Compound K: A systematic review of its anticancer properties and probable mechanisms



Anticancer properties of compound K

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## Abstract~~ABSTRACT~~

*Panax ginseng* is a common natural product~~s~~, which is well-known to have a wide range of pharmacological activities in cancer. Its metabolite, compound K (CK), has been reported to have anticancer activity. We aimed to systematically review the literature for evidence of anticancer effects of CK. We conducted a systematic search in eight databases. We included all in vitro and in vivo studies investigating the anticancer effects of CK with no restrictions. Quality assessment was applied by ToxRTool. Fifty-four ~~54~~ articles were included in our study. The purity of CK in our included studies was at least 95%. The in vitro studies reported that CK

had a potential anticancer activity on several cell lines including human lung cancer cell lines (A549, PC-9), nasopharyngeal carcinoma cell line (Hk-1), liver cancer cell line (BEL 7402), and pediatric acute myeloid leukemia cell lines (Kasumi-1, MV4-11). The in vivo studies reported a significant decrease in tumor volume in mice treated with CK. CK is a potential supplementary treatment in cancer chemotherapies. The safety and further clinical trials of CK should be explored for future drug development.

## Keywords

anticancer
compound K
ginsenosides
in vivo
in vi~~v~~tro
systematic review

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AIF | a~~A~~poptosis-inducing factor |
| Akt | Ak strain transforming |
| AMPK | AMP-activated protein kinase |
| AP | a~~A~~scorbyl palmitate |
| AP-1 | a~~A~~ctivator protein (AP)-1 |
| ATF-6 | ~~Of~~ activating transcription factor-6 |
| BAX | Bcl-2-associated X protein |
| Bcl-2 | B-cell CLL/lymphoma 2 |

| | |
|---|---|
| CAMK-IV | $Ca^{2+}$ p/calmodulin-activated protein kinase-IV |
| CDK | cCyclin-dependent kinase |
| CHOP | CCAAT/enhancer-binding protein-homologous protein |
| CK | cCompound K |
| COX | cCyclo-oxygenase enzyme |
| EGFR | eEpidermal growth factor receptor |
| eIF-2α | eEukaryotic initiation factor-2α |
| ER | eEndoplasmic reticulum |
| ERK | eExtracellular signal-regulated kinase |
| FGFR3 | fFibroblast growth factor receptor 3 |
| GRP-78 | gGlucose-regulated protein-78 |
| HCC | hHepatocellular carcinoma |
| HDAC | hHistone deacetylase |
| $IC_{50}$ | mMedian inhibition concentration |
| IRE-1 | iInositol requiring kinase-1 |
| JAK1 | Janus activated kinase 1 |
| JNK | ——C-Jun $NH_2$-terminal kinase |
| MAPK | mMitogen-activated protein kinases |
| MMP | mMatrix metalloproteinase |
| MSNP | mMesoporous silica nanoparticles |

| | |
|---|---|
| NYAM | New York Academy of Medicine Grey Literature Report |
| PEG-PCL | p~~P~~oly-(ethylene glycol)-poly(ε-caprolactone) |
| PERK | p~~P~~rotein kinase-like endoplasmic reticulum kinase |
| PI3K | p~~P~~hosphatidylinositol 3-kinase |
| PRISMA | Preferred Reporting Items for Systematic Review and Meta-Analysis |
| ROS | r~~R~~eactive oxygen species |
| RUNX3 | Runt-related transcription factor 3 |
| SDF-1 | s~~S~~tromal cell-derived growth factor 1 |
| STAT3 | s~~S~~ignal transducer and activator of transcription 3 |
| TNF | t~~T~~umor necrosis factor |
| TRAIL | TNF-related apoptosis-inducing ligand |
| VHL | v~~V~~irtual health library |
| WHO GHL | WHO global health library |
| WHO | World Health Organization |
| XBP | X-box transcription factor |
| XIAP | X-linked inhibitor of apoptosis protein |
| XP | x~~X~~eroderma pigmentosum |



## 1. INTRODUCTION~~INTRODUCTION~~

Cancer refers to a group of diseases involving the uncontrollable growth and differentiation of cells that potentially invade and affect any parts of the body [1]. It has been a huge health burden

to humanity for a long time. In 2018, cancer caused approximately 9.6 million deaths worldwide and was the second leading cause of mortality [2]. Currently, there are 35 cytotoxic medicines for cancer treatments in the World Health Organization (WHO) Lists of Essential Medicines [3]. These drugs inhibit the survival of cancer cells via various mechanisms such as arresting cell cycles, causing apoptosis, or affecting signaling pathways [4]. However, most chemotherapeutic treatments result in severe side effects. For example, common cancer drugs such as 5-fluorouracil, doxorubicin, bleomycin, or cyclophosphamide are toxic to the heart, kidney, liver, bone marrow, and skin [5–8]. Therefore, scientists have sought new medicines that have a similar efficiency but fewer adverse reactions on patients. Phytochemicals derived from plants are common sources of compounds to be discovered and constituted new treatments for cancer. Approved drugs include~~ing~~ vinblastine and vincristine from the vinca alkaloids,~~;~~ etoposide from the epipodophyllotoxins,~~;~~ paclitaxel and docetaxel from the taxanes, and irinotecan from the camptothecin derivatives [9].

Ginseng is a perennial plant in the genus *Panax* and the family *Araliaceae*. ~~–~~To present, the three common species are *Panax ginseng* (commonly called ginseng or Korean ginseng), *P.* ~~-~~*quinquefolius* (commonly called American ginseng), and *P.* ~~-~~*notoginseng* (commonly called Chinese notoginseng or Sanqi) [10]. Traditionally, ginseng is used in medical practices to treat various diseases including dementia, diabetes, gastric ulcer, and cancer [11]. The pharmacological activities of ginseng are thought to be driven by its unique triterpene saponins called ginsenosides, several of which have proven in vitro and in vivo benefits [12–15]. Although reports of anticancer activity of ginseng have been presented in both traditional and modern literature, there is a lack of knowledge about the involved active ingredients as well as their modes of action [11, 16]. Among ginsenosides, Rh1, Rh2, and compound K (CK) have been reported to have antitumor activity [12, 17, 18]. Recent systematic reviews also revealed that the antitumor activity of ginsenosides Rh1 and Rh2 ~~are~~ is mediated via mitogen-activated protein kinases (MAPK) signaling pathway or Ak strain transforming (Akt)/mTOR signaling pathway [12, 17]. However, there is no systematic review of the anticancer activity of CK and its mechanism. CK is a potential anticancer candidate with a variable effect on different cancer cells. For instance, this compound seemed to be very active on the gastric carcinoma cell line BCG823 ($IC_{50}$ value of 5 ~~-~~μM) [19, 20]. On the other hand, its efficiency was controversial on

human lung carcinoma cell line A529 with $IC_{50}$ values ranging from 20 μM to 125 μM [21, 22]. Therefore, we conduct this systematic review to comprehensively summarize current evidence concerning its anticancer activity and therapeutic characteristics to give an insight for possible application in future studies.



# 2. METHODS

## 2.1. Search strategy and study selection

This study was conducted according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Statement recommendations [23]. The PRISMA checklist is presented in Supplementary Table S1. The protocol of our study was registered in PROSPERO International prospective register of systematic reviews under number CRD42016049511.

We performed an initial literature search in 2015, and later an updated search in 2020 on eight databases: PubMed, Virtual Health Library (VHL), WHO Global Health Library (WHO GHL), POPLINE, ISI Web of Knowledge, Scopus, New York Academy of Medicine Grey Literature Report (NYAM), and Google Scholar. Detailed search keywords for nine databases were represented in Supplementary Table S2. A manual search was performed by looking at the references of the included studies, related articles in PubMed and Google Scholar.

## 2.2. Selection criteria and title/abstract screening

Endnote X7 was used to remove duplicated reports retrieved from the above-mentioned databases. Three reviewers independently screened the titles and abstracts for potential relevance. Original publications reporting anticancer activity of ginseng CK with no restriction for language, area, publication year, and age of patients were included in the present study. Exclusion criteria were: (1) unreliably extracted data, (2) overlapping datasets, and (3) data that cannot be extracted. Any disagreement was resolved by a consensus discussion among authors.

Included papers from titles and abstracts screening were retrieved for full-text evaluation and then included in our study if finally eligible.



## 2.3. Data extraction

Five randomly included studies were selected to perform a pilot extraction. Data were extracted by three independent authors, and any discrepancies were resolved by the senior investigator (NTH). The following data were extracted: basic information (title, author, publication year, journal name), study characteristics (name of the study, study design, country), participants/population characteristics (sample size, age, gender, race/ethnicity for human studies, species, the weight of animals, ages for animal studies, cancer cell lines for in vitro studies), assessment of exposures, and outcomes. Regarding in vitro studies, we extracted values of $IC_{50}$ of CK on cell lines, survival/death percents after treatment, the percent of metastatic cells, the percent of invaded cells, the percent of relative migration, and the percent of apoptosis. For in vivo studies, we collected data of the experimental cancer cells, tumor size, and volume of tumor after treatment of the animals. We re-calculated the percent of cell death from the percent of cell survival or cell proliferation (the formula: 100% - the percent of cell survival [(%)]) to make an appropriate comparison. When the $IC_{50}$ values were presented as weight/volume concentration (μg/mL), we converted these values into molecular concentration (μM) (the molecular weight of CK is 622.68 g/mol). Studies for which we could not obtain clarification from authors (after at least two attempts via E-mail) were listed as "no data available." All the extracted data were double-checked.

## 2.4. Quality assessment

The risk of bias of the included studies was independently evaluated using the software-based tool ToxRTool [24], which is specially designed for toxicological research. It is a two-part tool addressing in vivo and in vitro data, including assessment of five criteria: (i) test substance identification, (ii) test system characterization, (iii) study design description, (iv) study results

documentation, and (v) plausibility of study design and data. The detail of each criterion was outlined in ~~Supplementary~~ Table S3. The judgment of each author on each domain was categorized as reliable without the restriction of bias (if an in vitro study gets 15–18 points or an in vivo study gets 18–21 points), reliable with restriction of bias (if an in vitro study get 11–14 points or an in vivo study gets 13–17 points), or not reliable of bias (if an in vitro study get <11 points or an in vivo study gets <13 points). Each paper was appraised by three independent authors, and any different conclusion was revised by discussion to reach a final consensus.

## 3. RESULTS

Our search initially retrieved 1866 studies. Screening for duplicates subsequently excluded 231 articles. After the title and abstract screening phase, 311 articles were included for full-text screening. Finally, 54 studies were enrolled in the qualitative synthesis. The details of the search strategy were provided in the PRISMA flow diagram (Figure 1). The structure of CK was presented in Figure 2.

### 3.1. Baseline characteristics of the included studies

A total of 54 studies were included in our review (~~Supplementary~~ Table S3). All studies used pure CK for anticancer activity experiments. In addition, three of them tested CK mixed micelles (CK-M) that were formed by loading CK in a liposome system using tocopheryl polyethylene glycol 1,000 succinates (TPGS) combined with ascorbyl palmitate (AP) or poly-(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) –[22, 25, 26]. One study combined CK with activated mesoporous silica nanoparticles (MSNP), which is a carrier system to produce a form named MSNPs-CK [27]. CK used in most included studies was supplied from commercial sources (31 of 54 studies). Other studies prepared CK by treating ginseng extract or protopanaxadiol ginsenosides with bacteria (human intestinal bacteria *Sulfolobus solfataricus*, *Bifidobacterium* K-103 or K-506, *Bacteroides* JY-6, *Penicillium oxalicum*, *Fusobacterium* K-60) or β-glycosidase

(21 of 54 studies). Two studies did not report the source of the tested CK [18, 22]. ~~12~~ Twelve studies reported the purity of the CK, which was at least 95%.

