

Potential of Catechins in the Inhibition of Hepatocellular Carcinoma Progression: A Systematic Review and Meta-Analysis of *In Vivo* Studies

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death, and effective therapeutic options remain limited. Catechins, particularly epigallocatechin gallate (EGCG), possess anticancer activity with potential to inhibit HCC progression through multiple molecular mechanisms, but preclinical evidence has not been systematically synthesized. **Objective:** To analyze the potential of catechins to inhibit HCC progression through molecular mechanisms, histopathological changes, and macroscopic and clinical outcomes in animal models of HCC. **Methods:** This systematic review followed PRISMA 2020 (PROSPERO CRD420251064844). PubMed, Sage, Scopus, Springer, and Web of Science were searched through September 2025. Reporting quality and risk of bias were assessed with ARRIVE Guidelines 2.0 and SYRCLE's Risk of Bias Tool. Data were synthesized narratively and by meta-analysis in Review Manager 5.4.1. **Results:** Of 1587 records identified, 18 *in vivo* studies (all testing EGCG) were included, and 13 were meta-analyzed. EGCG decreased proliferation biomarkers, increased cell death biomarkers, inhibited metastasis, and improved histopathological findings. Meta-analysis showed that EGCG significantly reduced tumor volume (MD=-313.28 mm³, 95%CI: [-616.75, -9.82], p=0.04) and relative liver weight (MD=-1.22 g/100 g, 95%CI: [-1.81, -0.62], p<0.0001), and improved clinical outcome, comprising increased survival rate (RR=3.68, 95%CI: [1.50, 9.04], p=0.004), in animal models of HCC. **Conclusion:** EGCG, the only catechin evaluated in the included studies, shows potential to inhibit HCC progression in animal models through consistent effects on molecular biomarkers, histopathological findings, and macroscopic and clinical outcomes.

Keywords: catechins; epigallocatechin gallate (EGCG); hepatocellular carcinoma; cancer; non-communicable diseases

INTRODUCTION

Liver and intrahepatic bile duct cancer ranks seventh worldwide in incidence and third in cancer-related mortality [1]. Hepatocellular carcinoma (HCC) accounts for approximately 80% of primary liver cancers and has a poor prognosis, with a 5-year survival rate of approximately 18% [2,3]. HCC development is a multistep process commonly associated with liver cirrhosis and influenced by the underlying causes of liver disease [4]. Hepatitis B and C remain the dominant global etiologies, but their incidence is projected to decline as metabolic

dysfunction-associated fatty liver disease (MAFLD) emerges as a key driver [5,6].

The molecular pathogenesis of HCC is highly heterogeneous, involving chronic inflammation, genomic instability, and dysregulation of the MAPK/ERK, PI3K/Akt/mTOR, Wnt/ β -catenin, JAK/STAT, and Hedgehog pathways, which collectively drive proliferation, apoptosis resistance, and metastasis [7–9]. The lack of consensus on optimal combination therapies, sequencing, and timing, alongside the adverse effects of conventional

treatments, highlights the need for additional therapeutic strategies, including traditional herbal medicines as potential sources of anticancer agents [10,11].

The principal catechins in *Camellia sinensis* are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), of which EGCG is the most potent anti-inflammatory and anticancer congener [12,13]. Catechins exhibit anticancer activities through anti-inflammatory, antiproliferative, antimetastatic, antioxidant, pro-oxidant, antiangiogenic, and DNA methylation-inhibitory mechanisms [13]. Green tea polyphenols, particularly EGCG, show potential as HCC adjuvants by inhibiting tumor growth and inducing cancer cell death while sparing healthy cells through autophagy modulation and antiangiogenic effects [14]. Individual animal studies suggest that EGCG reduces HCC nodule formation, lowers alpha-fetoprotein, attenuates fibrosis, and prolongs survival [15,16]. However, this preclinical evidence remains fragmented, with substantial variation in dosing, animal models, and treatment duration. Systematic review and meta-analysis of preclinical data are essential to bridge the translational gap between bench research and clinical trial design [17].

This study presents a systematic review and meta-analysis evaluating the potential of catechins to inhibit HCC progression in animal models, encompassing molecular biomarkers, histopathology, and macroscopic and clinical outcome.

METHODS

Study Design and Protocol Registration

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD420251064844 [18,19].

Eligibility Criteria

Eligibility followed the PICO framework: (P) *in vivo* animal models of HCC induced chemically, dietarily, or by tumor implantation; (I) any purified green tea catechin (EC, ECG, EGC, or EGCG) by any route; (C) placebo, vehicle, or no treatment; (O) biomarkers of proliferation, cell death, or metastasis; macroscopic outcomes (tumor volume and relative liver weight), histopathological findings; or clinical outcome of survival rate. Exclusion criteria consisted of: (1) human studies; (2) *in vitro*, *ex vivo*, or *in silico* studies; (3) review articles, case reports, letters, and comments; (4) duplicate publications; (5) incomplete datasets or non-disclosed data; (6) unretrievable full texts; (7) ongoing studies; and (8) non-English articles.

Search Strategy

Three reviewers (NAA, NNN, and AII) independently searched PubMed, Scopus, Sage, Springer, and Web of Science from inception to September 2025 using combinations of terms “catechin”, “epicatechin”,

“epicatechin gallate”, “epigallocatechin”, “epigallocatechin gallate”, “EC”, “ECG”, “EGC” or “EGCG” with terms for hepatocellular carcinoma (“hepatocellular carcinoma”, “hepatoma”, “liver cell carcinoma”, or “HCC”), together with Medical Subject Headings (MeSH) terms, without year restriction. Records were managed in Rayyan.ai for deduplication and title/abstract screening, then continued to full text screening, with disagreements resolved by consensus.

