Antitumour activity of oleanolic acid: A systematic review and meta-analysis

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Abstract. Oleanolic acid (OA), a compound known for its potent antitumour properties, has been the subject of investigations in both cell and animal models. Although OA has good biological activity, its low water solubility and bioavailability limit its therapeutic use, and therefore translating the potential of OA into the clinical oncology setting remains challenging. The present systematic review and meta-analysis utilized evidence from animal model studies to gain insights into the antitumour mechanisms of OA to address the gap in understanding, and to provide guidance for future research directions and potential clinical applications. The guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses were applied in the present study and a comprehensive search was conducted across the PubMed/MEDLINE, Web of Science, Cochrane Library and Embase databases, with a cut-off date of June 30, 2023. The primary focus was on randomized controlled trials that used animal models to assess the antitumour effects of OA. The methodological quality appraisal was conducted using the Systematic Review Centre for Laboratory Animal Experimentation risk of bias tool, and tumour volume and weight served as the principal outcome measures. Data were analysed using the RevMan (version 5.3) and Stata SE11 software packages, with an assessment of heterogeneity conducted using the I^2 statistical test, sensitivity analysis conducted using the leave-one-out approach, and evaluation of publication bias performed using Egger's test and funnel plot analysis. The present study demonstrated a significant inhibitory effect of OA intervention on tumour growth and a decrease in tumour weight in animal models. Despite the broad spectrum of antitumour effects exhibited by OA, further investigations are warranted to optimize the dosage and administration routes of OA to maximize its efficacy in clinical cancer treatment.

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Introduction

Cancer has been deemed a notable public health issue by the World Health Organization due to its prominent contributions to global morbidity and mortality (1,2). Moreover, the incidence and mortality rates of cancer are rapidly increasing worldwide. By 2040, the number of cancer cases worldwide is expected to reach 30.2 million, and the number of cancer-related deaths is expected to reach 16.3 million (3). Cancer is a heterogeneous disease and its development involves multiple biological processes and multiple factors, including environmental pollution or immune dysfunction (4,5).

At present, surgery, immunotherapy, and hormone, gene, radiation, laser and targeted therapies are the primary cancer treatment methods (6-8). Despite advances in cancer treatment, drug resistance remains a notable cause of relapse and poor survival in most tumor patients (9). Natural products can be used as important substrates to overcome drug resistance and improve the efficacy of cancer therapy (8). Since the late 1930s, natural products and their derivatives have been recognized as sources of antitumour drugs (10) due to properties such as preventing and delaying tumour growth (11,12). Oleanolic acid (OA; 3β-hydroxyolean-12-en-28-oic acid), as a representative natural product, has shown marked effects in this field (13).

OA is a pentacyclic triterpene compound that is widely found in the plant kingdom; it exists both as a free acid and as a triterpenoid saponin when joint with sugar chains (14). The richest source of OA is the leaves of the olive plant (Fig. 1A) (15). OA has a diverse range of biological and pharmacological activities, including liver protection (16,17), anti-inflammatory (18,19), antidiabetic (20,21), antiviral (22,23), bidirectional immunity (24) and antitumour effects (14,25-27). The anticancer effects of OA have been demonstrated at the cellular level and in animal models in numerous types of cancer (Fig. 1B), including osteosarcoma, liver, lung and breast cancer (26,28-44). The mechanisms underlying the antitumour activity of OA are multifaceted and include the inhibition of cellular proliferation, the promotion of apoptosis, the induction of autophagy, the modulation of cell cycle regulatory proteins, the inhibition of tumour cell

migration and invasion, and the suppression of angiogenesis (44-46). Furthermore, OA increases the sensitivity of tumour cells to radiation (47). OA, akin to numerous other triterpenes, exhibits a broad spectrum of pharmacological activities, coupled with low toxicity and favourable tolerance profiles (48). However, high doses or prolonged administration of OA are reported to induce hepatotoxicity (49). According to the Biopharmaceutical Classification System (BCS), OA is categorized as a BCS Class IV drug characterized by exceedingly low aqueous solubility and suboptimal intestinal permeability, which collectively constrain its absorption and bioavailability (50). With further research, the chemical synthesis of OA derivatives and novel dosage forms may markedly improve the water solubility and bioavailability, thus strengthening their antitumour effects and ensuring their biosafety (15,16).