All included studies performed in vitro experiments from which 15 studies conducted in vivo experiments and other two studies did in silico experiments. Anticancer activity of CK was tested on various cancer cell lines, including colon carcinoma cell lines (15 of 54 studies), lung cancer cell lines (10 of 56), liver cancer cell lines (8 of 56), and leukemia cell lines (7 of 54). The in vitro dosage of CK was varied, ~~;~~ ranging in concentration from 0.1 to 250 µM and in duration from 24 ~~hours~~ to 96 h~~ours~~. Most studies examined the ability to inhibit cancer cells through $IC_{50}$ (µM), cell survival or proliferation rates (%), and apoptosis (%). ~~22~~ Twenty-two studies measured the interference of CK with the cell cycle to identify its mechanism or apoptotic activity. Only one study studied the protective effect of CK on the keratinocyte cell line from cancer trigger (ultraviolet rays) [28]. Another study investigated the role of CK in lung cancer treatment in association with gamma-ray radiation [29]. For assessment of the anti-migratory and metastatic effect, number of migrating cells, relative migration (%), and number of colonies and invaded cells (%) were mainly used.

Regarding in vivo models, mice were used in these experiments. The dosage ranged from 0.2 to 50 mg/kg/day for 5 ~~—~~40 days by different routes (gastrointestinal perfusion, intraperitoneal injection [~~(~~i.p.~~)~~], or intravenous injection [~~(~~i.v.~~)~~]). Most experiments measured the volume of the tumor before and after CK treatment to assess its anticancer activity.

## 3.2. Effect of CK and its productions on the viability or proliferation of cancer cell lines

Generally, CK showed a weak or moderate cytotoxic effect on cancer cells that was time and concentration dependent. Other pharmacological forms, such as MSNP-CK and CK mixed micelles, showed a more promising effect.

Hk-1 cells (nasopharyngeal carcinoma cell line) were the most sensitive to the exposure of CK, as 41.1 ~~—~~88.0% of cell death was observed under low doses (10 ~~—~~20 µM) [30]. On the other

hand, the cytotoxic activity of CK on colon cancer cell lines and human lung carcinoma cell lines was mostly modest, as shown by $IC_{50}$ values (Table 1). The $IC_{50}$ values of CK application on HT-29 and HCT-116 cell lines (colon cancer cell lines) for 24–48 hours ranged from 32.1 to 51.4 μM [31–33]. At the dose of 20–64 μM, CK caused 46.41–70.92% cell death of the HT-29 cell line, when measured at 24 hours or 72 hours. In contrast, Yao et al. [20] reported that CK strongly inhibited cell proliferation of HT-29 cell line at high doses (>90% at 60–100 μM), while Zheng et al. [34] stated that even at 250 μM, CK continued to show a weak activity. On HCT-116 cell line, the anticancer activity of CK seemed to be dose- and time-dependent. For instance, the modest doses (40–50 μM) could cause 60.3–85.9% cell death following 24 hours of testing whereas 75.8–100% of cell death could be reached at 60–100 μM during the same duration [20, 32, 35]. Also, the modest doses (40–50 μM) resulted in strong inhibition of cell viability (above 96.0%), and the low dose of 20 μg/ml could cause 60.2–78.8% of cell death when the duration was extended to 48 and 72 hours, respectively [32, 35]. Three reports pointed out that CK had a weak effect on HCT-116 cell line [34, 36, 37]. While the study of Chen et al. [37] revealed only 20.2%of cell death caused by the modest dose (50 μM) after 24 hours of testing. Wang et al. [36] also showed that only 40.0–53.3% of cell death was observed at the modest doses (40–50 μM) following 48 hours of the experiment. Finally, less than 15% of cell death was observed at a very high dose of 250 μM after a long duration (72 hours) by Zheng et al. [34]. Moreover, CK also had a moderate effect on DLD and SW480 cell lines. Modest doses ranging from 25 to 50 μM caused 61% of DLD-1 cells death [37]. For the SW480 cell line, at the modest dose of 40 μM, CK inhibited >75% of cell viability after 24–48 hours of experiment, while 100% inhibition was reached with 50 μM after 72 hours [35, 36]. 20% of cell deaths were seen on Colo205 cancer cell line when treated with 50 μM of CK [37]. A strong effect of CK was noted on CT-26 cell lines with 79.52% cell death at 10 μM, and the effect did not change much when the dose increased to 40 μM (80.48% cell death) [38]. Additionallty, CK at 20 μM seemed to be safe on the normal cell line, as only 1.3–10.3% cell death was recorded even after 72 hours of testing [32, 33]. Details of the percent of cell death caused by CK were presented in Table 2.

On human lung carcinoma, CK showed the strongest inhibitory effect on the PC-9 cell line, moderate effect on PC-14 cell line, and the weakest effect was on LLC cell line with $IC_{50}$ value

of 23.2 μM, 40.1—41.6 μM, and 39.00 μM, respectively (Table 1) [26, 39–41]. Interestingly, the $IC_{50}$ values of CK on A549 cell line were much varied across studies, ranging from 25.9 to 52.9 μM [22, 25, 26, 42]. The differences seemed to be attributed to the wavelength used to measure these data where the longer wavelength (570 nm) gave lower $IC_{50}$ values compared to the shorter wavelength (490 nm). Nevertheless, the moderate efficacy of the compound generally seemed to be consistent between studies; 43.0—89.6% of cell death was caused by concentrations from 20—60 μM [22, 27, 43, 44]. Only high doses (80 μM—249 mg/l) resulted in a strong inhibitory effect (70—97.2% cell death) [21, 22, 43, 44]. The moderate efficacy was also seen on H1975 cell line with 53.7—61.9% of cell death resulting from 20—80 mg/mL of CK [43]. Other lung cancer cell lines, namely, NCI-H460, H460, CRT-MG, and H1299, appeared to be not sensitive to CK, as it caused less than 50% cell death even at high doses (30—50 mg/L) [15, 29, 45, 46]. Finally, it should be highlighted that CK may have a strong effect on T24, B16-B16, and PC-14 cell lines [40, 47, 48]. The compound inhibited 78.2—84.3% of T24 cells viability at modest doses of 20—25 μM [47]. Meanwhile, low doses from 10—20 μM inhibited over 60% of the cell viability of B16-B16 cells [48]. Nearly 100% of PC-14 cells were also inhibited, but at a very high dose of 100 μM [40].

On the other hand, the inhibitory effect of CK appeared to be weak on hepatocellular carcinoma (HCC) cell lines, breast cancer cell lines, metastatic human fibrosarcoma (HT1080) cell line, and blood cancer cell lines, as most $IC_{50}$ values observed were above 56.2 μM (Table 1). Some exceptions with $IC_{50}$ ranging from 31.2 to 40.0 μM were seen on Hep-G2 (HCC) cell line (540 nm wavelength was used), Jurkat (human T lymphoblastic leukemia) cell line, U937 (human monocytic leukemia cell line), and HL-60 (human myeloid leukemia) cell line. For gastric carcinoma cell lines, CK showed a weak effect on MKN-45 and MKN28 cells, with $IC_{50}$ values of 56.6 and 31.1 μM, respectively. However, the compound was quite effective on BCG823 and SGC7901 cells showing >76% cell death with low doses (7.5—10 μM) [19]. In the case of blood cancer cell lines, pediatric acute myeloid leukemia cell lines (Kasumi-1 and MV4-11) were the exception that showed a modest effect, as the moderate doses (10—20 μM) inhibited over 50% of cell viability [49]. Meanwhile, the high efficacy (>70% of cell death) was only shown on HL-60 and U266 cells at high or very high doses (50—100 μM) [39, 40, 50].

Regarding the liver cancer cell lines, the inhibitory efficacy of CK on Hep-G2 cells apparently depended on doses and test duration. For instance, 20 μM caused 46.47% cell death after 24 hours of exposure [27]. The percent reached over 90% when the test duration increased to 48 hours even at the lower dose of 10 μM [51]. Only one study of Shin et al. [52] indicated that the low dose of 5 μM was still quite weak even at 72 hours of exposure, and the strong inhibition (>90%) was only observed after 96 hours of exposure for both low and high doses. On other HCC cell lines (MHCC97-H, SMMC7721, Hep3B), an effective inhibition (>90% cell death) was observed, but almost at high doses for MHCC97-H and Hep3B cell lines (50—75 μM), or at an extremely high dose (100 μM) for SMMC7721 cell line [53–57]. One study reported that low doses (7.5—15 μM) of CK resulted in relatively high efficacy on SMMC7221 cells (73—96% cell death) if the exposure time was 76 hours [58]. Also, this study demonstrated that CK had a promising effect on BEL 7402 cells with 73% cell death caused by the low dose (15 μM) [58]. In contrast, Huh 7 cell line was considered not really sensitive to CK as only 16.54% of cell death was observed after exposure to 20 μM of this compound [56]. For the safety of CK on normal liver cells, CK seemed to be tolerated at quite low doses (20—40 μM), whereas the high dose of 60 μM resulted in 25.33% of cell death [56].

The cytotoxicity of CK on breast cancer cell lines was quite weak. The strong inhibition of cell growth (59—100%) was only shown in case high doses (50—100 μM) or long duration of exposure (>72 hours) [57, 59–62]. At low doses (10—20 μM) and short duration of exposure (24—48 hours), CK only suppressed 45—55% of the growth of MCF-10A1a cells and 40% of the growth of MCF10DCIS cells [61]. In a combination with cisplatin, CK (50 μmol/L, 72—96 hours) significantly enhanced the cytotoxicity of cisplatin to 55.9—65.1% compared to cisplatin alone (29.8—40.0%) [62].

Interestingly, CK under other forms such as MNSPs-CK was more promising than pure CK. The inhibitory effect was much improved on colon cancer cell line (HT-29), lung cancer cell line (A549), and liver cancer cell line (Hep-G2). At 20 μM, MNSPs-CK caused the death of 88.27—97.5% of these cells that was almost double the effect of pure CK on respective cells [27]. Besides, CK could enhance the anticancer activity of gamma-ray (1.5 Gy/min) and cisplatin on

human lung cancer cells (5 µM) [44, 45]. The percent of cell death of NCI-H460 cell line increased a little bit from 32% to 45% when adding 30 mg/L of CK for 48 hours, while the percent of cell death of H460 cell line almost doubled when treated with a combination of CK (20 µM) and cisplatin (5 µM) compared to cisplatin alone.

## 3.3. The anti-metastatic activity of CK on cell lines

CK showed anti-migratory effect on lung cancer cell lines (A549, PC-9) [25, 44], glioblastoma cell lines (U87MG, U373MG) [63], and bovine aortic endothelial cells (BAE) [41]. On A549 cell line, CK at 50 µg/mL after 24 hours of treatment significantly reduced transportation areas to 28.3% compared to 63.1% shown by negative control [44]. Meanwhile, CK at 21.97 µg/mL dramatically increased gap length in the scratch-wound test indicating its prevention of A549 cell line migration compared to the negative control (270 nm compared to 170 nm, respectively) [25]. A similar result was observed on PC-9 cell line when testing CK at 14.46 µg/mL, as the gap length between cell scratches on cells incubated with CK was 100 nm larger than on cells of the control group (300 nm compared to 200 nm, respectively) [25]. These results implied a promising anti-migratory activity on lung cancer cells. On glioblastoma cells, CK seemingly showed stronger anti-migratory activity on U87MG cell line than on the U373MG cell line [63]. For instance, on U87MG cell line, CK at 25 and 50 µg/mL diminished the migration of cells to 80—82% and 32—67% compared to negative control after 24 and 48 hours of treatment, respectively. Meanwhile, a reduction of U373MG migrating cells by 91% or 77% was only observed at 50 µg/mL after 24 or 48 hours, respectively. The anti-migratory activity of CK was also shown on BAE cell line, as it could lower the migrating cells to 56.8% at 20 µg/ml after 24 hours of treatment. This effect was 25 times stronger than the positive control (suramin) [41].