Data Extraction

Extracted data comprised first author and year, country, study design, animal characteristics, HCC induction method, catechin type, route, dose and duration, control type, sample size, and outcomes. Graphically presented data were digitized using WebPlotDigitizer [20].

Reporting Quality and Risk of Bias Assessment

Reporting quality and risk of bias were assessed with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) Guidelines 2.0 and the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias Tool [21,22]. ARRIVE items were scored 2 (fully reported), 1 (partially reported), or 0 (not reported), summed and normalized over 42 points; coefficients of 0.8–1.0, 0.5–0.8, and <0.5 denoted high, moderate, and low quality, respectively [23,24]. SYRCLE's tool appraised selection, performance, detection, attrition, reporting, and other biases across ten domains with additional signaling questions: “yes” indicates a low risk of bias, “no” indicates a high risk of bias, and “unclear” indicates an unclear risk of bias. If any relevant signaling question is answered with “no,” this indicates a high risk of bias for that specific entry [22].

Statistical Analysis

Meta-analyses were performed in Review Manager 5.4.1 (Cochrane Collaboration). Mean differences (MD) were used for continuous outcomes and risk ratios (RR) for the clinical outcome, all with 95% confidence intervals. Heterogeneity was quantified with Higgins' I^2 (negligible 0–25%, low 25–50%, moderate 50–75%, high >75%). A fixed-effect model was used when $I^2 < 50\%$ and a random-effects model otherwise. For high heterogeneity, sensitivity analyses excluding studies with extreme effect estimates were performed. Standard errors were converted to standard deviations ($SD = SE \times \sqrt{N}$) [25].

RESULTS AND DISCUSSION

Study Selection

The search retrieved 1587 records (PubMed 246, Sage 45, Scopus 155, Springer 461, Web of Science 680). After removal of 327 duplicates, 1260 titles and abstracts were screened and 77 reports were sought for retrieval, of which one could not be retrieved. Seventy-six full-text reports were assessed for eligibility, and 58 were excluded (wrong population, $n=16$; wrong intervention, $n=28$; different outcome, $n=9$; wrong study model, $n=4$; review article, $n=1$). Eighteen studies were included in the qualitative synthesis, of which 13 provided sufficient data for meta-analysis (Figure 1).

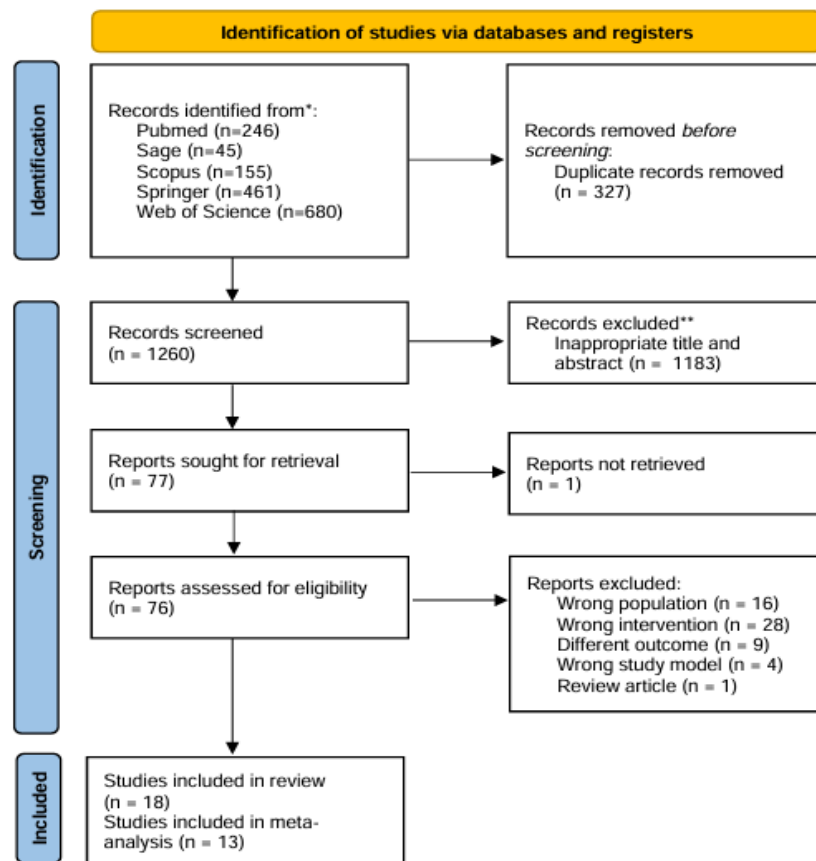


FIGURE 1: PRISMA 2020 flow diagram of literature selection.

Reporting Quality and Risk of Bias Assessment

ARRIVE 2.0 appraisal classified 14 studies as moderate quality (0.5–0.8) and 4 as low quality (<0.5); no study met the high-quality threshold (Figure 2). Reporting was strong for study design, outcome measures, objectives, and generalisability, but weak for sample size, inclusion and exclusion criteria, randomization, blinding, experimental animal, housing and husbandry, animal care and monitoring, protocol registration, data access, and declaration of interest.

SYRCLE assessment (Figure 3) found sequence generation, allocation concealment, random housing, performance bias, and random outcome assessment as unclear risk of bias because of sparse reporting, whereas baseline characteristics (72.22%), incomplete outcome data (66.67%), selective outcome reporting (100%), and other sources of bias (83.33%) were rated low risk (Figure 3). Blinding of outcome assessors was rated low risk in only 16.67% of studies and unclear in the remainder. No domain in either tool was rated uniformly high risk, the dominant limitation was incomplete methodological reporting rather than confirmed bias, and all studies were therefore retained in the synthesis.

