similar responses in reducing the expression of lipid metabolism-related genes, especially PPAR-γ and PPAR-γ-regulated genes, such as CD36, which positively correlated with fatty acid uptake in the liver (Takahashi et al., 2014) (Fig. 5).

Vitaglione et al. (2010) compared decaffeinated coffee treatment with combinations of polyphenols or melanoidins individually (Table 5). In general, all treatments showed similar responses in reducing liver fat accumulation through increased PPAR-α mRNA and protein expression, which is responsible for lipid β-oxidation and clearance, decreased oxidative stress (decreased MDA levels and increased GSH axis) and inflammation (increased levels of IL-4 and IL-10 and decreased mRNA and protein of TNF-α and TGF-β) (Fig. 5). Decaffeinated coffee treatment individually, presumably containing chlorogenic acid and trigonelline, also counteracted NAFLD development in high fat diet (HFD)-fed rats (Brandt et al., 2019; Salomone et al., 2014; Takahashi et al., 2014) (Table 5). Salomone et al. (2014) proposed that these protective effects may be attributed to the reduction of lipid peroxidation and DNA oxidative damage through the upregulation of antioxidant agents (peroxiredoxin 1, glutathione S-transferase α2, and D-dopachrome tautomerase) and chaperones that maintain endoplasmic reticulum or mitochondrial homeostasis (GRP78, PDI-A3, mtHSP70, and DJ-1) (Fig. 5). Decaffeinated coffee reduced the protein expression of electron transfer flavoprotein subunit α, which is part of the mitochondrial respiratory chain and directly relates to *de novo* lipogenesis (Salomone et al., 2014). Alterations in the intestinal barrier permeability with subsequent translocation of bacterial endotoxins to the liver are proposed to trigger inflammatory responses and insulin resistance in the liver, contributing to the multifactorial development of NAFLD (Miele et al., 2009). Decaffeinated coffee treatment is also suggested to attenuate NAFLD development by maintaining small intestine barrier integrity, reducing endotoxin translocation to the liver and subsequently decreasing proinflammatory response (reduced granulocyte infiltration and IL-1b mRNA) and insulin resistance (decreased insulin receptor mRNA) (Brandt et al., 2019).

The administration of the most common and highly bioavailable coffee compounds alone unraveled both distinct (autophagy induction) and similar (PPAR-γ axis downregulation) mechanisms implicated in experimental NAFLD attenuation compared to whole or decaffeinated coffee approaches. Caffeine treatment alone enhanced lipids autophagy by abrogating mTOR negative regulation (enhanced LC3-II protein and lipase A gene expression) (Fig. 5), which was followed by β-oxidation of fatty acids (increased carnitine palmitoyltransferase I α gene expression and ketones and ATP levels) that culminated in lipid clearance and NAFLD attenuation (Sinha et al., 2014). Interestingly, trigonelline alone showed similar results as caffeine in inducing liver autophagy by the modulation of the mTOR pathway (Sharma et al., 2018) (Fig. 5). In addition to autophagy, caffeine also reduced oxidative stress (reduced malondialdehyde, nitrogen oxide and increased GSH levels) and *de novo* lipogenesis (fatty acid synthase and acetyl CoA carboxylase genes), while increasing lipid β-oxidation (gene expression of PPAR-α) (Helal, Ayoub, Elkashefand, & Ibrahim, 2018) (Fig. 5). Similarly to whole caffeinated or decaffeinated coffee regimens, chlorogenic acid alone reduced proinflammatory responses by downregulating macrophage-related genes and decreasing PPAR-γ axis (Ma, Gao, & Liu, 2015) (Fig. 5). The downregulation of PPAR-γ pathway was also featured when trigonelline was individually administered to high-fat diet-fed rats (Sharma et al., 2018) (Fig. 5).

7. Conclusions and future perspectives

Taken together, epidemiological (Tables 1–3), *in vitro* (Figs. 3 and 4) and *in vivo* (Tables 4–6, and Fig. 5) findings predominantly address protective effects of coffee beverages and of its most common and bioavailable individual compounds in gastrointestinal and liver cancer development. Some mechanistic glimpses on their antiproliferative, antioxidant, proapoptotic and antifibrotic actions are also described, mostly *in vitro* (Figs. 3 and 4). It is also noteworthy mentioning that caffeine, chlorogenic acid, and trigonelline, when individually administered, modulate common molecular targets directly implicated in key cancer hallmarks, suggesting that the combination of coffee compounds (as seen in whole coffee beverages), may account for the beneficial effects of coffee consumption. It should be stressed that *in vivo* studies are sometimes mechanistically limited. Hopefully, translational approaches using different preclinical rodent models, such as genetically modified and patient-derived xenograft (PDX), will overcome these limitations. As far as *in vitro* approaches are concerned, caution should be taken in data interpretation, as supraphysiological and non-translatable concentrations are often used. Therefore, more physiologically relevant administration based on coffee compound metabolism and bioavailability should be considered in these studies. Considering that naturally-occurring polyphenols (as chlorogenic acid) and alkaloids (as trigonelline and caffeine) are proposed to target epigenetic processes (Bishayee & Bhatia, 2018), the modulation of the epigenetic machinery by coffee or selected coffee compounds, including DNA methylation, histone modifications and non-coding RNAs, should be definitely investigated in further experiments on gastrointestinal and liver carcinogenesis.

In general, the consumption of common and/or espresso coffee brews is considered a safe and popular dietary habit, especially among

adult and older people. Moreover, coffee bean production and consumption displayed remarkable 70% and 160% increases in the past 3 decades (1990–2018) (International Coffee Organization, 2018), respectively, eliciting the need and relevance of future research on the beneficial effects of coffee on cancer development. On the other hand, some concern on the presence of heat-derived contaminants in coffee beverages (polycyclic aromatic hydrocarbons, furan, acrylamide), as well as the potential risk of hot-beverage intake on esophageal cancer, have been recently raised (Loomis et al., 2016). Nonetheless, according to the International Agency for Research on Cancer (IARC) report, there is lack of evidence for carcinogenicity in humans, and along with experimental data, coffee intake was classified into group 3 (not classifiable as to its carcinogenicity to humans) (Loomis et al., 2016). Although coffee consumption can be incorporated as a healthy habit for most of the adult population, side effects of coffee consumption should be considered in potential "sensitive" populational subgroups. Poole et al. (2017) described harmful associations between high coffee ingestion and pregnancy outcomes (increased risk of low birth weight, pregnancy loss, and 1st and 3rd semester preterm birth). Indeed, EFSA (2015) indicates that caffeine consumption in pregnant women should be limited to 200 mg/day (corresponding to 1–2 cups of common or espresso brews), half the habitual caffeine consumption (400 mg/day). Other health consequences should be considered, since coffee consumption is proposed to cause sleep disruption (Clark & Landolt, 2017) and to slightly increase peripheral arterial stiffness (Echeverri, Pizano, Montes, & Forcada, 2017).

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Declaration of competing interests

The authors have no conflict of interest to report.

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