In addition, CK revealed its anti-invasive activity on BAE cell line and lung cancer cell lines (A549, PC-9) at low doses, on human glioblastoma cell line (U87MG), and human astrocytoma cell line (U373MG) at high doses [26, 41, 63]. The percentage of cell invasion was reduced to 60% after incubating A549 cell line with 21.97 µg/mL and to 61% in PC-9 cell line after incubation with 14.46 µg/mL [26]. The results were more prominent on BAE cell line, as very

low doses (1 and 3.2 µM) diminished cell invasion to 45—73%, while no invasion was observed from the dose of 10 µM [41]. Nevertheless, CK at 50 µM caused a significant reduction of the invading cells to 20% and 40% in U87MG and U373MG cell lines [63]. Similarly, the anti-invasive activity of CK on glioblastoma stem-like cells was observed at 50 µM as 15—20% reduction of the invading cells was seen [63]. On HCC cell line (MHCC97-H), CK showed its anti-invasive activity at very high doses (50—75 µM) [54]. A dramatic depletion of the number of transmigrating cells by 33.91—67.82 was seen compared to 136.33 shown in the control group. Similarly, the anti-adhesive activity of CK on MHCC97-H cell line was only observed at very high doses, as shown by 40.19—61.4% of adhesive cells after treatment. These results indicated its weak anti-metastatic on such cell lines.

On the other hand, Yang et al. [25] revealed a promising effect of CK-M on lung cancer cell lines (A549, PC-9). CK-M increased the gap length between the wound space more than CK pure form when the same doses were applied (270 nm compared to 208 nm, respectively). The invasive cells were nearly double reduced when treating cells with CK-M compared to CK pure form (28.3% compared to 60.0%, respectively). A similar result was also detected on PC-9 cell line (29.3%, 61.7% of invasive cells in CK-M and CK pure groups, respectively).

## 3.4. Other effects

Furthermore, CK was reported to have a cell protective effect, and it can inhibit the self-renewal capacity of cancer stem-like cells [28, 63].

CK at low doses (5 or 15 µg/mL) and high dose (45 µg/mL) could protect 64—79% and 100% of keratinocyte cell line (HaCaT) from death caused by UV rays (a cancer trigger) [28]. The effect was attributed to prevention of apoptosis resulting from the UV exposure. When HaCaT cell line was treated with CK at 5 or 45 µg/ml, 8—14.6% reduction of apoptotic cells in CK groups was observed compared to 24.4% in the UV exposed group without CK treatment. The inhibition of self-renewal capacity of CK was observed on stem-like cells derived from glioblastoma cell lines (U87MG, U373MG) as only 8.6—17.4% of cells were visible after 7

days of exposure to 50–100 μM [63]. In addition, almost no neurosphere was formed in these cell lines after treatment with 50 μM, which strengthened the ability of CK to prevent the self-renewable capacity of stem-like cells.



## 3.5. Anti-tumor activity of CK~~compound K~~ on in vivo models

A total of 14 in vivo studies were included in our study (Table 3). Experiments were conducted on numerous cancer cells~~-~~lines such as human lung cancer, Lewis lung carcinoma, human lung carcinoma, human colon carcinoma, gastric carcinoma, nasopharyngeal carcinoma, HCC, and human breast cancer. Mice were used as the animal model, with CK concentrations from 0.05 to 37.5 mg/kg/day, and a follow-up period of ~~five~~ 5 ~~to~~ 40 days. The target outcomes were the tumor volume and size~~,~~ and the number of metastases. Indeed, all results showed a significant decrease in tumor volume compared with the control group. The lowest reduction was 18.2%, while the highest was 96.4% regardless of the administration way of CK. According to Yang et al. [25], the CK dose of 15 mg/kg/day after 15 days of follow-up caused a 40.8% decrease in tumor volume compared to the negative control. Noteworthy, CK-liposome induced the best response by reducing the tumor volume up to 67.3% compared to the negative control (on day 15). However, when CK dose reached the highest range (30 ~~mg/kg/d~~ or 37.5 mg/kg/day), a reverse effect was observed with lower effectiveness in tumor volume reduction compared to the lower dose (35–44.3% vs. 29.3%) [21, 22]. Besides, when compared to the negative control, CK prepared in the form of liposomes (CK/parthenolide type-1 liposomes) resulted in a higher tumor reduction than CK alone on day 15 (52.4~~%~~ ~~–~~67.3% vs. 29.3~~%~~ ~~–~~40.8%) [21, 25]. On the other hand, the tumor volume decreased significantly to 67.2–83.9% when combining CK + γ-ray in comparison to the negative control (on days 26–40) [29]. In addition, two in vivo studies using human colon carcinoma showed that increasing CK dose from 0.2 ~~mg/kg/d~~ ~~–~~to 1.0 mg/kg/day significantly promoted the reduction of tumor volume (32.1% to 39.1–60.9%) after 28 days [38]. However, when CK dose reached a high level of 30 mg/kg, the tumor reduction effect did not improve (57.7–75.7% on days 10–20) [64]. Regarding gastric

carcinoma, high dose of CK (10.0 mg/kg) was proved to significantly reduce tumor size compared to the low dose (2.5 mg/kg) (73.3% 96.4% vs. 30.7% 52.7%) [19].

Also, CK showed promising results in preventing metastasis. On day 35, the number of metastasis in mice caused by BEL-7402 cell line was reduced significantly up to 84.6% with a dose of 10 mg/kg/day and up to 69.2% with the dose of 5 mg/kg/day; both administered via i.p. route. The anti-metastasis efficiency of cisplatin (positive control) at a high dose of 20 mg/kg/day showed a decrease in the number of metastases by 69.5% compared to the negative control on day 35; meanwhile, 5 and 10 mg/kg/day doses of CK significantly decreased the number of metastases by 69.2% and 84.6%, respectively [58]. The same effect was described when mice with MHCC97-H cell line were treated with CK at doses of 50 and 100 µg/kg/day. The compound reduced the number of metastases by 87% and 95.2% compared to the negative control [54].

## 3.6. Mechanism of anticancer activity of CK

The anticancer activities of CK are seemingly related to apoptosis, autophagy, necrosis, interference with the cell cycle, and some signal pathways.

CK induced the arrest of cell cycles of many cancer cell lines including leukemia, HCC, colon cancer, non-small cell lung cancer, gastric cancer, breast cancer, and nasopharyngeal cancer with the minimum dose (5 µM for at least 24 hours) (Table 4) [19, 20, 25, 30–32, 35, 36, 43, 49, 53, 55, 57, 61, 63, 65, 66]. CK caused the arrest at G0/G1 for all cancer cell lines except human gastric carcinoma cell lines and MHCC97-H (HCC cell line), which were arrested at phase G2/M [19, 53]. This effect was weaker on human glioblastoma cell lines (U87MG, U373MG) than on leukemia cell line (U937), HCC cell line (Hep-G2), human colon cancer cell (HCT-116), and breast cancer cell lines (MCF10CA1a, MDA-MB-231).

The arrest of G1 phase was attributed to the increase in p21 expression resulting in the inactivation of cyclin D1/3 and the cyclin-dependent kinase (CDK)4/6 expression (activation at the early G1 phase) as well as cyclin E (activation at the late G1 phase) (Figure 3) [31, 35, 65]. The expression of c-Jun $NH_2$-terminal kinase (JNK), c-Jun, or activator protein (AP)-1 was

raised in the presence of CK that implied the activation of JNK/c-Jun/AP-1 signal pathway contributing to the upregulation of p21 [32, 65]. In addition, CK-induced inhibition of histone deacetylase (HDAC) activity may be an indirect cause of the increased p21 expression [31]. The decreased activity of HDAC led to accumulation of H3 and H4 histones, which enhanced their interference with Runt-related transcription factor 3 (RUNX3), thus, raising the level of p21 [31]. Furthermore, the induction of $p27^{Kip1}$ and consequent inhibition of CDK2-cyclin E activity (activation at the late G1 phase) with increasing the hypophosphorylation of Rb and p130 also caused G1 phase arrest [57]. Interestingly, Zhang et al. [35] suggested that the mechanism of G1 cell cycle arrest was associated with multiple pathways via the elevation of Smad3, p53/p21, and p15, and the increase in FoxO3a-meditated p27. In the meanwhile, the arrest of G2 phase is related to the suppression of cyclin B1 and cdc2 levels as well as the elevation of p21 expression [19].

The inhibition of cell growth by CK was possibly associated with apoptosis (Figure 4) [22, 25, 30–32, 43, 53, 67, 68]. The treatment with CK augmented the level of DR5 protein on colon cancer cell line (HCT-116), which is a protein that mediates apoptosis via binding with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) [37]. The increase in DR5 level was supposed to be associated with an activation of reactive oxygen species (ROS)-JNK-autophagy pathway and p53-CCAAT/enhancer-binding protein-homologous protein (CHOP) pathway [37]. Particularly, CK-produced ROS resulted in the activation of JNK and c-Jun phosphorylation paralleled with the decrease in extracellular signal-regulated kinase (ERK) or p38 phosphorylation. The augmentation of CHOP level by CK also induced the DR5 level [37]. Nevertheless, on astrocytoma cells and bladder cancer cells (T24), there was an activation of p38 MAPK [15, 47]. No effect of JNK or ERK was observed in astrocytoma cells, which implied that CK caused apoptosis of astrocytoma cells through p38 MAPK, and this did not relate to the JNK pathway [15].

Moreover, CK raised levels of some pro-apoptotic proteins (tBid, and cytochrome c) and tumor suppressor (p53), which could lead to the apoptosis on cancer cells [19, 37, 39, 45, 55]. Additionally, downregulation of Bid expression was observed by CK [19, 58]. These studies showed that Bid translocated from the nuclear to the mitochondria where it transferred to its

activated form tBid and interacted with apoptotic proteins, thus, causing apoptosis. Also, the expression of other anti-apoptotic proteins such as Mcl-1, X-linked inhibitor of apoptosis protein (XIAP), survivin, and cFLIP decreased when cells were treated with CK [37].

The apoptotic induction of CK possibly resulted from the activation of some caspases such as caspase-3, caspase-8, and caspase-9 as well [15, 22, 25, 30, 32, 33, 36, 37, 39, 50, 51, 68, 69]. The elevation of these caspases was probably promoted by the release of cytochrome c from mitochondria to the cytosol, the translocation of apoptosis-inducing factor (AIF) from mitochondria to nucleus, the inhibition of Akt phosphorylation, the upregulation of Fas and Fas ligand, or the inhibition of NF-κB activation and its downstream genes [15, 30, 33, 37, 51, 53, 70]. In an in silico experiment, Wang et al. [51] reported that the prevention of NF-κB activation caused by CK was attributed to the interaction between CK and Annexin A2 at Lys302 [51]. Kim et al. [70] also suggested that cytochrome c and caspases were induced by the activation of AMP-activated protein kinase (AMPK) through the $Ca^{2+}$ p/calmodulin-activated protein kinase-IV (CAMK-IV). Moreover, CK also caused the depolarization of the mitochondrial membrane ~~which~~ that was considered a critical point of its anticancer activity, because this enhanced the translocation of AIF through the mitochondrial membrane and released cytochrome c [30, 33, 53, 60].