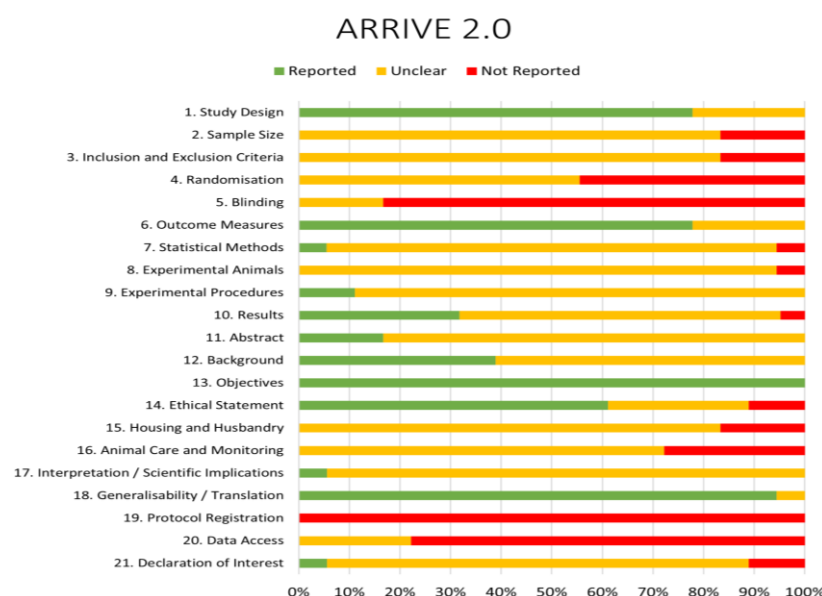


FIGURE 2: Reporting quality assessment using ARRIVE Guidelines 2.0.

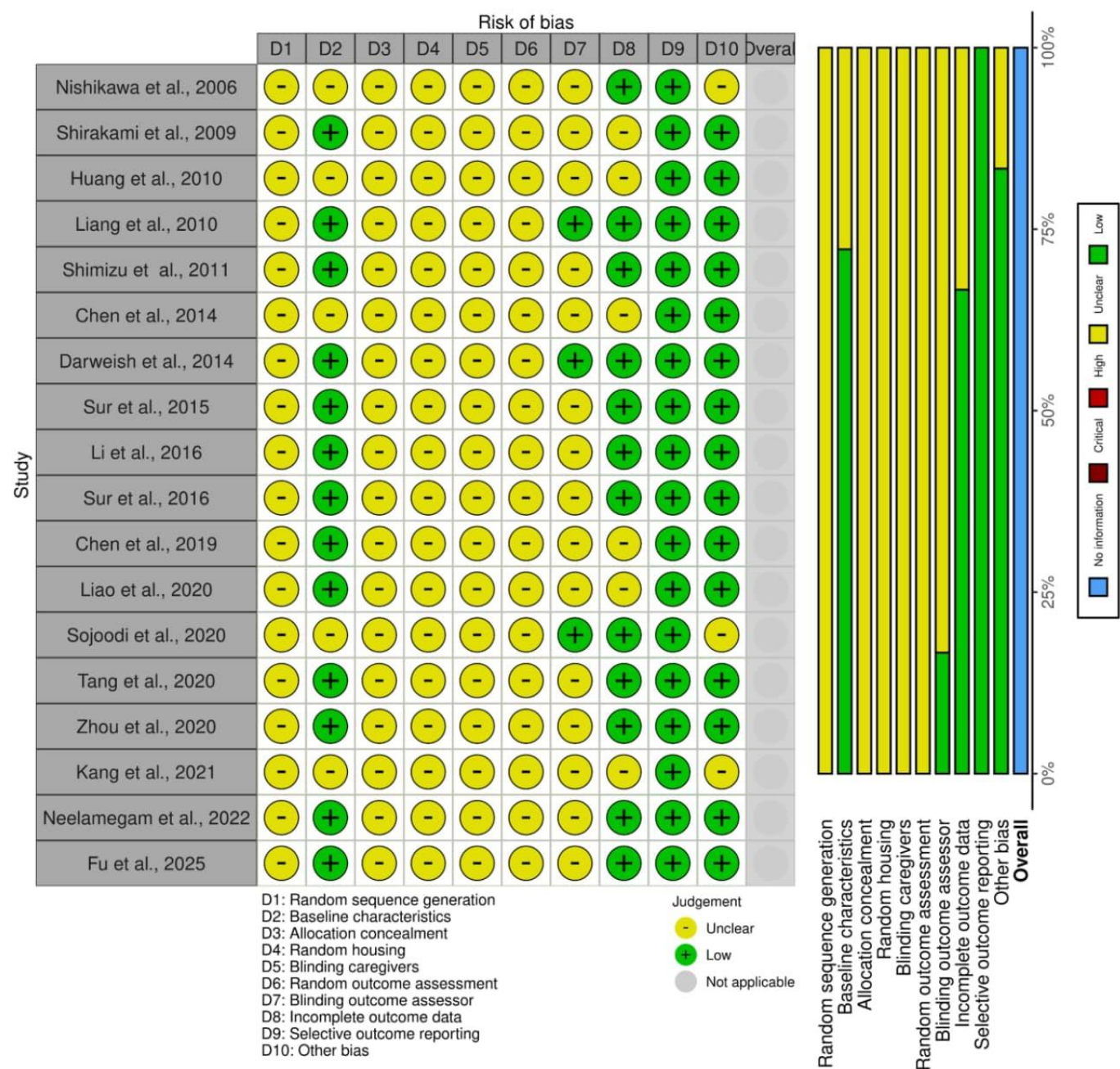


FIGURE 3: Risk of Bias assessment using SYRCLE’s Risk of Bias Tool.