At present, numerous studies have reported the antitumour effects of OA in cell and animal experiments (13,25,27,34,51-53). However, research on the antitumour effects of OA in clinical applications is currently limited. The present systematic review aimed to evaluate the potential antitumour effects of OA by collecting and analysing data from animal model experiments to provide guidance for further clinical research on the application of OA in cancer.

Materials and methods

Search strategy. The present systematic review and meta-analysis were performed in accordance with the Preferred Reporting Project for Systematic Review and Meta-Analysis guidelines (54). The PubMed/MEDLINE, Web of Science, Cochrane Library and Embase databases were comprehensively searched up to June 30, 2023, using the following key words: 'Oleanolic acid', 'cancer', 'tumor' and 'tumour'. A total of 12 studies with 190 animals were included in the present study. A specific search strategy was devised for each database based on the Population, Intervention, Condition and Outcome framework and Medical Subject Heading terms (Table SI) (55,56). The present review protocol was not registered.

Study selection

Inclusion criteria. The inclusion criteria were as follows: i) The study type was a randomized controlled trial using animals; ii) experimental subjects consisted of tumour model mice, without any specific limitations on disease models or modelling methods; iii) the interventions involved the use of OA alone; iv) the outcome indicators included tumour weight and/or tumour volume; v) the studies were published in English; and vi) any type of solid tumour was included in the present study.

Exclusion criteria. The exclusion criteria were as follows: i) The target disease was a non-malignant tumour or cancer; ii) no control group was included; iii) OA was used in combination with other drugs; iv) duplicate publications; v) observational or non-interventional studies, clinical studies, case reports, reviews, conference papers, systematic reviews, meta-analyses, editorial/letters or patent results; vi) unpublished dissertations; and vii) the full text could not be obtained or the data were incomplete.

Literature screening and data extraction. Based on the inclusion and exclusion criteria, two researchers independently screened the studies using EndNote (version X20; Clarivate plc). Data were from the included studies using Microsoft Excel (Microsoft Corporation) software and a subsequent cross-check was conducted to ensure accuracy. Any disagreements were resolved by consulting a third researcher. The following data were extracted: i) First author, year and country of journal publication; ii) animal species, weight, age, sample size and cancer type; iii) OA dosage, administration method and intervention time; and iv) tumour weight and tumour volume. In studies where the original publication presented the tumour sizes in only a graphical format, the Graph Grabber (version. 2.0.2; Quintessa Ltd.) program was used.

Risk of bias assessment. The Systematic Review Centre for Laboratory Animal Experimentation risk of bias tool (57) was used to evaluate the risk of bias of the included studies. The tool assessed 10 items across different domains, including selection bias, experimental bias, measurement bias, untracking bias and selective reporting bias (57). The analysis was independently performed by YZ and ZW. Any disagreements were resolved by consulting a third investigator. The tool offered three options: 'Yes' for a low bias risk, 'No' for a high bias risk and 'Unclear' when assigning bias was not possible.

Statistical methods. The data (mean ± standard deviation) from multiple intervention groups were merged in accordance with the Cochrane Handbook for Systematic Reviews of Interventions (58,59). The merged data were then analysed using RevMan (version 5.4; The Cochrane Collaboration) and Stata SE11 (StataCorp LP) software. Sensitivity and subgroup analyses were then performed to explore potential sources of heterogeneity and to assess the reliability of the results. A random-effects model was employed for the meta-analysis. $P<0.05$ was considered to indicate a statistically significant difference (60). The I^2 statistical test was used to quantify the degree of heterogeneity among the studies. An I^2 value of ≥50% was considered to indicate significant heterogeneity (58). Publication bias was assessed using Egger's test and funnel plots if ≥10 studies were included for an outcome.

Results

Literature retrieval results. The present search strategy produced a total of 3,654 articles (Fig. 2). Following the preliminary literature screen, 72 articles remained and the comprehensive review ultimately only included the results from 12 studies (27,46,47,51-53,61-66), with a total of 190 animals. The full texts of the 12 articles were read by two independent researchers who evaluated them according to the inclusion criteria.