In addition, the modulation of proteins in B-cell CLL/lymphoma 2 (Bcl-2) regulated apoptosis pathway was a contributing factor to the anticancer mechanism of CK. The elevation of the level of Bcl-2-associated X protein (BAX) expression and the decrease in the level of Bcl-2 were observed when treating some cell lines with CK [19, 22, 25, 32, 37, 53, 58, 69]. Results from another study revealed that the downregulation of Bcl-2 might relate to the inactivation of signal transducer and activator of transcription 3 (STAT3) and its upstream activator (Janus activated kinase 1 [~~(~~JAK1[~~)~~]) [50, 56]. This inhibition also resulted in the decrease in the level of STAT3 target genes such as Bcl-$x_L$, survivin, cyclin E, and cyclin D1 in human multiple myeloma U266 cells [50]. Meanwhile, the expression of Bcl-2 and Bcl-$x_L$ did not significantly change under the treatment of CK in human HCC cells, whereas the level of BAX was still upregulated [55]. CK also did not affect the level of Bcl-2 in human myeloid leukemia cell line (HL-60) and ~~–~~Bcl-$x_L$ in gastric carcinoma cells [19, 39].

Finally, the endoplasmic reticulum (ER) stress caused by CK contributed to the apoptosis of cancer cells [56, 67, 71]. In particular, CK promoted the phosphorylation of proteins involved in the ER stress-induced apoptosis, namely protein kinase-like endoplasmic reticulum kinase (PERK), eukaryotic initiation factor-2α (eIF-2α) (which is also phosphorylated by activated PERK), inositol requiring kinase-1 (IRE-1), and spliced X-box transcription factor (XBP) (its pre-mRNA is also matured by activated IRE-1). During the ER stress caused by CK, the concentration of calcium in the cytosol increased via RyR channel [71]. This might implicate stored calcium in ER that was released into the cytosol. The accumulation of calcium in cytosol might activate caspase-12. CK also resulted in the induction of activating transcription factor-6 (ATF-6) cleavage, which activates the transcription of caspase-12, glucose-regulated protein-78 (GRP-78), and CHOP, therefore, raising their expressions. CHOP and caspase-12 are pro-apoptotic factors that play an important role in ER stress-induced apoptosis pathway.

The mechanism of apoptosis caused by CK on breast cancer cell lines (MCF-7, MDA-MB-231) was varied [57, 60, 62]. In MCF-7 cell line, Kim et al. [60] suggested that apoptosis occurred via the activation of AMPK pathway, which consequently decreased the levels of cyclo-oxygenase enzyme (COX)-2 and prostaglandin E2. In the meanwhile, Zhang ~~et al.~~and Li [62] demonstrated that CK inactivated the phosphatidylinositol 3-kinase (PI3K)/protein B –pathway via the decrease in phosphorylation of Akt. Nevertheless, Yim et al. [57] proposed that the mechanism in MDA-MB-231 involved the activation of ERK. Increased levels of COX-2 and PEG2 were observed as a protective response of cells [57]. In addition, Kwak et al. [59] suggested that CK caused cell death in breast cancer cells (MCF-7) via programmed necrosis, not apoptosis or autophagy. This effect resulted from the dephosphorylation of GSK3β ~~which~~ that led to the decrease in β-catenin and cyclin D1 (oncogenic proteins).

On the other hand, CK also caused autophagy in colon cancer cell lines [32]. The presence of CK increased the level of LC3-II (a protein involved in the formation of autophagosomes)~~,~~; autophagy proteins~~,~~, namely Atg5, Atg6, and Atg7; ~~as well as~~ and the level of ROS through the activation of JNK pathway [32, 37].

The anti-proliferation activity of CK possibly implicates the inhibition of epidermal growth factor receptor (EGFR), h-Ras, and fibroblast growth factor receptor 3 (FGFR3) expressions [44,

68]. CK bonded with EGFR and h-Ras via the active site Met379 and Thr35, respectively [44]. This binding was stronger than the positive control (erlotinib). Meanwhile, the migratory and invasive activities may be associated with the suppression of downstream signals including protein kinase C (PKC)α, ERK, matrix metalloproteinase (MMP)-2,~~)~~ and MMP-9 that were activated by stromal cell-derived growth factor 1 (SDF-1) (a factor promoting the migration of glioma cells) [72]. Besides, CK also decreased the expression of MMP-2 and MMP-9 that participate in the migration of cancer cells in other studies [25, 46, 73]. The inhibition of MMP-9 expression might relate to the reduction of AP-1 activity, which was supposed as a transcription factor of MMP-9 [46].

Regarding the protection of keratinocytes from ultraviolet (UV)-induced apoptosis under the treatment of CK, Cai et al. [28] demonstrated that CK contributed to DNA repair via the increase in expression of xeroderma pigmentosum (XP)-C and ERCC1-, which are elements of nucleotide excision repair (NER).

## 3.7. Quality assessment results

ToxRTool was used to evaluate the quality of the extracted data. According to the scoring system, the quality assessment was scored and categorized as (i) not reliable, (ii) reliable with restriction, or (iii) reliable without restriction based on how the experiments were conducted. In 53 in vitro studies, there were 30 studies classified as reliable without restriction (~~Supplementary~~ Table S4) and 20 studies as reliable with restriction due to the lack of a control arm. The remaining three studies were considered as not reliable due to the unavailability of both control arms and replicated experiments, the absence of purity value, and the problem of methodology. Regarding in vivo studies, 12 among 15 studies (80%) were classified as reliable without restriction (~~Supplementary~~ Table S5). Three studies were not reliable due to lacking information regarding the purity, number of animals, animal sex, strain~~,~~, weight, and age.

# 4. DISCUSSION

Our review showed that CK mainly had a weak to moderate cytotoxic effect on cancer cells in a time and concentration-dependent manner. In particular, CK had considerable cytotoxicity on lung cancer cell lines (A549, PC-9), nasopharyngeal carcinoma cell line (Hk-1), liver cancer cell line (BEL 7402), and pediatric acute myeloid leukemia cell lines (Kasumi-1, MV4-11) as > 50% of cell growth inhibition was observed by approximately 20 μM. A moderate effect was observed on colon cancer cell lines, human T lymphoblastic leukemia cell line (Jurkat), human monocytic leukemia cell line (U937), and HCC cell line (Hep-G2). Meanwhile, the compound weakly inhibited the cell viability of metastatic human fibrosarcoma cells (HT1080), gastric cancer cells, myeloma, and breast cancer cells. It also demonstrated anti-migratory activity on lung cancer cell lines (A549, PC-9), glioblastoma cell lines (U87MG, U373MG), and bovine aortic endothelial cell lines. CK also improved the anticancer activity of cisplatin (a well-known anticancer drug), so it was suggested as a supplementary to ~~to~~ enhance the efficacy of anticancer medicines and reduce their side effects. Furthermore, our data showed that CK may protect cells against death caused by UV radiation and inhibit the self-renewal capacity of cancer stem-like cells. Additionally, all in vivo models showed a significant decrease in tumor volume in mice treated with CK that highlighted its anticancer effect.

The purity of CK used in the included studies was not less than 95%. However, the differences between results were considerable. The inhibitory effect of CK on colon cancer cell lines (HCT-116, HT-29) presented by Chen et al. [37] was significantly weaker than other studies. This might be because Chen et al. used WST-1 assay for their cell growth measurement whereas others used MTT assay. On top of that, the sources of CK seemingly did not affect the anticancer activity of CK, as efficacy conflicts on the same cell line ~~was~~ were still observed even when using the same extraction method [39, 40, 42]. Instead, we realized that the variances in the MTT assay used to measure the cell viability may contribute to the differences in results. First of all, the ratio between the number of cancer cells and the volume of MTT could affect the result [25, 42]. Limin et al. [42] used a 10-fold higher number of A549 cells but ~~5~~five-fold less volume of MTT compared to Yang et al. [25], whereas they used the same measuring wavelength. That led to a 1.5-fold weaker efficacy observed in the results of Limin et al. compared to Yang et al. [25]. A similar result on Hep-G2 cell lines was shown when higher numbers of cells to the volume of MTT gave a weaker effect when measuring with the same wavelength [39, 40, 42, 56]. If the

volume of MTT assay is too small, that means not all viable cells transfer MTT into formazan crystal, which could result in an incorrect measured number of viable cells. Moreover, the wavelength used in the experiment may be another contributing factor that caused the conflict between results. For instance, on A549 cell line, the measurement at 570 nm gave a more promising effect than at 490 nm even when the authors used a four~~4~~-fold higher ratio between cells number and volume of MTT [22, 25, 42]. The wavelength of 570 nm was also commonly recorded in MTT assay instead of 490 nm [74]. However, we could not conclude which wavelength should be applied throughout the current data. Yet, this should be deeply investigated in further studies. Briefly, we recommend that studies in the future should pay more attention to the ratio between cell~~s~~ number~~:~~ the volume of MTT~~,~~ and also the wavelength used in their experiments in order to obtain more exact results.

The problems limiting CK application in clinical studies include its poor solubility, significant efflux, and medium bioavailability [44, 75]. Thus, some carriers such as MSNP, AP/TGPS, or TGPS/PEG-PCL were used to improve its poor solubility characteristic and enhance its accumulation in cells [22, 25, 27]. MSNP-CK and AP/TGPS-CK showed more promising inhibition of cell viability compared to pure CK; TGPS/PEG-PCL-CK was quite less effective. This was attributed to the slow release of CK from the micelle form that delayed the inhibitory effect. However, this characteristic of TGPS/PEG-PCL-CK may benefit when there is a need for an extended-release form of CK for maintenance of CK concentration in cells. In the meanwhile, MSNP-CK and AP/TGPS-CK were advanced forms that enhance the anticancer activity of CK by improving drug delivery [22, 27].

On human colon cancer cell lines (HCT-116, Colo205, DLD-1, SW480, CT-26), liver cancer cell lines (BEL 7402, MHCC97-H, Hep3B, Huh7), human myeloid leukemia cell line (HL-60), and multiple myeloma (U266), CK only showed good effect with high doses (40—100 μM). However, the compound seems to be safe on normal cells with lower doses (<40 μM) [56]. This raised a caution when using this compound for treating those types of cancer in clinical studies. In addition, there was no report revealing the selective index value of the compound on any cancer cell line, which should be investigated in further studies before moving forwards with the application of CK. Instead, MSNPs-CK ~~which~~ that showed 88.27—97.46% cell growth

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inhibition at a low dose (20 µM) on HT-29 or Hep-G2 cell lines could be an alternative for such types of cells. On the other hand, Song et al. [58] reported that CK at low dose could inhibit > 50% of the growth of liver cancer cell line SMMC7721 (7.5—15 µM). This finding disagreed with other results, despite their measurement method was relatively similar [55, 56]. That means the efficacy of CK on this cell line should be studied more to obtain a conclusion relating to CK's effect. Moreover, on liver cancer cell lines (SMMC7721, Hep-G2) and breast cancer cell line (MCF-10A1a), low doses of CK (5—20 µM) could cause >55% of cell growth inhibition [58, 61] if the time of exposure was 72—96 hours. This implied that the anticancer activity on these types of cells demanded a long exposure to show its benefit. This point should be carefully considered in clinical studies as there should be a maintenance of CK concentration in the body for a long time to reach the expected effect.