Study Characteristics and Main Findings

The 18 included studies (2006–2025) predominantly originated from Asia (Japan, China, India), with single studies from Egypt and the USA (Table 1). Nude mice were the most frequently used animals, and most studies used males. Only EGCG was evaluated in eligible studies, although the search

included EC, ECG, and EGC as well. Administration was oral, intragastric, or intraperitoneal, under preventive, simultaneous, or therapeutic protocols. HCC was induced by xenograft implantation, the most common model, or by the chemical carcinogens diethylnitrosamine (DEN), carbon tetrachloride (CCl₄), and thioacetamide (TAA).

TABLE 1: Characteristics and main findings of the included studies.

Study	Animal model	HCC model; comparison	EGCG intervention	Main findings
Nishikawa <i>et al.</i> , 2006 (Japan) [26]	Nude mice (BALB/c Slc-nu/nu), 8 wk, male; n = 5/dose group	HLE xenograft; drinking water	0.8, 2.5, 7.5 mg/mL in drinking water; oral; ad libitum; therapeutic	↑ Apoptosis (TUNEL); ↓ Bcl-2 and Bcl-xL (p NR). Reduced tumor volume.
Shirakami <i>et al.</i> , 2009 (Japan) [27]	Nude mice (BALB/c), 5 wk, male; n = 8/dose group	HuH7 xenograft; drinking water	0.01%, 0.1% in drinking water; oral; 3x/wk; therapeutic	↓ p-ERK/ERK, p-Akt/Akt (p NR); ↓ Bcl-xL (p NR); ↓ p-VEGFR-2 (p NR); ↓ VEGF mRNA (p<0.05, 0.1% dose). Reduced tumor volume.
Huang <i>et al.</i> , 2010 (China) [28]	NR; n = 8 or 9/group	SMMC7721 and Hep3B xenografts; no treatment	NR	Reduced tumor volume. Attenuated doxorubicin-associated cardiotoxicity (↓ oxidative stress, improved cTnT).
Liang <i>et al.</i> , 2010 (China) [29]	BALB/c nu/nu mice, 4–5 wk, male/female; n = 10/group	BEL-7404/DOX xenograft; PBS injection	80 mg/kg/day; intragastric; 33 days; therapeutic	↓ HIF-1α mRNA (NS). EGCG alone did not reduce tumor volume; EGCG + doxorubicin outperformed doxorubicin alone. ↓ P-gp and MDR1 mRNA (p<0.01 and p<0.05), ↑ intracellular doxorubicin.
Shimizu <i>et al.</i> , 2011 (Japan) [30]	db/db mice, 4 wk, male; n = 10/group	DEN; no treatment	0.1% in drinking water; oral; ad libitum; 34 wk; therapeutic	↓ p-ERK, p-Akt, p-GSK-3β, p-STAT3, p-JNK, IGF-1/2, IGF-1R, ↑ p-AMPK. Fewer foci of cellular alteration (14.9 ± 4.2 vs 7.7 ± 3.0/cm ² , p<0.001). Relative liver weight not significantly different.
Chen <i>et al.</i> , 2014 (China) [31]	Nude mice, 4–6 wk, male; n = 6/group	Hep3B xenograft; no treatment	50 mg/kg; intragastric; 15 days; therapeutic	TUNEL apoptosis change NS. Reduced tumor volume.
Darweish <i>et al.</i> , 2014 (Egypt) [15]	Sprague–Dawley rats, male; n = 10/group	TAA; no treatment	20 mg/kg; IP; 16 wk (2x/wk); simultaneous	↓ Caspase-3 (NS); ↓ FGF-2 (p NR), MMP-9, syndecan-1 (p<0.05); ↑ HSPG (p<0.05). Improved lobular architecture, less inflammation and fibrosis. Survival rate 30% → 80%. ↓ AFP (p<0.05).
Sur <i>et al.</i> , 2015 (India) [32]	Swiss albino mice, 5–6 wk, female; n = 12/group	CCl ₄ + DEN; no treatment	0.008 mg/kg; oral; preventive, simultaneous, or therapeutic schedules	↓ β-catenin, Gli1, SMO, BrdU+, PCNA, c-Myc, Cyclin D1, EGFR, ↑ sFRP1, APC, PTCH1 (p<0.05 to p<0.001); ↑ apoptosis (TUNEL) (p<0.05); ↑ E-cadherin mRNA (p<0.001), ↓ CD44 (p NR). Slowed dysplasia progression, no progression to HCC. ↓ AFP (p<0.001).
Li <i>et al.</i> , 2016 (China) [33]	BALB/c nu/nu athymic mice, 4 wk, male; n = NR	HCC-LM3 xenograft; 95% ethanol and water	10 mg/kg/day; IP; 30 days; therapeutic	↑ Apoptosis (TUNEL), p<0.05.
Sur <i>et al.</i> , 2016 (India) [34]	Swiss albino mice, 5–6 wk, female; n = 12/group	DEN; no treatment	0.008 mg/kg; oral; preventive, simultaneous, or therapeutic schedules	↓ β-catenin, Cyclin D1, Gli1, SMO, c-Myc, EGFR, BrdU+, ↑ sFRP1, APC (all p<0.05); ↑ apoptosis (TUNEL) (p<0.05); ↑ E-cadherin, ↓ CD44 (p<0.05). Slowed dysplasia progression, no progression to HCC.
Chen <i>et al.</i> , 2019 (China) [35]	Mice, 7 wk, female; n = 5/dose group	Hep3B xenograft; sterile water	2, 10, 50 mg/kg; IP; every 2 days; therapeutic	Dose-dependent tumor necrosis.
Liao <i>et al.</i> , 2020 (China) [36]	BALB/c nude mice, 4–6 wk, male/female; n = 10/group	HepG2 xenograft; no treatment	40 mg/kg/day; intragastric; 20 days; therapeutic	↓ p-ERK1/2 (p<0.05), ↓ p-AKT (NS); ↓ MVD/CD34 and VEGF (p<0.01), ↓ HIF-1α (p<0.05). Reduced tumor volume.