Basic characteristics of the included studies. The present review encompassed 12 studies that addressed seven distinct types of cancer, with contributions from publications across six countries (Fig. 3A and B). Colorectal cancer emerged as the most prevalent form of malignancy within the present dataset, accounting for 34% of all cases. Notably, the journals

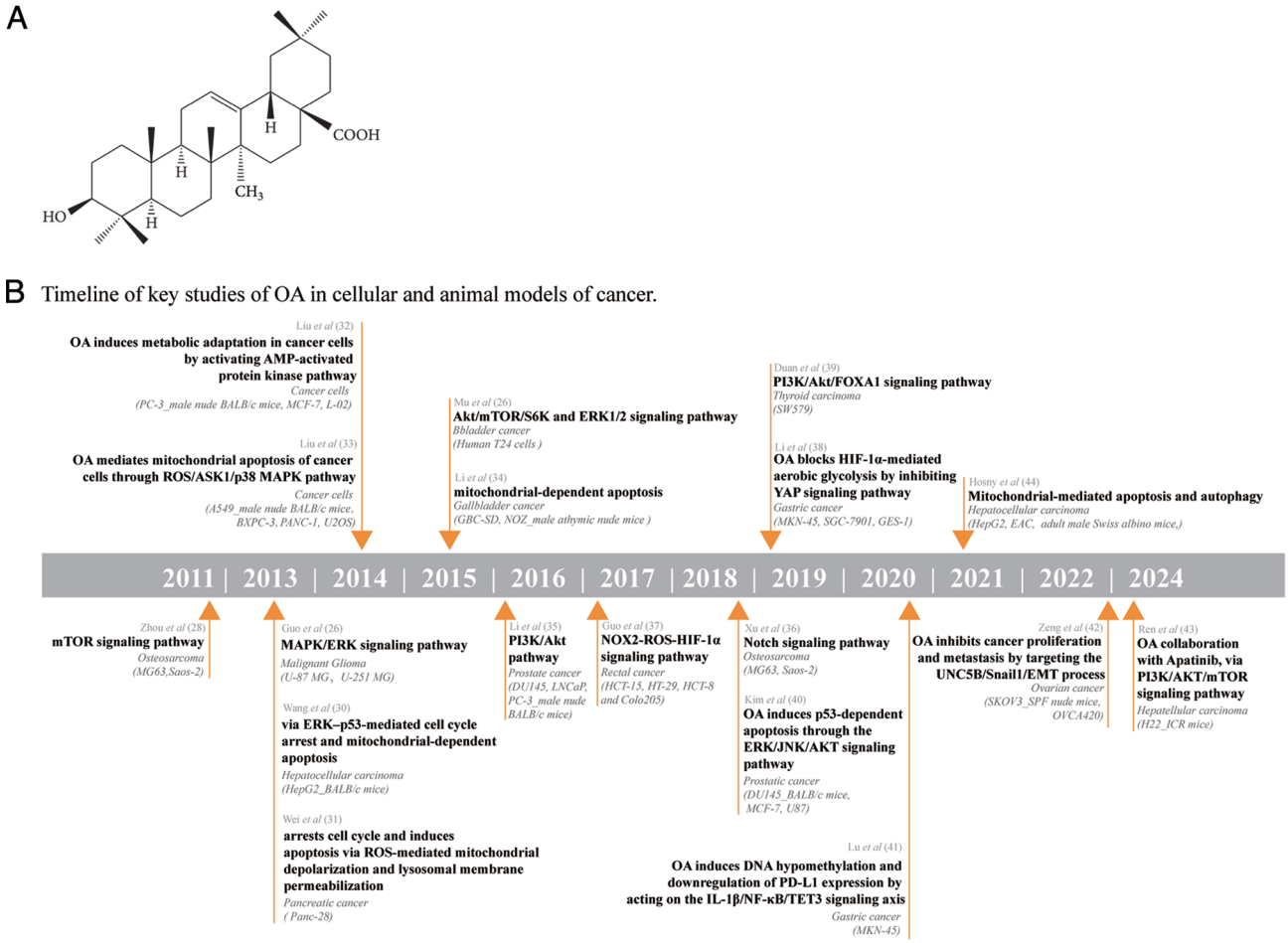


Figure 1. Chemical structure of OA and its antitumour development process. (A) Chemical structure of OA. (B) Timeline of key studies of OA in cellular and animal models of cancer. The figure provides a summary of the first author (Ref.), the molecular mechanisms through which oleanolic acid exerts its antitumor effects, the specific types of cancer investigated, and the cell and animal models utilized in the study. The literature describing only cell lines did not carry out animal studies. Cell lines: MG63, Saos-2 (osteosarcoma); U-87 MG, U-251MG (glioblastoma); HepG2, H22 (hepatoma); Panc-28 (pancreatic cancer); PC-3, DU145, LNCaP (prostate cancer); MCF-7 (breast cancer); T24 (bladder cancer); GBC-SD, NOZ (gallbladder cancer); HCT-15, HT-29, HCT-8, Colo 205 (rectal cancer); MKN-45, SGC-7901 (gastric cancer); SW579 (thyroid carcinoma); SKOV3, OVCA420 (ovarian cancer). OA, oleanolic acid; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinases; ERK, extracellular signal regulated kinases; ROS, reactive oxygen species; NOX2, NADPH oxidase 2; HIF-1α, hypoxia inducible factor-1α; YAP, yes-associated protein; FOXA1, forkhead box A1; PD-L1, programmed cell death-ligand 1; EMT, epithelial to mesenchymal transition.

from the United States and the Netherlands each contributed to 25% of the included studies.