Regarding the mechanism, all included studies revealed that CK caused cell death via arresting cell cycles (at phase G1 or G2), resulting in autophagy, necrosis, or apoptosis. CK-induced cell cycle arrest seemed to be different between types of cells. While most cancer cell lines were arrested at G0/G1, the arrest at phase G2/M was observed in human gastric carcinoma cell line [19, 53]. The weak effect on cycle arrest was concordant with the weak growth inhibition in human glioblastoma cell lines, which seemingly indicate that the cell cycle effect could be the main mode of action of CK on this cell line. In general, CK regulated the cell cycle via its effect on CDK-cyclin complexes that are necessary for the activation of cell cycle phases [76]. Cyclin D1/3-CDK4/6 and cyclin E-CKD2 complexes involved in the cell progression at phase G1 were mainly implicated [76]. Inhibition of the binding between both proteins caused by the increase in p21 expression and the activation JNK/c-Jun/AP-1 pathway could induce cycle arrest [76, 77]. Although the cyclin-CKD complexes suppression may be mediated via the phosphorylation of Rb, all included studies in our review did not exhibit clear evidence of whether the induction of phosphorylated-Rb resulted in the suppression of cyclin-CDK complexes in the presence of CK [57, 78]. On the other hand, CK induced arrest at phase G2 in gastric cancer cells was associated with the inhibition of cyclin B1, which mainly plays a role in phase M [19]. Consequently, these cells passed through the phase G2 but could not start the mitosis. This mode of action was also supported by the suppression of cdc2 expression, which is critical for cells moving towards mitosis [79]. Although Zhang et al. [35] suggested that FoxO3a-mediated p27/p15 and Smad3

contributed to cell cycle arrest in colon cancer cell line, no direct relationship elucidated the role of these factors in cell cycle arrest. Nevertheless, FoxO3a ~~which~~ that is involved in the PI3K/Akt pathway possibly affected the expression of p27, which plays a critical role in cycle arrest at phase G2/M [57, 80]. Meanwhile, Smad3 was thought to regulate the expression of cyclin D1 that participates in the G1 phase arrest as known before [76, 81]. These are the probable suggestions explaining the roles of FoxO3a-mediated p27/p15 and Smad3;~~,~~ however, further investigations are recommended to obtain a conclusion.

Most of our included studies indicated that the inhibitory effect of CK on cancer cells was attributed to promotion of apoptosis. Only two studies revealed the involvement of autophagy, and one study suggested the role of necrosis [32, 37, 59]. In general, the induction of apoptotic caspases and the ratio between BAX and Bcl-2 were reported in many of these studies as the main mechanisms of anticancer activity. Besides, Chen et al. [37] also realized that the increase in DR5 expression via the activation of (ROS)-JNK-autophagy pathway had a relationship with autophagy. The DR5-induced apoptosis has recently been reported in other studies as well [82, 83]. Thus, the inhibitory mechanism of CK on colon cancer cells in some cases was possibly a combination of apoptosis and autophagy. In addition, no effect on JNK pathway was seen in astrocytoma cells and bladder cancer cells although p38 expression was raised in the presence of CK [15, 47]. It seemed that CK did not induce autophagy in astrocytoma cells and bladder cancer cells since JNK pathway was not activated, and the onset of apoptosis was just caused by p38 MAPK only.

There was also a disagreement about the mechanism of action of CK in breast cancer cell line (MCF-7) [59, 60]. While Kim et al. [59] suggested that CK resulted in apoptosis in this cell line, Kwak et al. [59] demonstrated that necrosis played the main role instead of apoptosis. These two articles used the flow cytometry method to detect apoptotic cells but obtained different results. Therefore, we suggest that more experiments should be performed to confirm that mechanism.

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EGFR and Ras family proteins play crucial roles in the survival, proliferation, and differentiation of cells [84, 85]. Several EGFR inhibitors were approved for the treatment of non-small-cell lung cancer decades ago [86]. In our review, we recorded a report that revealed the downregulation of mRNA of EGFR and h-Ras genes via the strong binding with EGFR and h-Ras at Met769 and

Thr35, respectively [44]. This binding was even stronger than the erlotinib (positive control) [44]. Thus, this could be the rationale why CK had a promising effect on A549 cell line in in vitro studies reported in our review. Additionally, the mode of the inactivation of NF-κB caused by CK on Hep-G2 cells was also clarified by in silico study [51]. CK had a strong binding with Annexin A2 at Lys302. Annexin A2 protein regulates the activation of NF-κB by the interaction with subunit p50 of NF-κB, thus~~,~~ activates NF-κB boosting cell survival [87, 88]. Nevertheless, the antiproliferative activity of CK on Hep-G2 cells was also varied between studies in our review, which needs more experiments to confirm [51, 52, 56]. Especially, the anticancer activity of CK on Hep-G2 may demand a long time of exposure (72 ~~—~~92 h~~ours~~) to show its highest effect.

In vivo studies' results confirmed the in vitro anticancer activity of CK on various cancer cell lines through the decrease in volume or size of tumors. In mice implanted with human lung cancer cell lines (A549, NCI-H460), CK was used at moderate doses (15 ~~—~~37.5 mg/kg/day) [21, 22, 25, 29, 41]. The compound caused a significant effect after at least 12 days after treatment, which implicated the need for prolonged exposure in these types of cells. The effect of CK in in vivo studies for liver cancer cell lines (SMMC-7721 and BEL-7402) or nasopharyngeal carcinoma cell line (Hk-1) also seemed to be concordant with the in vitro experiments, which exhibited a promising effect at low doses (5 ~~—~~20 mg/kg/day) following a long time of treatment~~;~~ 11 ~~—~~35 days in case of liver cancer cells or short treatment duration (3 ~~—~~5 days) in case of nasopharyngeal carcinoma cell line [30, 56, 58]. Meanwhile, the weak effect of CK on MHCC97-H (HCC~~hepatocellular carcinoma~~) was also consistent between in vitro and in vivo studies as it demanded high doses (100 μg/m/day via gastric perfusion) for 35 days to result in a significant decrease in metastases in mice model [53, 54]. Briefly, the in vitro anticancer activity of CK on some human lung cancer cell lines, liver cancer cell lines, and nasopharyngeal carcinoma cell line was supported by in vivo experiments. For mice with CT-26 cell line (human colon carcinoma), CK gave a considerable decrease in the volume of tumor at low doses (0.5 ~~—~~1.0 mg/kg/d, i.p.) from week ~~two~~2, whereas its in vitro efficacy was shown at moderate-high doses [38]. Meanwhile, mice with HCT-116 (another colon carcinoma cell line) required a modest dose (30 mg/kg/d. i.p.) to diminish tumor size from day 10 [64]. The efficacy of CK on

colon carcinoma cell lines was not noteworthy in in vitro studies. Thus, the potential of CK on colon cancer cell lines should be deeply investigated in further studies.

The in vivo experiments also revealed that the dose of 15 mg/kg/day via intravenous injections was possibly safe in mice, as no abnormalities were recorded during the treatment [25]. However, the sides effect of CK must be studied further. Previously, Kim et al. [70] reported the effect of CK on CAMK-IV that resulted in AMPK activation playing an important role in the anticancer activity of CK. This also implied an effect on the intracellular concentration of calcium. Despite the presence of no report addressing the disorder of intracellular concentration of calcium relating to activators of CAMK-IV, future studies should be performed to clarify and confirm the safety of CK. Similarly, the increase in COX-2 and PEG2 on MDA-MB-231 cells (human breast cancer cell lines) by CK might intimate side effects relating to inflammation or cardiovascular diseases [57, 89].

One of the limitations in this review was the high heterogeneity among the results. Many different cancer cell lines were used in different papers, and the efficacy of CK was inconsistent and hard to be concluded. It is still hard to specify an appropriate concentration of CK for clinical studies via the current in vitro and in vivo results. Nonetheless, our review included a large number of related studies that comprehensively studied the anticancer activity of CK on different cancer cell lines as well as its mechanism of action.

## 5. CONCLUSIONS

In conclusion, our review revealed that CK had a promising anticancer activity on some human lung cancer cell lines (A549, PC-9), nasopharyngeal carcinoma cell line (Hk-1), liver cancer cell line (BEL 7402), and pediatric acute myeloid leukemia cell lines (Kasumi-1, MV4-11). The efficacy of CK could be improved using micelle forms that enhanced its solubility and the possibility of membrane delivery. CK could be used as a supplement in chemotherapies as it promoted the efficacy of such medicines. The mechanism of CK inhibition of cell growth varied but mostly related to the cell cycle arrest and the regulation of apoptotic proteins via different pathways. The safety of CK was not confirmed and required further investigations.

## AUTHOR CONTRIBUTIONS

Nguyen Tien Huy~~NTH~~ proposed the idea, designed, and supervised the study. All authors contributed to screening, extracting data, writing manuscript, and making figures and tables. Dao Ngoc Hien Tam~~DNHT~~ and Nguyen Hai Nam~~NHN~~ revised the manuscript. Nguyen Tien Huy ~~NTH~~ checked and made the final version.

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## CONFLICT OF INTEREST STATEMENT~~CONFLICT OF INTEREST~~

All authors declared that ~~we~~ they have no interest to conduct this study.

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FIGURE 1. The PRISMIA flow diagram.

FIGURE 2. The structure of CK.

FIGURE 3. Compound K caused cell cycle arrest at phase G1 and phase G2 via signal pathways.

FIGURE 4. Compound K caused cell death via apoptosis, necrosis, and autophagy.

TABLE 1. $IC_{50}$ values showing the inhibitory effect of compound K on the growth of varied cancer cell lines.

**Commented [RA6]:** AUTHO captions are correct.

| | | Design | | | Outcomes |
|---|---|---|---|---|---|
| Ref | Intervention (purity) | Duration of experiment (hours) | Method of detection (density of cell, volume of MTT, wavelength of absorption) | Cell lines | $IC_{50}$ of compound K (positive control) (μM) |
| Colon cancer cells | | | | | |
| Kang et al. and Lee et al. [31, 33] | Compound K (N/R) | 48 | MTT assay ($10^4$ cells/well, 50 μL, 570 nm) | HT-29 (human colon cancer) | 32.1–51.4 μM |
| Kim et al. [32] | Compound K (N/R) | 24 | MTT assay ($10^4$ cells/well, 50 μL, 570 nm) | HCT-116 (human colon cancer) | 32.1 μM |
| Human lung carcinoma | | | | | |
| Yang et al. [26] | Compound K (> 98%) | 24 | MTT assay ($5 \times 10^3$ cells/well, 100 μL, 490 nm) | A549 (human lung cancer) | 35.3 ± 2.4 μM |
| Liao et al. [42] | Compound K (> 95%) | 72 | MTT assay ($5 \times 10^4$ cells/well, 20 μL, 490 nm) | | 52.9 μM |
| Zhnag et al. and Yang et al. [22, 25] | Compound K (> 98%) | 24 | MTT assay ($1 \times 10^5$ cells/well, 10 μL MTT, 570 nm) | | 25.9–40.0 μM |
| Yang et al. [25] | Compound K-liposomes | 24 | MTT assay ($10^5$ cells/well, 10 μL MTT, 570 nm) | | 26.2 μM |
| Yang et al. [26] | Compound K mixed micelles (TPGS/PEG-PCL) | 24 | MTT assay ($5 \times 10^3$ cells/well, 100 μL, 490 nm) | | 40.8 μM |
| Zhang et al. [22] | Compound K mixed micelles (AP/TPGS) | 24 | MTT assay (570 nm) | | 16.5 μM |