Study	Animal model	HCC model; comparison	EGCG intervention	Main findings
Sojoodi <i>et al.</i> , 2020 (USA) [16]	Wistar rats, male; n = NR	DEN; no treatment	25 mg/kg/day; oral; 6 wk; simultaneous	↓ PCNA; ↑ SA-β-gal+ cells with HSC inactivation (p<0.001); ↓ α-SMA (p<0.01). Collagen type I 15.78 ± 1.326 vs 2.64 ± 0.40 (p<0.001); Ishak score 5.8 vs 4.4 (p<0.05). Relative liver weight reduced (p<0.05).
Tang <i>et al.</i> , 2020 (China) [37]	Sprague–Dawley rats, 4 wk, male; n = 15 control, 10 EGCG	DEN; saline	25 mg/kg/day; intragastric; 10, 20, or 30 wk; simultaneous	↓ CDC25A (p<0.01), ↑ p21/Waf1/Cip1 (p<0.05). Survival rate 6.67% → 50%.
Zhou <i>et al.</i> , 2020 (China) [38]	BALB/c mice, 4–6 wk, male/female; n=8/group	HepG2 xenograft; CMC-Na	40 mg/kg/day; intragastric; 21 days, therapeutic	↓ p-ERK1/2, p-AKT (p NR); ↓ HIF-1α mRNA (p<0.001) and protein (p<0.05). Reduced tumor volume. ↓ CBR1 mRNA (p<0.001).
Kang <i>et al.</i> , 2021 (China) [39]	BALB/c nude mice, 4–6 wk, female; n = 5/dose group	miR-483-3p-overexpressing HepG2 xenograft; EGCG 0%	0.1%, 0.5% in drinking water; oral; ad libitum; 2 wk before to 2 mo after cell injection; simultaneous	Inhibited lung metastasis, about 2-fold stronger at 0.5% (p<0.01) than 0.1% (p<0.05).
Neelamegam <i>et al.</i> , 2022 (India) [40]	Wistar albino rats, 12 wk, male; n=8/group	DEN; no treatment	100 mg/kg; route and duration NR; NR	Reduced hepatocyte degeneration and hyperchromatism. Relative liver weight reduced (p<0.01). ↓ AFP (p<0.001).
Fu <i>et al.</i> , 2025 [41]	BALB/c nude mice, 5–6 wk, male; n=6/group	HepG2 xenograft; no treatment	10 mg/kg/day; IP; 21 days; therapeutic	↓ ATP7B and MTF1 (pNR), increasing sensitivity to cuproptosis. Reduced tumor volume

NR, not reported; NS, not significant; n, number of animals; wk, weeks; mo, months; IP, intraperitoneal; DEN, diethylnitrosamine; CCl₄, carbon tetrachloride; TAA, thioacetamide; ↑, increased; ↓, decreased. Values given as “a vs b” are control vs EGCG.

Effect on Molecular Biomarkers

Biomarker reporting varied widely and few studies reported quantitative data, which precluded meta-analysis of molecular outcomes.

Antiproliferative effects

EGCG generally inhibited tumor cell proliferation, induced cell death, and suppressed metastasis in *in vivo* HCC models. At the molecular level, EGCG decreased phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), Akt, and glycogen synthase kinase-3 β (GSK-3 β), and downregulated signal transducer and activator of transcription 3 (STAT3), c-Jun N-terminal kinase (JNK), and the insulin-like growth factor (IGF)-1/IGF-1 receptor (IGF-1R) axis [27,30,36,38]. These findings indicate that the antiproliferative activity of EGCG involves modulation of signaling pathways regulating tumor growth, cell-cycle progression, and cell survival. Reduced IGF-1, IGF-2, IGF-1R, and epidermal growth factor receptor (EGFR) expression and reduced ERK and Akt phosphorylation [30,32,34] suggest inhibition of the RAS/RAF/MEK/ERK and PI3K/Akt/mTOR pathways [9]. In obesity-associated HCC, EGCG also suppresses STAT3, JNK, GSK-3 β , and IGF-1/IGF-1R signaling, while increased AMP-activated protein kinase (AMPK) activation may inhibit mTOR-mediated protein synthesis and tumor growth [30,42].