The main characteristics of the included studies were summarised (Table I), including the following: i) First author and year; ii) species, strain, sex and age of the animals, of which BALB/c mice (n=10) were the most common, with 7 studies of female mice and 5 studies of male mice; iii) tumour types, which included colorectal (n=4), cervical (n=2), gastric (n=2), breast (n=1) and lung (n=1) cancer, hepatocellular carcinoma (n=1) and melanoma (n=1); iv) dosage of OA (range, 2-150 mg/kg); v) route of administration, including oral (n=2), intraperitoneal (n=6), intragastric (n=3) or subcutaneous (n=1) injections; vi) duration of drug intervention (range, 10-30 days); vii) type of control, with normal saline used in 50% of the included studies; and vii) country of journal publication which included the United States (n=3), Netherlands (n=3), England (n=2), Greece (n=2), United Arab Emirates (n=1) and Brazil (n=1).

This study evaluated the antitumor effects of oleanolic acid by tumor weight and tumor volume. But in addition to the information presented in Table I, further *in vitro* and *in vivo* tests using OA had been performed in the included studies of the present systematic review. A total of 5 studies had 2 intervention groups, in which two doses of OA were tested. Furthermore, 7 studies had only 1 intervention group. The most common *in vitro* tests were cytotoxicity tests (n=9) and flow cytometry (n=3). The most frequent *in vivo* assessments were tumour volume (n=12), tumour weight (n=8) and animal weight (n=8) measurements.

Results of the risk of bias assessment. All studies included in the present review were evaluated using the RoB guideline's assessment of the risk of bias (Table SII). Numerous parameters were described as 'unclear', which indicated that the information reported in the reviewed articles was incomplete or unclear. This lack of clarity was predominantly related to sequence generation, baseline features and covert grouping. Of note, in none of the included studies, the evaluators were blinded regarding the results; however, this deficiency did not affect the accuracy of the results due to the objectivity of the measured results. As a result, these studies were identified as low-risk in the bias risk assessment.