| | | | | | |
|---|---|---|---|---|---|
| Hasegawa et al. [41] | Compound K (N/R) | 48 | MTT assay (N/R) | LLC (Lewis lung carcinoma) | 39.00 μM (13)[a] |
| [26] | Compound K (> 98%) | 24 | MTT assay ($5 \times 10^3$ cells/well, 100 μL, 490 nm) | PC-9 (human non-small cell lung cancer) | 23.2 μM |
| | Compound K Mixed Micelles (TPGS/PEG-PCL) | | | | 29.5 μM |
| Lee et al. [39, 40] | Compound K (> 99%) | 24–96 | MTT assay ($1 \times 10^4$–$8 \times 10^4$ cells/well, 50 μL, 550 nm) | PC-14 (human pulmonary adenocarcinoma cells) | 40.1—41.6 μM |
| Hepatocellular carcinoma cell line | | | | | |
| Liao et al. [42] | Compound K (> 95%) | 72 | MTT assay ($5 \times 10^4$ cells/well, 20 μL, λ = 490) | Hep-G2 (human liver cancer) | 101.762 μM |
| Zhang et al. [56] | Compound K (≥ 98%) | 48 | MTT assay ($5 \times 10^3$ cells/well, 20 μL, 490 nm) | | 40.45 μM |
| Lee et al. [39, 40] | Compound K (> 99%) | 72–96 | MTT assay ($10^4$–$8 \times 10^4$ cells/mL, 50 μL, 540 nm) | | 24.3—24.9 μM |
| Zheng et al. [53] | Compound K (> 98%) | 48 | MTT assay ($5 \times$ ~~×~~$10^3$ cells/well, 200 μL of MTT, 570 nm) | MHCC97-H (human liver cancer) | 49.8 ± 2.5 (71.3 ± 3.7 μM)[b] |
| Yim et al. [57] | Compound K (N/R) | 72 | MTT assay ($10^4$–$10^6$, 50 μL, 570 nm) | Hep3B (human liver cancer) | 36.1 μM |
| Metastatic human fibrosarcoma | | | | | |
| Hasegawa et al. [41] | Compound K (N/R) | 48 h | MTT assay (N/R) | HT1080 (human fibrosarcoma) | 55 μM |
| Gastric carcinoma cell lines | | | | | |
| Lee et al. [39] | Compound K | 72 | MTT assay ($10^4$ cells/well, 50 μL of MTT, 550 nm | MKN-45 (human gastric cancer) | 56.6 μM |
| Yim et al. [57] | Compound K | 72 | MTT assay ($10^4$–$10^6$, 50 μL of MTT, 570 nm) | MKN28 (human gastric cancer) | 33.1 μM |

| | | | | | |
|---|---|---|---|---|---|
| Bood cancer cell lines | | | | | |
| Kang et al. [69] | Compound K (N/R) | 96 | MTT assay ($10^4$–$10^5$ cells/well, 50 µL, 540 nm) | H9 (human T lymphoblastic leukemia cell lines) | 102.7 µM |
| | | | | CEM-C3 (human T lymphoblastic leukemia cell lines) | 57.8 µM |
| | | | | Molt4 (human T lymphoblastic leukemia cell lines) | 70.6 µM |
| | | | | Jurkat (human T lymphoblastic leukemia cell lines) | 41.7 µM |
| | | | | U937 (human monocytic leukemia cell line) | 32.1 µM |
| Lee et al. [39, 40] | Compound K (> 99%) | 72—96 | MTT assay ($1 \times 10^4$–$8 \times 10^4$ cells/well, 50 µL, 550 nm) | HL-60 (human myeloid leukemia cell line) | 24.3 µM |
| Choi et al. [68] | Compound K (N/R) | 24 | MTT assay ($10^4$–$10^6$ cells/well, 50 µL, 570 nm) | KMS-11 (multiple myeloma) | 75.56 µM |
| | | | | ARH-77 (multiple myeloma) | 39.37 µM |
| | | | | HS-Sultan (multiple myeloma) | 39.67 µM |
| | | | | IM-9 (multiple myeloma) | 38.58 µM |
| | | | | MC/CAR (multiple myeloma) | 38.22 µM |
| | | | | NCI-H929 (multiple myeloma) | 37.46 µM |
| | | | | KMS-18 (multiple myeloma) | 42.5 µM |
| | | | | U266 (multiple myeloma) | 46.72 µM |

[a]-Positive control: 5-FU.

[b]-Normal cell lines.

TABLE 2. The inhibition of cell survival of compound K on varied cancer cell lines.

| Design | | | | | Outcomes | | |
|---|---|---|---|---|---|---|---|
| Intervention (Purity of compound K) | Cell Lines | Cell survival method | Duration of Experiment | Concentration of compound K | Cell death (%) | Apoptosis (%) | Ref |
| Colon cancer cell lines | | | | | | | |
| Compound K (NR) | Fetal human colon cells | MTT (570 nm) | 24–72 hours | 20 μM | 1.3–10.3 | | Kim et al. and Lee et al. [32, 33] |
| Compound K (>98%) | HT-29 | MTT (490–570 nm) | 6 h | 60–70 μM | 87.14–99.31[a] | | Yao et al. [20] |
| | | | 24 hours | 20 μM | 70.92 | | Singh et al. [27] |
| | | | 24 hours | 100 μM | 86.47[a] | | Yao et al. [20] |
| | | | 48 hours | 20–30 μM | 46.41–63.18 | | Lee et al. [33] |
| | | | 48 hours | 32–64 μM | 49.54–57.39 | | Kang et al. [31] |
| | | | 48 hours | 40 μM | 74.89 | | Lee et al. [33] |
| | | | 48 hours | 70 μM | | 54.37 | Yao et al. [20] |
| | | | 48–72 hours | 80–100 μM | 93.56-100[a] | | Yao et al. [20] |
| | | | 72 hours | 20 μM | 67 | | Lee et al. [33] |
| | | | 72 hours | 50–250 μM | 6.53–22.20[a] | | Zheng et al. [34] |
| | | WST-1 (450 nm) | 24 hours | 50 μM | 26.5 | | Chen et al. [37] |
| | | N/A | 24 hours | 10–50 μM | | 1.93–4.96 | Zhang et al. and Kim et al. [35, 70] |
| | | N/A | 72 h hours | 30–50 μM | | 0.73–4.81 | Zhang et al. [35] |
| | | N/A | 48 hours | 70–80 μM | | 27.0–50.6 (early stage); 17.5–26.8 (late stage) | Yao et al. [20] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Compound K (>95%) | HCT-116 | MTT (490, 540, 570 nm) | 6 hours | 60—70 μM | 65.83—99.65[a] | | Yao et al. [20] |
| | | | 24 hours | 20 μg/mL | 6.5—47.25 | | Kim et al. and Zhang et al. [32, 35] |
| | | | 24 hours | 40—50 μM | 60.3—85.9 | | Kim et al. and Zhang et al. [32, 35] |
| | | | 24 hours | 60—100 μM | 75.8—100.0[a] | | Yao et al. [20] |
| | | | 48 hours | 40—50 μM | 96.0—99.5 | | Zhang et al. [35] |
| | | | 48 hours | 40—50 μM | 40.0—53.3[a] | | Wang et al. [36] |
| | | | 48 hours | 60—100 μM | 98.2—100.0[a] | | Yao et al. and Wang et al. [20, 36] |
| | | | 72 hours | 20 μg/mL | 60.2—78.8 | | Kim et al. [32] |
| | | | 72 hours | 30—50 μM | 94—100 | | Yao et al. and Zhang et al. [20, 35] |
| | | | 72 hours | 50—250 μM | 3.0—14.7[a] | | Zheng et al. [34] |
| | | WST-1 (450 nm) | 24 hours | 50 μM | 20.2 | 2.4—98.05 | Chen et al. [37] |
| Compound K (NR) | Colo205 | WST-1 (450 nm) | 24 hours | 50 μM | 19 | | Chen et al. [37] |
| | DLD-1 | WST-1 (450 nm) | 24 hours | 25—50 μM | 61 | | |
| Compound K (>95%) | SW480 | MTT (490—570 nm) | 24—48 hours | 40—50 μM | 90.7—100 | | Zhang et al. [35] |
| | | | 48 hours | 40—50 μM | 75—100[a] | | Wang et al. [36] |
| | | | 72 hours | 30—50 μM | 45—100 | 4.9—20.7 (early stage); 13.1—59.5 (late stage) | Zhang et al. [35] |
| | | WST-1 (450 nm) | 24 hours | 50 μM | 20 | | Chen et al. [37] |
| Compound K | CT-26 | MTT (570 nm) | 24 hours | 10—40 μM | 79.52—80.48 | | Hwang et al. [38] |
| MSNPs-CK | HT-29 | MTT (570 nm) | 24 hours | 1—20 | 30.26—97.46 | | Singh et al. [27] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Human lung carcinoma | | | | | | | |
| Compound K (>98%) | T24 | MTT (450 nm) | 24 hours | 20—25 μM | 78.2—84.3 | | Wang et al. [47] |
| | B16-Bl6 | NR | 48–120 hours | 40 μM | 86.6—100[a] | | Wakabayashi et al. [48] |
| | | | 120 hours | 10—20 μM | 61.9—79.5[a] | | |
| | PC-14 | MTT (540 nm) | 24 hours | 100 μM | 99.5 | | Lee et al. [40] |
| | A549 | MTT (570 nm) | 24 hours | 20–25 μM | 43.0—60.0 | | Previous studies [22, 43, 44] |
| | | | | 40—60 μM | 55.3—89.6 | | Previous studies [22, 43, 44] |
| | | | | 80 μM | 72.6—93 | | Zhang et al. and Li et al. [22, 43] |
| | | | | 100 μM | 97.2 | | Veronica et al. [44] |
| | | | 48 hours | 20 μM | 58.7 | | Singh et al. [27] |
| | | | 72 hours | 125–249 mg/L | 81.0–94.2 | | Wei et al. [21] |
| | H1975 | MTT (570 nm) | 24 hours | 20—80 mg/L | 53.7—61.9 | 40.15–59.07 | Li et al. [43] |
| | NCI-H460 | MTT (540 nm) | 48 hours | 30 mg/L | 37.0 | | Chae et al. [29] |
| | H460 | MTT (450 nm) | 72 hours | 20—40 μM | 24.2—35.6 (43.7[b]) | | Li et al. [45] |
| | CRT-MG | MTT (450 or 550 nm) | 24–48 hours | 5—50 mg/L | 4.3—58.6 | | Choi et al. and Jung et al. [15, 46] |
| | H1299 | MTT (450 nm) | 24–72 hours | 20 μm | 10 | 3 | Li et al. [45] |
| | A549 | N/A | 24 hours | 10 μg/mL | | 7.34 | Zhang et al. [22] |
| | | | | 20–80 mg/mL | | 44.62–64.31 | Yang et al. and Li et al. [26, 43] |
| | | | 48 hours | 15 μM | | 42.71 | Shin et al. [71] |
| | Sk-Mes-1 | | 48 hours | 15 μM | | 59.85 | Shin et al. [71] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pc-9 | | 24 hours | 14.46 μg/mL | | 31.79 | Yang et al. [26] |
| | H460 | | 24 hours | 20 μM | | 4.28 | Li et al. [45] |
| MSNPs-CK | A549 | MTT (570 nm) | 48 hours | 10 | 61.7 | | Singh et al. [27] |
| | | | | 15—20 μM | 95.7—97.5 | | |
| CK-M (AP/TPGS) | Pc-9 | N/A | 24 hours | 10 μg/mL | | 16.59 | Zhang et al. [22] |
| CK-M (TPGS/PEG-PCL) | Pc-9 | N/A | 24 hours | 21.97 mg/mL | | 40.6 | Yang et al. [26] |
| | | | | 18.35 μg/mL | | 43.48 | |
| Compound K + Gy | NCI-H460 | MTT (540 nm) | 48 hours | 30 mg/L | 45 (32°) | | Chae et al. [29] |
| Compound K + cisplatin (2.5 μM) | H460 | N/A | 72 hours | 20—40 μM | | 13.46 | Li et al. [45] |
| Compound K + cisplatin (5 μM) | | MTT (450 nm) | 24 hours | 20 μM | 78.2—84.9 (43.7[b]) | | |
| Liver cancer cells | | | | | | | |
| Compound K (>98%) | L02 (normal liver cell line) | MTT (490 nm) | 48 hours | 20—40 μM | 3.95—4.69 | | Zhang et al. [56] |
| | | | | 60 μM | 25.33 | | |
| Compound K (>95%) | Hep-G2 | MTT (540 or 570 nm) | 24—96 hours | 50 μM | 74.06—91.81 | | Shin et al. [52] |
| | | | 24 hours | 20 μM | 46.47 | | Singh et al. [27] |
| | | | 48 hours | 10—50 μM | 96—97 | | Wang et al. [51] |
| | | | 48 hours | 40—60 μM | 45.24—81.84 | | Zhang et al. [56] |
| | | | 72 hours | 5 μM | 59.94 | | Shin et al. [52] |
| | | | 96 hours | 5–100 μM | 90.85–96 | | Lee et al. and Shin et al. [40, 52] |
| Compound K (>98%) | BEL 7402 | MTT (570 nm) | 24 hours | 15 μM | 73 | | Song et al. [58] |
| | | | 72 hours | 5—15 μM | 57—90 | | |
| | MHCC97-H | MTT (570 nm) | 6—12 hours | 75—100 μM | 96—99.72 | | Zheng et al. [53] |
| | | | 24 hours | 25—75 μM | 62.5—92.6[a] | | Ming et al. [54] |
| | | | 48 hours | 75—100 μM | 90.5—100 | | Zheng et al. [53] |
| | SMMC7721 | MTT (570 nm) | 6—12 hours | 100 μM | 96.92—97.12 | | Ming et al. [55] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | MTT (570 nm) | 24 hours | 7.5—15 μM | 56—75 | | Song et al. [58] |
| | | MTT (490 nm) | 24 hours | 40—60 μM | 37.34———64.57 | 23.98———52.18 | Zhang et al. [56] |
| | | MTT (570 nm) | 24 hours | 75—100 μM | 56.73—97.5 | | Ming et al. [55] |
| | | MTT (570 nm) | 48 hours | 75—100 μM | 75.96———97.12 | | Ming et al. [55] |
| | | MTT (570 nm) | 72 hours | 75—100 μM | 93.65 | | Ming et al. [55] |
| | | MTT (570 nm) | 76 hours | 7.5—15 μM | 73—96 | | Song et al. [58] |
| | Hep3B | MTT (490 nm) | 48 hours | 40—60 μM | 41.87———68.08 | | Zhang et al. [56] |
| | | MTT (570 nm) | 72 hours | 50—100 μM | 93.31—100 | | Yim et al. [57] |
| | Huh7 | MTT (490 nm) | 24 hours | 20—60 μM | 16.54———73.79 | | Zhang et al. [56] |
| MSNPs-CK | Hep-G2 | MTT (570 nm) | 24 hours | 10—20 μM | 61.5—88.27 | | Singh et al. [27] |
| Nasopharyngeal carcinoma cells | | | | | | | |
| Compound K (>98%) | Hk-1 | MTT (540 nm) | 24 hours | 10—20 μM | 41.1—88.0 | | Law et al. [30] |
| Gastric carcinoma cell lines | | | | | | | |
| Compound K (NR) | BCG823 | MTT (550 nm) | 24 hours | 5 μM | 64.5—69.9 | 16.9—29 | Hu et al. [19] |
| | | | 24 hours | 7.5—10 μM | 86.5 | 95.5 | |
| | | | 36———48 hours | 5 μM | 93.7—97.2 | 44.8—68.6 | |
| | SGC7901 | | 24 hours | 7.5 μM | 76.0 | 18 | |
| | | | 24———48 hours | 10 μM | 87—98.6 | 37.2—88.8 | |
| Compound K (>99%) | MKN-45 | MTT (540 nm) | 24 hours | 100 μM | 93 | | Lee et al. [40] |
| Compound K (NR) | MKN28 | MTT (570 nm) | 72 hours | 50—100 | 100 | | Yim et al. [57] |
| Breast cancer cell lines | | | | | | | |
| Compound K (NR) | MCF-7 | MTT (540 or 570 nm) | 24 hours | 70 μg/mL | 59 | | Kwak et al. [59] |
| | | | 48 hours | 40———70 μg/mL | 73.1—96.1 | | Kwak et al. and Kim et al. [59, 60] |
| | | | 72 hours | 35 μg/mL | 78.3 | | Kim et al. [60] |
| | | MTT | 72 hours | 50 μg/mL | 37.9 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | (492 nm) | 96 hours | 50 μg/mL | 50.0 | | Zhang and Li [62] |
| | MCF-10A | MTT (540 nm) | 24—72 hours | 35 μg/mL | 7.7—20 | | Kim et al. [60] |
| | MCF-10A1a | MTT (570 nm) | 24—48 hours | 10—20 μM | 45—55 | | Lee et al. [61] |
| | | | 72 hours | 10—20 μM | 55—70 | | |
| | MDA-MB-468 | MTT (570 nm) | 72 hours | 50—100 | 100 | | Yim et al. [57] |
| | Hs578T | MTT (570 nm) | 72 hours | 50—100 | 100 | | |
| | MDA-MB-231 | MTT (570 nm) | 72 hours | 50—100 | 100 | | |
| | MCF10DCIS | MTT (570 nm) | 24 hours | 20 μM | 40 | | Lee et al. [61] |
| | | | 48—72 hours | 20 μM | 70—80 | | |
| Compound K (98%) + cisplatin (10 mg/L) | MCF-7 | MTT (492 nm) | 72—96 hours | 50 μg/mL | 55.9—65.1 | | Zhang and Li [62] |
| Blood cancer cell lines | | | | | | | |
| Compound K (99%) | Kasumi-1 (pediatric acute myeloid leukemia cell lines) | MTT (550 or 570 nm) | 48—72 hours | 10 μM | 41.7—52.6 | | Chen et al. [49] |
| | | | 48—72 hours | 20 μM | 60—85 | | |
| | | | 20 hours | 20 μM | 65.8[a] | | |
| | MV4-11 (pediatric acute myeloid leukemia cell lines) | | 48—72 hours | 10—20 μM | 50.19—70.57 | | |
| Compound K (>99%) | HL-60 (human myeloid leukemia cell line) | | 24—96 hours | 50—100 | 83—100 | | Lee et al. [39, 40] |
| Compound K | U266 (mutiple myeloma) | | 24 hours | 100 μM | 71 | | Park et al. [50] |