This inhibition was accompanied by reduced proliferating cell nuclear antigen (PCNA), Cyclin D1, c-Myc, cell division cycle 25A (CDC25A), and the bromodeoxyuridine-positive (BrdU+) proliferative index, alongside upregulation of the cyclin-dependent kinase inhibitor p21, indicating cell-cycle arrest [16,32,34,37]. Cyclin D1 and CDC25A participate in the activation of cyclin-dependent kinases, and c-Myc is a transcription factor regulating cell growth and proliferation [43,44], whereas p21 reinforces cell-cycle arrest by inhibiting cyclin-dependent kinase complexes [45]. Reduced fibroblast growth factor-2 (FGF-2) was also reported [15]. EGCG also modulates the Wnt/ β -catenin and Hedgehog pathways by decreasing β -catenin, Gli1, and smoothened (SMO) while enhancing secreted frizzled-related protein 1 (sFRP1), adenomatous polyposis coli (APC), and patched 1 (PTCH1) expression [32,34]. The Wnt/ β -catenin pathway is aberrantly activated in many HCC cases, promoting proliferation, differentiation, and cancer stem cell maintenance, while Hedgehog signaling supports tumor cell proliferation and survival [46,47].

Induction of Cell Death

Apoptosis induction was supported by increased terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)-positive indices [26,32–34] and downregulation of the anti-apoptotic proteins Bcl-2 and Bcl-xL, supporting activation of the intrinsic mitochondrial apoptotic pathway [26,27]. However, the evidence is not uniform. Chen *et al.* [31] found no change in TUNEL-positive cells with EGCG alone, but apoptosis increased significantly when EGCG was combined with doxorubicin ($p < 0.01$), suggesting that EGCG may act more

effectively as a chemosensitizer than as monotherapy. Likewise, the reduction in caspase-3 activity reported by Darweish *et al.* [15] was not significant, which may be attributed to the presence of normal hepatic tissue adjacent to the tumor and the limited bioavailability of EGCG resulting from its absorption, metabolism, and excretion. EGCG also induced cellular senescence, marked by elevated senescence-associated β -galactosidase (SA- β -gal) staining with hepatic stellate cell (HSC) inactivation, which may limit proliferation, fibrosis, and modulate the tumor microenvironment [16,48,49]. In addition, EGCG increased the sensitivity of HCC cells to cuproptosis through downregulation of ATP7B and MTF1, potentially promoting intracellular copper accumulation [41].

Antimetastatic Effects

For metastasis, EGCG restored cell-cell adhesion by upregulating E-cadherin and decreased α -smooth muscle actin (α -SMA), CD44, and matrix metalloproteinase-9 (MMP-9) [15,16,32,34]. These changes suggest suppression of epithelial-mesenchymal transition (EMT), HSC activation, and tumor-cell migration and invasion [50]. Reduced CD44 expression suggests diminished HCC cell migration and invasion, as CD44 is a cancer stem cell marker involved in tumor initiation, progression, and the regulation of MMP-2 and MMP-9 expression [51]. EGCG may further limit extracellular matrix degradation through MMP-9 inhibition and modulation of syndecan-1 and heparan sulfate proteoglycan (HSPG) [15]. EGCG also inhibited lung metastasis of miR-483-3p-overexpressing xenografts in a dose-dependent manner [39]. Angiogenesis was blunted, as shown by downregulation of vascular endothelial growth factor (VEGF), VEGF receptor-2 (VEGFR-2), phosphorylated VEGFR-2, and hypoxia-inducible factor-1 α (HIF-1 α), and reduced microvessel density (MVD/CD34) [15,27,29,36,38]. This indicates inhibition of tumor angiogenesis, potentially restricting the oxygen and nutrient supply required for HCC growth and dissemination [52].

Histopathological Findings

Histopathological improvements paralleled the molecular and macroscopic findings, including restoration of liver architecture, reduced fibrosis, inhibition of pre-cancerous lesion progression, and increased tumor necrosis. These findings complement the molecular biomarker data, suggesting that the antiproliferative, pro-cell-death, and antimetastatic effects of EGCG are reflected in morphological changes within liver tissue. Antifibrotic effects were demonstrated by Sojoodi *et al.* [16], who reported reduced collagen type I deposition and lower Ishak fibrosis scores. The decrease in Ishak score indicates reduced fibrosis severity, a major risk factor for HCC in humans [53]. Fibrosis also contributes to the tumor microenvironment through altered cell-matrix and cell-cell interactions, promoting hepatocarcinogenesis, metastasis, and immune evasion [54]. Thus, EGCG may both restore liver structure and mitigate a microenvironment conducive to tumor progression.

Darweish *et al.* [15] reported improved lobular architecture with less inflammatory infiltration and tissue damage, whereas Neelamegam *et al.* [40] reported decreased hepatocyte degeneration and hyperchromatism, indicating preserved hepatocyte morphology. In pre-neoplastic lesions, Shimizu *et al.* [30] reported fewer foci of cellular alteration (FCA). Sur *et al.* [32,34] found that EGCG slowed dysplasia progression in the preventive group and maintained or improved dysplasia grades in the simultaneous and therapeutic groups, with no progression to HCC in EGCG-treated groups. In a xenograft model, Chen *et al.* [35] reported dose-dependent tumor necrosis, demonstrating a dose-response relationship and direct tumor tissue damage, consistent with the pro-cell-death activity of EGCG.