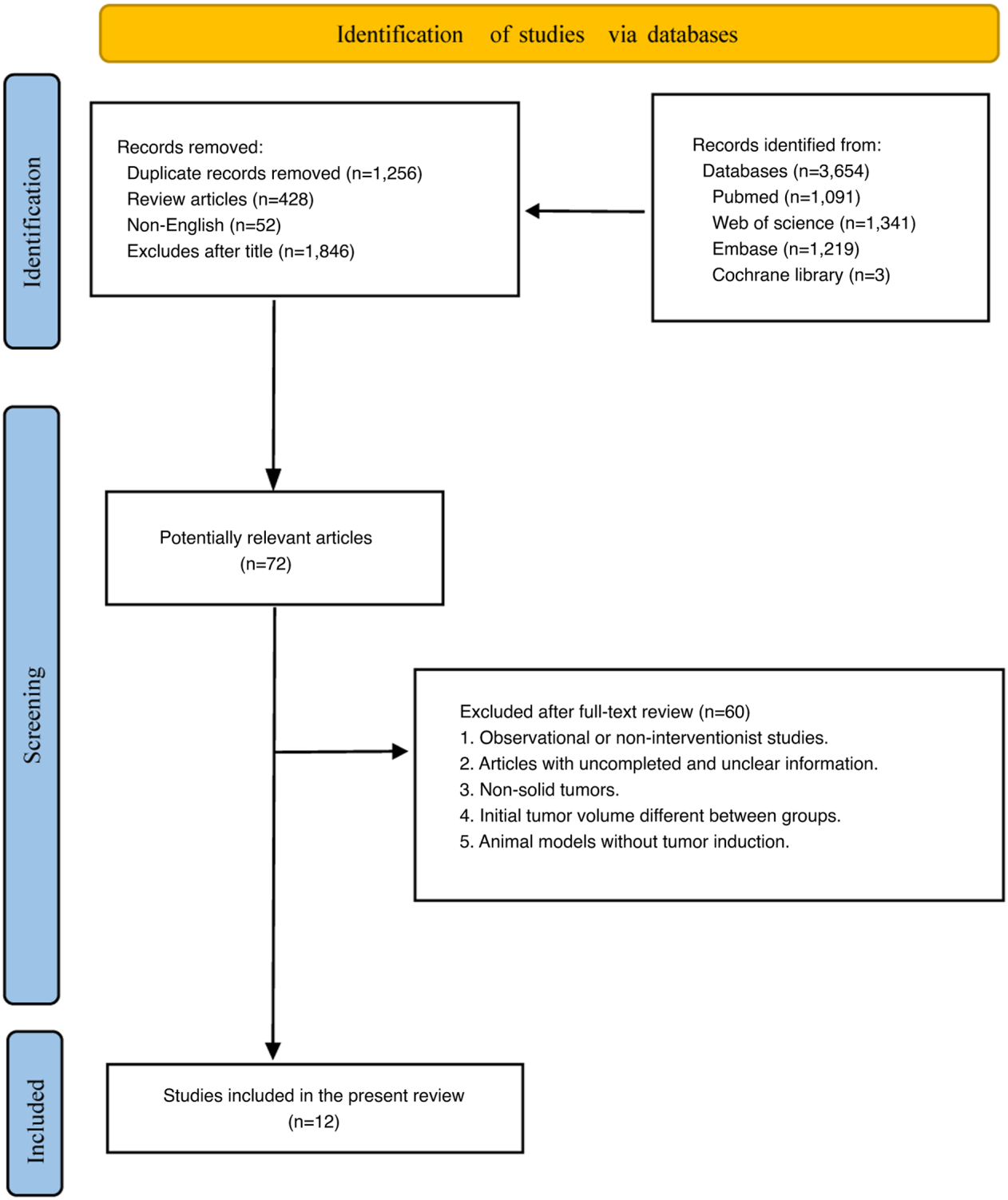
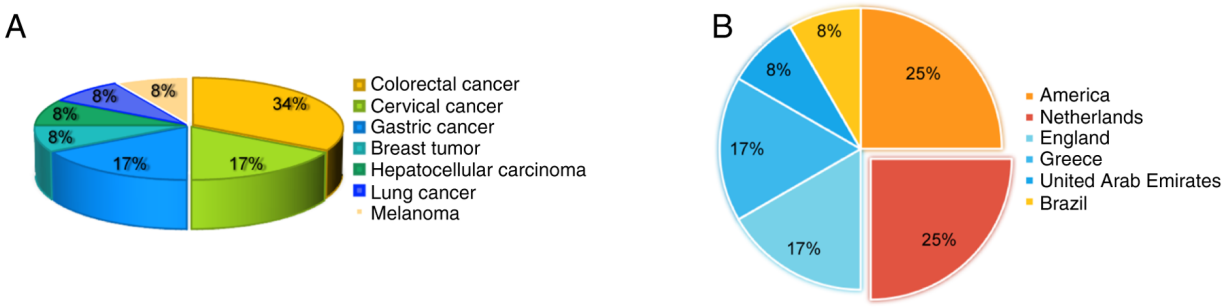


Figure 2. Flow diagram of the present systematic literature search strategy.

Meta-analysis. Tumour volume and tumour weight were extracted as outcome measures for subsequent analysis to evaluate the antitumour effects of OA in animal experiments. No additional studies were included in this assessment.

Tumour volume. Forest plot analysis demonstrated that 10 studies reported the tumour volumes from 159 animals (Fig. 3C). Statistically significant differences were observed between the OA groups and control groups (mean difference, -0.64; 95% CI, -0.89 to -0.39; P<0.00001). These results suggest that treatment with OA significantly inhibited tumour growth compared with the control treatment.