Abbreviations: CK-M (AP/TPGS), cCompound K mix micelles using ascorbyl palmitate (AP)/D-α-tocopheryl polyethylene glycol 1000 succinate monoester; MSNPs-CK, mesoporous silica nanoparticles-compound K.

[a]Anti-proliferation activity.

[b] Positive control (cisplatin at 5 μM).

[c] Positive control (gamma ray).



TABLE 3. The anticancer activity of CK via in vivo studies.

| Ref | Treatment | Dose (method of drug delivery) | Duration of treatment | Animal used | Cancer cell used | Volume of tumor ($mm^3$) | Tumor size (mg) | Number of metastases |
|---|---|---|---|---|---|---|---|---|
| Yang et al. [25] | CK | 15 mg/kg/day (s.c.) | 15 days | Athymic nude mice | A549 (human lung cancer) | ↓ 40.8%** compared to negative control (on day 15) | | |
| | CK-liposomes | | | | | ↓ 67.3%** compared to negative control (on day 15) | | |
| Yang et al. [26] | CK | 15 mg/kg/day (s.c.) | 15 days | Athymic nude mice | A549 (human lung cancer) | ↓ 28.9%** compared to negative control (on day 15) | | |
| | CK-M (TPGS/PEG-PCL) | | | | | ↓ 48.9%** compared to negative control | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | (on day 15) | | |
| | Cisplatin (positive control) | 2 mg/kg | | | | ↓ 62.2%** compared to negative control (on day 15) | | |
| Zhang et al. [22] | CK | 30 mg/kg/day (tail vein injection) | 12 days | Nude mice | A549 (human lung cancer) | ↓ 35.0—44.3% compared to negative control (on days 9—15) | | |
| | CK-M (AP/TPGS) | | | | | ↓ 50.4—66.7% compared to negative control (on days 9—15) | | |
| Wei et al. [21] | CK | 37.5 mg/kg/day (i.v.) | 15 days | Nude mice | A549 (human lung cancer) | ↓ 29.3% compared to negative control (on day 15) | | |
| | CK and parthenolide | 37.5 ~~mg/kg/d~~ and 7.5 mg/kg/day (i.v.) | | | | ↓ 43.5%** compared to negative control | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | (on day 15) | | |
| | CK/parthenolide PEG liposomes | | | | | ↓ 45.7%** compared to negative control (on day 15) | | |
| | CK/parthenolide tLyp-1 liposomes | | | | | ↓ 52.4%** compared to negative control (on day 15) | | |
| | Cisplatin | 1 mg/kg (i.v.) | | | | ↓ 52.9%** compared to negative control (on day 15) | | |
| Hasegawa et al. [41] | CK | 10 mg/kg/day (p.o.) | 14 days | C57BL/6 | LLC (Lewis lung carcinoma) | ↓ 55.3%** compared to negative control (on day 14) | | |
| | FU (p-Positive control) | | | | | ↓ 48.2%** compared to negative control | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | (on day 14) | | |
| Chae et al. [29] | γ-ray (positive control) | 30 mg/kg/day (s.c.) | 40 days | BALB/cathymic nude mice | NCI-H460 cells (hHuman lung carcinoma) | ↓ 37.0––46.0% compared to negative control (on days 26–40) | | |
| | CK | | | | | ↓ 36.9––54.8% compared to negative control (on days 26–40) | | |
| | CK + γ-ray | | | | | ↓ 67.2––83.9%* compared to negative control (on days 26–40) | | |
| Hwang et al. [38] | CK | 0.2 mg/kg/day (i.p.) | 28 days | BALB/c mice | CT-26 (hHuman colon carcinoma) | ↓ 32.1%** compared to negative control (on week 4) | | |
| | | 0.5 mg/kg/day (i.p.) | | | | ↓ 28.0––54.8%** | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | compared to negative control (on weeks 2 – week 4) | | |
| | | 1.0 mg/kg/day (i.p.) | | | | ↓ 39.1 – 60.9%** compared to negative control (on weeks 2 – week 4) | | |
| Dougherty et al. [64] | CK | 30 mg/kg/day (i.p.) | 20 days | Nu/nu mice | HCT-116 (human colon carcinoma) | | ↓ 57.7 – 75.7%** compared to negative control (on days 10 – 20) | |
| Song et al. [58] | Cis-diaminodichloroplatinum (DDP) (positive control) | 10 mg/kg/day (i.p.) | 35 days | Balb/c nude mice | BEL-7402 cells ($2 \times 10^6$/mice) (human hepatocellular carcinoma) | | | ↓ 44.2%** compared to negative control (on day 35) |
| | | 20 mg/kg/day (i.p.) | | | | | | ↓ 69.5%** compared to negative |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | control (on day 35) |
| | CK | 5 mg/kg/day (i.p.) | | | | | | ↓ 69.2%** compared to negative control (on day 35) |
| | | 10 mg/kg/day (i.p.) | | | | | | ↓ 84.6%** compared to negative control (on day 35) |
| Zhang et al. [56] | CK | 5 mg/kg/day (i.v.) | 15 days | BALB/c mice | SMMC-7721 (liver cancer cell) | ↓ 26.2–35.6%** compared to negative control (on days 11–15) | ↓ 37.7%** compared to negative control (on day 15) | |
| | CK | 10 mg/kg/day (i.v.) | | | | ↓ 39.3–47.5%** compared to negative control (on days 11–15) | ↓ 43.5%** compared to negative control (on day 15) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CK | 20 mg/kg/day (i.v.) | | | | ↓ 39.3–43.6%** compared to negative control (on days 11–15) | ↓ 50.7%** compared to negative control (on day 15) | |
| Hu et al. [19] | CK | 2.5 mg/kg/day (s.c.) | 27 days | Balb/c nude mice | SGC7901 (gastric carcinoma) | ↓ 30.7–52.7% compared to negative control (on days 12–21) | ↓ 60.5% compared to negative control (on day 27) | |
| | | 5.0 mg/kg/day (s.c.) | | | | ↓ 60.1–73.3% compared to negative control (on days 12–21) | ↓ 73.0% compared to negative control (on day 27) | |
| | | 10.0 mg/kg/day (s.c.) | | | | ↓ 73.3–96.4% compared to negative control (on days 12–21) | ↓ 89.2% compared to negative control (on day 27) | |
| Law et al. [30] | CK | 10 mg/kg/day (s.c.) | 5 days | BALB/c nude mice | HK-1 (nasopharyngeal carcinoma) | ↓ 18.2–26.2% compare | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | d to negative control (on days 3–5) | | |
| Ming et al. [54] | CK | 50 μg/m/day (p.o.) | 35 days | BALB/c athymic nude mice | MHCC97-H (hHepatocellular carcinoma) | | | ↓ 87%** compared to negative control (on day 35) |
| | | 100 μg/m/day (p.o.) | | | | | | ↓ 95.2%** compared to negative control (on day 35) |
| Lee et al. [61] | CK | 0.2 mg/kg/day (i.p.) | 21 days | Nude mice | MCF10DCIS.com cells (hHuman breast cancer) | ↓ 63.8–66.7% compared to negative control (on days 14–21) | | |
| | CK | 1 mg/kg/day (i.p.) | | | | ↓ 66.7–69.0% compared to negative control (on days 14–21) | | |