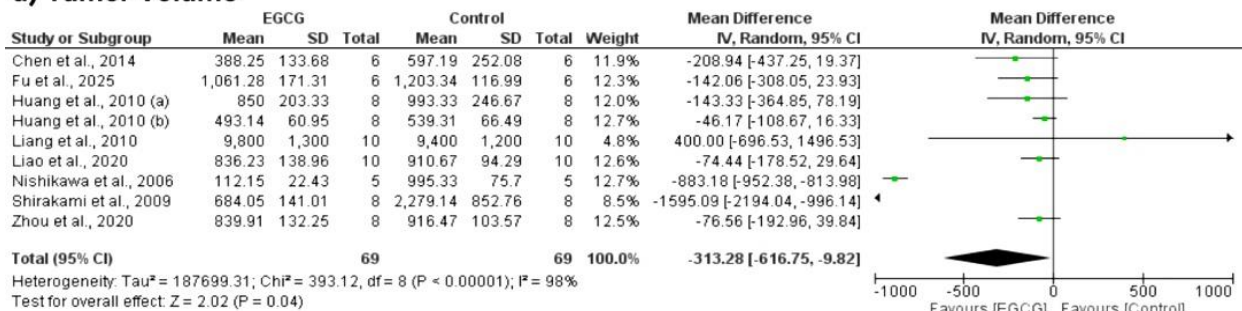
Macroscopic Outcomes

The macroscopic outcomes analyzed to assess the inhibitory effects of catechins on HCC progression were tumor volume and relative liver weight.

Tumor Volume

Eight xenograft studies reporting tumor volume (mm^3) were meta-analyzed [26–29,31,36,38,41] (nine comparisons, as Huang *et al.* [28] reported two xenograft models). Tang *et al.* [37] was excluded because of its markedly longer duration (30 weeks vs 15–33 days) and chemically induced model. For studies with multiple dose groups [26,27], the highest dose was used. EGCG significantly reduced tumor volume overall (MD=−313.28 mm^3 , 95%CI:[−616.75, −9.82], $p=0.04$; $I^2=98\%$; Figure 4a). Because Nishikawa *et al.* [26] and Shirakami *et al.* [27] showed disproportionately large effects, a sensitivity analysis excluding both eliminated heterogeneity ($I^2=0\%$) while preserving significance (MD=−72.36, 95%CI:[−117.14, −27.59], $p=0.002$; Figure 4b), indicating that these two studies inflated heterogeneity without reversing the direction of effect. A further sensitivity analysis using the upper bound of the sample size range reported by Huang *et al.* [28] ($n=9$ instead of $n=8$) gave a consistent estimate (MD=−71.13, 95%CI:[−114.43, −27.83], $p=0.001$, $I^2=0\%$).

a) Tumor Volume



b) Sensitivity Analysis of Tumor Volume

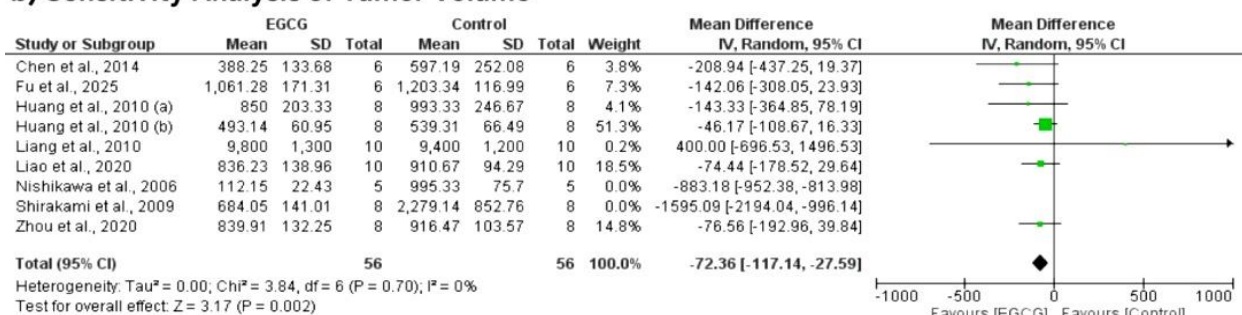


FIGURE 4: Forest plots of tumor volume: (a) all included xenograft studies; (b) sensitivity analysis.

The significant pooled effect was consistent with the qualitative synthesis, which showed that EGCG inhibits proliferation, induces cell death, and suppresses metastasis through multiple molecular pathways, thereby limiting tumor growth. Because all studies in this analysis used xenograft models, which lack a complete immune system, the findings may not adequately reflect interactions between tumor and immune cells in the tumor microenvironment [55]. Liang *et al.* [29] were the only study showing an opposite trend. It used a doxorubicin-resistant BEL-7404/DOX xenograft model and is therefore biologically distinct from the other HCC models. In that model, EGCG monotherapy did not significantly reduce tumor volume, whereas EGCG combined with doxorubicin produced greater tumor growth inhibition than

doxorubicin alone, suggesting that in multidrug-resistant models EGCG may function more as a chemosensitizing agent than as monotherapy.

Relative Liver Weight

Sojoodi *et al.* [16] and Neelamegam *et al.* [40] reported significant reductions in relative liver weight ($p<0.05$ and $p<0.01$, respectively), whereas Shimizu *et al.* [30] reported no significant difference, possibly because the degree of hepatic pathology or the intervention duration was insufficient for detectable changes. Pooled analysis of these three studies confirmed a significant reduction (MD=−1.22 g/100 g, 95%CI:[−1.81, −0.62], $p<0.0001$; $I^2=62\%$; Figure 5), consistent with a reduced hepatic pathological burden.

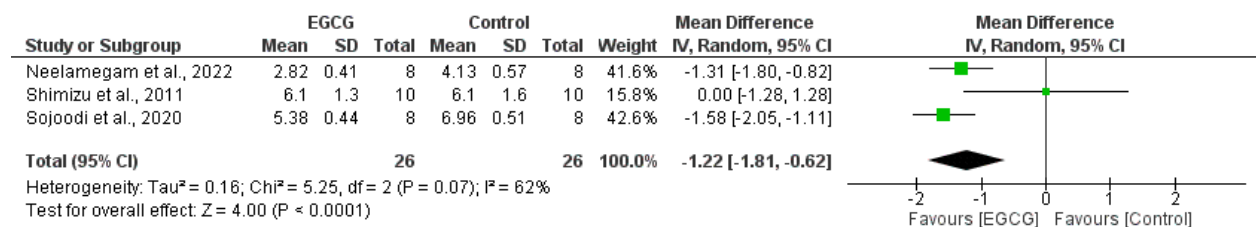


FIGURE 5: Forest plot of relative liver weight.