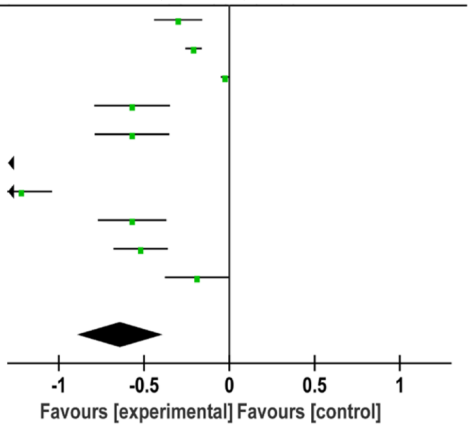
Tumour weight. In total, 9 articles assessed differences in tumour weight between the control and OA groups (Fig. 3D). The differences in tumour weight between the OA and control groups were statistically significant (mean difference, -0.43; 95% CI, -0.58 to -0.28; P<0.00001). By the end of the experiments evaluated in the present review, OA-treated animals had significantly lower tumour weights



C

Study or Subgroup	OA group Mean	SD	Total	Control group Mean	SD	Total	Weight (%)	Mean difference IV, Random, 95% CI
Gao et al, 2016	0.63	0.12	10	0.93	0.13	5	10.1	-0.30 [-0.44, -0.16]
Jiang et al, 2021	0.38	0.07	12	0.59	0.02	6	10.4	-0.21 [-0.25, -0.17]
Lee et al, 2021	0.048	0.014	5	0.073	0.018	5	10.5	-0.02 [-0.04, -0.01]
Li et al, 2015	0.79	0.14	10	1.36	0.32	10	9.7	-0.57 [-0.79, -0.35]
Li et al, 2016	0.79	0.15	10	1.36	0.31	10	9.7	-0.57 [-0.78, -0.36]
Nie et al, 2016	0.26	0.05	8	2.52	0.28	8	9.8	-2.26 [-2.46, -2.06]
Niu et al, 2018	0.26	0.06	5	1.48	0.19	5	10.0	-1.22 [-1.39, -1.05]
Potočnjak et al, 2022	0.89	0.22	10	1.46	0.16	5	9.8	-0.57 [-0.77, -0.37]
Wang et al, 2019	0.89	0.16	10	1.41	0.19	10	10.1	-0.52 [-0.67, -0.37]
Woo et al, 2021	0.78	0.23	10	0.97	0.13	5	9.9	-0.19 [-0.37, -0.01]
Total (95% CI)			90			69	100.0	-0.64 [-0.89, -0.39]

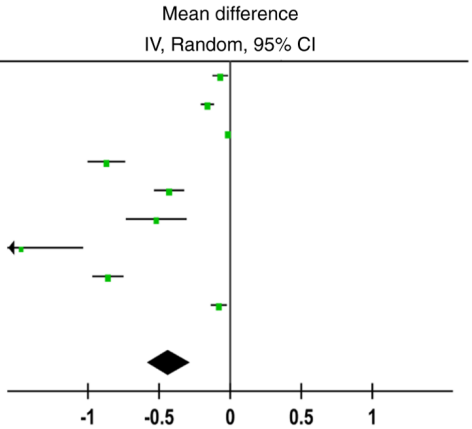
Heterogeneity: Tau² = 0.16; Chi² = 794.69, df = 9 (P < 0.00001); I² = 99%
Test for overall effect: Z = 4.99 (P < 0.00001)



D

Study or Subgroup	OA group Mean	SD	Total	Control group Mean	SD	Total	Weight (%)	Mean difference IV, Random, 95% CI
Gao et al, 2016	0.34	0.036	10	0.41	0.049	5	12.2	-0.07 [-0.12, -0.02]
Jiang et al, 2021	0.25	0.06	12	0.41	0.028	6	12.3	-0.16 [-0.20, -0.12]
Lee et al, 2021	0.021	0.004	5	0.037	0.007	5	12.4	-0.02 [-0.02, -0.01]
Liang et al, 2021	0.73	0.14	5	1.6	0.032	5	11.4	-0.87 [-1.00, -0.74]
Li et al, 2015	0.51	0.08	10	0.94	0.14	10	11.7	-0.43 [-0.53, -0.33]
Niu et al, 2018	0.37	0.11	5	0.89	0.21	5	10.0	-0.52 [-0.73, -0.31]
Potočnjak et al, 2022	1.3	0.4	10	2.77	0.4	5	6.2	-1.47 [-1.90, -1.04]
Tang et al, 2013	0.64	0.12	14	1.5	0.11	7	11.7	-0.86 [-0.96, -0.76]
Woo et al, 2021	0.33	0.04	10	0.41	0.05	5	12.2	-0.08 [-0.13, -0.03]
Total (95% CI)			81			53	100.0	-0.43 [-0.58, -0.28]