**Significant difference compared to γ-ray and compound K groups.

*Significant difference.

TABLE 4. The interference of CK on the cell cycle.

| Ref | Design: Intervention (purity) | Design: Dose/aAdministration route (dDuration of treatment) | Design: Type of cancer cells | Design: Cell lines | Outcomes: Cell cycle phase | Outcomes: | Probable mechanism |
|---|---|---|---|---|---|---|---|
| Leukemia cell lines | | | | | | | |
| Kang et al. [65] | Compound K (N/A) | 30 μM (12–72 hours) | Human monocytic leukemia cell line | U937 cells | G1 | ↑ 41–68% | ↑ p21 expression<br><br>Inactivating cyclin D, the cdk4 protein, and cyclin E<br><br>Activating JNK and<br><br>the transcription factor AP-1 |
| Chen et al. [49] | Compound K (99%) | 20 μM (12–20 hours) | Pediatric acute myeloid leukemia cell line | Kasumi-1 | G1 | ↑ 6.1–11.6%* | |
| | | | | | S | ↓ 33.9–52.2%* | |
| | | | | | G2 | ↓ 3.5–7.2% | |
| Human hepatocellular carcinoma cell lines | | | | | | | |
| Dong et al. [66] | Compound K (N/A) | 5 μM–10 μM (48 hours) | Human liver hepatocellular carcinoma cell line | Hep-G2 | G0/G1 | ↑ 36.1–55.3% | ↑ expression levels of the proapoptotic proteins cleaved-caspase-9, cleaved-caspase-3 and Bax.<br><br>↓ the antiapoptotic protein, Bcl-2, and of the inactive form of PARP. |
| | | | | | S | ↓ 56.7–69.4% | |
| | | | | | G2/M | ↓ 51.2–66.7% | |
| Zheng et al. [53] | Compound K (>98%) | 25–75 μM | Human hepatocellular carcinoma | MHCC97-H | G0/G1 | ↓ 12.1–37.7% | ↑ the expression of Fas/FasL and cleaved-caspase-8 |
| | | | | | S | ↓ 29.9–75.0% | |

Commented [RA7]: AUTHO table notes have been presen

Commented [RA8]: AUTHO "a" in Table 4.

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| | | | | | G2/M | ↑ 7.42–16.1% | ↓ downstream proteins pro-caspase-9, pro-caspase-3<br><br>Inhibited Akt phosphorylation<br><br>↑Bax/Bcl-2 ratio.<br><br>These results indicated that compound K-induced apoptosis may occur through Fas- and mitochondria-mediated caspase-dependent pathways. |
| Yim et al. [57] | Compound K | 40 μM (24–48 hours) | Hepatocellular carcinoma cells | Hep3B | G1 | ↑ 12.8–16.8% | ↑ expression of p27Kip1.<br><br>↓ CDK2 kinase activity |
| | | | | | S | ↓ 44.8–56.4% | |
| Ming et al. [55] | Compound K | 20–75 μM (24 hours) | Hepatocellular carcinoma cells | SMMC7721 | G0/G1 | ↑ 2.9–18.4% | ↑ cytochrome c, p53, and Bax expression<br><br>↓pro-caspase-3 and pro-caspase-9 expressions<br><br>Unchanged levels of Bcl-2 and Bcl-XL |
| | | | | | S | ↓ 9.2–73.5% | |
| Human gastric carcinoma cell lines | | | | | | | |
| Hu et al. [19] | Compound K (N/A) | 5 μM | Human gastric carcinoma cell lines | BCG823 | G0/G1 | ↓ 3.5% | ↑ expression of p21 and ↓ expression of cdc2 and cyclin B1 |
| | | | | | S | ↓ 62.7% | |
| | | | | | G2 | ↑ 3-fold | |
| | | | | SGC7901 | G0/G1 | ↓ 9.1% | |
| | | | | | S | ↓ 40% | |
| | | | | | G2 | ↑ 2.9-fold | |
| Colon cancer cell lines | | | | | | | |

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|---|---|---|---|---|---|---|---|
| Kim et al. [32] | Compound K (N/A) | 20 μg/mL | Human colon cancer | HCT-116 | G1 | ↑ 5.7-fold* | |
| Wang et al. [36] | Compound K (N/A) | 20—40 μM (48 hours) | Human colon cancer | HCT-116 | G1 | ↑ 9.6—23.2% | |
| | | | | | S | ↑5.9—18.1% | |
| | | | | | G2/M | ↓ 36.8—52.2% | |
| | | | | SW480 | G1 | ↑18.2—42.2% | |
| | | | | | S | ↓ 53.6—31.9% | |
| | | | | | G2/M | ↓ 3.1—45% | |
| Zhang et al. [35] | Compound K (N/A) | 30—50 μM (72 hours) | Human colon cancer | HCT-116 | G1 | ↑ 1.7—3.3-fold | |
| | | | | | S | ↓ 27.2—88.4% | |
| | | | | | G2/M | ↓ 18.2—72.0% | |
| Yao et al. [20] | Compound K | 40—50 μM (48 hours) | Human colon cancer | HCT-116 | G1 | ↑ 25.7—35.8 | |
| | | | | | S | ↓ 0.4—2.8% | |
| | | | | | G2/M | ↓ 34.3—57.9% | |
| | | | | HT-29 | G1 | ↑ 28.9—43.2% | |
| | | | | | S | ↑ 6.5% (40 μM)<br>↓ 14.2% (50 μM) | |
| | | | | | G2/M | ↓ 51.1—63.2% | |

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|---|---|---|---|---|---|---|---|
| Kang et al. [31] | Compound K (N/A) | 32 μM (6—12 hours) | Human colon cancer | | G0/G1 | ↑ 25.5—32.8% | Induced the transcription of RUNX3 and p21 transcription factors<br><br>Reduced DNA methylation of RUNX3 |
| | | | | | S | ↓ 39.4% (6)<br><br>↑ 2.6% (12 h) | |
| | | | | | G2/M | ↑ 19.6% (6 h)<br><br>↓ 36.7% (12 h) | |
| Breast cancer cell lines | | | | | | | |
| Lee et al. [61] | Compound K (N/A) | 10–20 μM (48 h) | Breast cancer cells | MCF10CA1a cells | G1 | ↑ 20.0—23.3% | |
| | | | | | S | ↓ 25—35% | |
| | | | | | M | ↓ 35—50 % | |
| Yim et al. [57] | Compound K | 40 μM (24–48 hours) | Breast Cancer cells | MDA-MB-231 | G1 | ↑ 63.0—76.0% | ↑ p27Kip1 mRNA and protein expression<br><br>↓ CDK2 kinase activity. |
| | | | | | S | ↓56.2—74.2% | |
| Nasopharyngeal carcinoma cell lines | | | | | | | |
| Law et al. [30] | Compound K (>98%) | 15 μM (24 hours) | Nasopharyngeal carcinoma | HK-1 | G0/G1 | ↓ 41.7% | |
| | | | | | S | ↑ 5.3-fold | |
| | | | | | G2/M | ↓ 72.7% | |
| Human glioblastoma cell lines | | | | | | | |
| Lee et al. [63] | Compound K | 50 μM (24 hours) | Human glioblastoma | U87MG | G0/G1 | ↑ 17.63% | |
| | | | | | S | ↓ 47.9% | |
| | | | | | G2/M | ↓ 51.4% | |
| | | | | U373MG | G0/G1 | ↑ 17.1% | |

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|---|---|---|---|---|---|---|---|
| | | | | | S | ↓ 13.2% | |
| | | | | | G2/M | ↓ 42.5% | |
| Lung cancer cell lines | | | | | | | |
| Yang et al. [26] | Compound K (>98%) | 21.97 μM (24 hours) | Non-small cell lung cancer | A549 | G1 | ↑ 13.1% | |
| | | | | | G2 | ↑ 61.3% | |
| | | | | | S | ↓ 47.3% | |
| | CK-M (TPGS/PEG-PCL) | 21.97 μM (24 hours) | | | G1 | ↑ 27.4% | |
| | | | | | G2 | ↑ 34.0% | |
| | | | | | S | ↓ 59.2% | |
| | Compound K (>98%) | 14.46 μM (24 hours) | | PC-9 | G1 | ↑ 17.2% | |
| | | | | | G2 | ↓ 11.5% | |
| | | | | | S | ↓ 15.8% | |
| | CK-M (TPGS/PEG-PCL) | 14.46 μM (24 hours) | | | G1 | ↑ 29.7% | |
| | | | | | G2 | ↓ 24.4% | |
| | | | | | S | ↓ 20.6% | |
| Li et al. [43] | Compound K | 20—80 μg/mL (24 hours) | Non-small cell lung cancer | A549 | G1 | ↑ 37.7—57.6% | |
| | | | | | S | ↓ 41.8—58.2% | |
| | | | | | G2/M | ↓ 16.9—32.7% | |
| | | | | H1975 | G1 | ↑ 29.8—58.4% | |
| | | | | | S | ↓ 37.4—56.0% | |
| | | | | | G2/M | ↓ 16.0—51.0% | |

[a]Compared to negative control.

*Significant differences.