In Sojoodi et al. [16] and Neelamegam et al. [40], the reduced relative liver weight more likely reflects decreased hepatomegaly associated with reduced fibrosis, inflammation, and tumor burden than hepatotoxicity. This interpretation is supported by the histopathological findings from the same studies, including reduced collagen deposition, improved hepatic architecture, and lower fibrosis scores. Only three studies contributed to this outcome, and heterogeneity was moderate (I²=62%).

Clinical Outcome

Two studies, Darweish et al. [15] and Tang et al. [37],

reported survival rate. EGCG significantly increased survival rate (RR=3.68, 95% CI:[1.50, 9.04], p=0.004; I²=0%; Figure 6). Darweish et al. [15] reported an increase in survival rate from 30% in the control group to 80% in the EGCG group in a TAA-induced HCC model, whereas Tang et al. [37] reported an increase from 6.67% to 50% in a DEN-induced model. Despite the small sample sizes in both studies, the magnitude and consistent direction of the effect support a potential survival benefit. Survival is considered the most clinically meaningful outcome in cancer because it comprehensively reflects disease progression, treatment efficacy, toxicity, and their interactions [56].

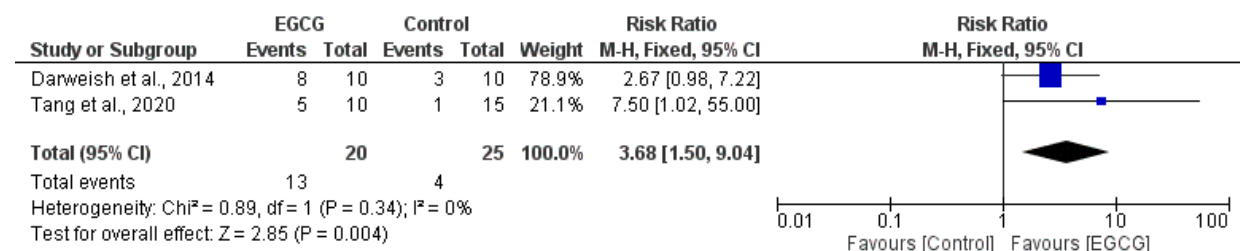


FIGURE 6: Forest plot of survival rate.

Additional Findings

In addition to the primary outcomes, several studies reported findings that broaden the understanding of the mechanisms and therapeutic potential of catechins in HCC. Alpha-fetoprotein (AFP), a standard HCC biomarker widely used for diagnosis, treatment monitoring, and prognostic assessment, was consistently reduced by EGCG [15,32,40], suggesting reduced tumor activity. EGCG may also overcome drug resistance, as shown in two studies. In the BEL-7404/DOX multidrug-resistant xenograft, EGCG reduced P-glycoprotein (P-gp) expression (p<0.01) and MDR1 mRNA expression (p<0.05), with increased intracellular doxorubicin accumulation, suggesting enhanced chemosensitivity [29]. This finding may have clinical relevance because chemoresistance remains a major challenge in HCC management [57]. Reduced carbonyl reductase 1 (CBR1) mRNA expression suggested that EGCG could enhance the efficacy of anthracycline chemotherapy while potentially reducing cardiotoxicity [38]. In addition, Huang et al. [28] reported that EGCG attenuated anthracycline-associated cardiotoxicity, as indicated by reduced oxidative stress and improved cardiac troponin T (cTnT) levels. Together, these findings suggest a chemosensitizing role alongside the antitumor effects of EGCG.

Strengths and Limitations

This study has several strengths. It combined qualitative and quantitative synthesis of in vivo evidence, used structured assessment of reporting quality and risk of bias (ARRIVE 2.0 and SYRCLE), and performed sensitivity analyses. These approaches improve transparency and characterise uncertainty, although they cannot eliminate limitations in the underlying studies.

However, several limitations persist. All 18 included studies tested EGCG, so the findings cannot be extrapolated to other catechins. Reporting quality was moderate in 14 studies and low in 4, and randomization, blinding, and outcome assessment were of unclear risk of bias in most studies. The tumor volume analysis was restricted to xenograft models, while relative liver weight and survival were each based on only three and two studies, respectively. Variation in dose, route, duration, and model, together with inconsistent biomarker and histopathological reporting, precluded quantitative synthesis of those outcomes.

CONCLUSION

EGCG, the only catechin evaluated in the 18 included in vivo studies, significantly reduced tumor volume (MD=-313.28 mm³, 95%CI:[-616.75, -9.82], p=0.04)

and relative liver weight (MD=-1.22 g/100 g, 95%CI: [-1.81, -0.62], $p<0.0001$) and improved survival (RR=3.68, 95%CI: [1.50, 9.04], $p=0.004$) in animal models of HCC, accompanied by antiproliferative, pro-cell-death, and antimetastatic effects and improved histopathology. High heterogeneity and incomplete methodological reporting limit confidence in these findings. EGCG warrants further investigation as a chemopreventive and adjunctive candidate for HCC, but adequately powered and well-reported in vivo studies are required before clinical translation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENTS

This systematic review and meta-analysis did not require formal ethical approval or participant consent, as it was conducted solely on data from previously published studies.

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