Heterogeneity: Tau² = 0.05; Chi² = 607.64, df = 8 (P < 0.00001); I² = 99%
Test for overall effect: Z = 5.62 (P < 0.00001)



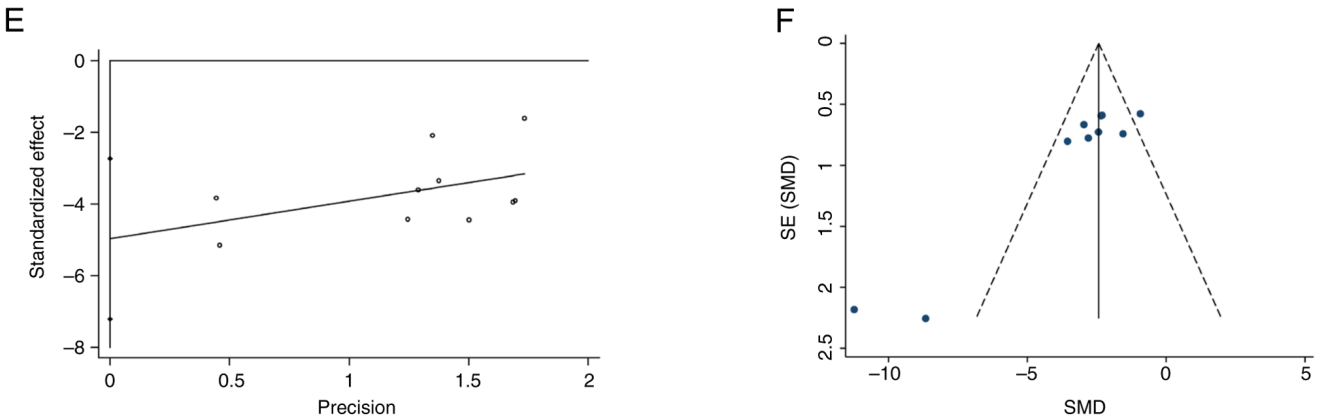


Figure 3. Analysis of the 12 included studies. Percentage distribution of the studies evaluating the antitumour effects of OA that met the inclusion criteria for this systematic review by (A) type of tumour model and (B) country of journal publication. Forest plots of the effects of OA on (C) tumour volume (cm^3) and (D) tumour weight (g). (E) Egger's test of publication bias in the present meta-analysis of tumour volume inhibition by OA treatment. (F) Funnel plot of included studies of tumour volume inhibition by OA treatment. SE, standard error; SMD, standardized mean difference; OA, oleanolic acid; SD, standard deviation; CI, confidence interval; IV, inverse variance; df, degrees of freedom.

Table I. Basic characteristics of the included literature.

First author, year	Animals				Type of cancer	Oleanolic acid			Control group	Country of journal publication	(Refs.)
	Mouse species	Sex	Age, weeks	Weight, g		Dosage groups, mg/kg (frequency)	Medication time, days	Route of administration			
Li *et al*, 2015	BALB/c athymic (nude)	Male	6	20-22	Colorectal	12.5 6 days (per week)	16	i.p.	Saline	Greece	(51)
Tang *et al*, 2013	Kunming	Female	-	-	Cervical carcinoma	37.5 and 70 (once daily)	10	p.o.	Saline	England	(61)
Liang *et al*, 2021	BALB/c	Female	4	-	Breast	30 (once daily)	14	i.g.	Corn oil	United States	(46)
Wang *et al*, 2019	BALB/c nu/nu	Male	6-8	-	Hepatocellular carcinoma	50 (once daily)	14	i.g.	Saline	United States	(47)
Li *et al*, 2016	BALB/c athymic (nude)	Male	6	20-22	Colorectal	12.5 (6 days a week)	16	i.p.	Saline	Greece	(52)
Niu *et al*, 2018	BALB/c nu/nu	Male	6-8	-	Colorectal	5 (once daily)	21	i.p.	DMSO	United Arab Emirates	(62)
Jiang *et al*, 2021	BALB/c nude	Male	5	20±2	Cervical	40 and 80 (once daily)	16	i.p.	Saline	United States	(53)
Potočnjak *et al*, 2022	Severe combined immune deficient	Female	12-15	24-28	Colorectal	2 and 10 (once daily)	21	i.g.	-	Netherlands	(27)
Lee *et al*, 2021	BALB/c nude	Female	4	18-22	Gastric	10 (once daily)	14	i.p.	DMSO	Netherlands	(63)
Gao *et al*, 2016	BALB/c nude mice	Female	-	20±2	Lung	50 and 100 (every other day)	14	s.c.	Saline	Brazil	(64)
Woo *et al*, 2021	BALB/c nude	Female	4	-	Melanoma	75 and 150 (5 times a week)	13	i.p.	-	Netherlands	(65)
Nie *et al*, 2016	BALB/c nude	Female	6	-	Gastric	100 (once daily)	30	p.o.	DMSO	England	(66)

-, no relevant information was provided in the study. Dosage was calculated as dosage=dose/(time interval of medication x medication days). i.p., intraperitoneal injection; i.g. intragastric administration; p.o., oral administration; s.c., subcutaneous; DMSO, dimethyl sulfoxide.

Table II. Sensitivity analyses of studies that reported tumor volume and weight.

A, Tumor volume

First author of omitted study, year	I^2, %	Pooled MD (95% CI)	P-value	(Refs.)
Niu *et al*, 2018	99	-0.57 (-0.81, -0.33)	<0.00001	(62)
Nie *et al*, 2016	98	-0.45 (-0.63, -0.28)	<0.00001	(66)
Wang *et al*, 2019	99	-0.65 (-0.91, -0.38)	<0.00001	(47)
Potočnjak *et al*, 2022	99	-0.64 (-0.91, -0.38)	<0.00001	(27)
Lee *et al*, 2021	98	-0.71 (-1.08, -0.34)	<0.00001	(63)
Jiang *et al*, 2021	99	-0.69 (-1.11, -0.27)	<0.00001	(53)
Woo *et al*, 2021	99	-0.69 (-0.95, -0.42)	<0.00001	(65)
Li *et al*, 2015	99	-0.64 (-0.91, -0.38)	<0.00001	(51)
Li *et al*, 2016	99	-0.64 (-0.91, -0.38)	<0.00001	(52)
Gao *et al*, 2016	99	-0.67 (-0.95, -0.40)	<0.00001	(64)

B, Tumor weight

First author of omitted study, year	I^2, %	Pooled MD (95% CI)	P-value	(Refs.)
Niu *et al*, 2018	99	-0.42 (-0.58, -0.26)	<0.00001	(62)
Potočnjak *et al,* 2022	99	-0.36 (-0.51, -0.21)	<0.00001	(27)
Lee *et al*, 2021	98	-0.51 (-0.71, -0.31)	<0.00001	(63)
Jiang *et al*, 2021	99	-0.48 (-0.67, -0.30)	<0.00001	(53)
Woo *et al*, 2021	99	-0.49 (-0.67, -0.31)	<0.00001	(65)
Li *et al*, 2015	99	-0.43 (-0.59, -0.27)	<0.00001	(51)
Tang *et al*, 2013	98	-0.35 (-0.48, -0.22)	<0.00001	(61)
Gao *et al*, 2016	99	-0.49 (-0.68, -0.31)	<0.00001	(64)
Liang *et al*, 2021	98	-0.36 (-0.50, -0.22)	<0.00001	(46)

MD, mean difference; CI, confidence interval.

compared with control animals, regardless of the tumour type.

Analysis of sources of heterogeneity

Sensitivity analysis. Of the included studies, 10 analysed the tumour volume. After excluding one study at a time, the heterogeneity remained unchanged. The I^2 of each pooled analysis remained between 98-99%, which indicated no significant change in heterogeneity. In the 9 studies that analysed tumour weight, similar results were observed after each study was excluded individually. The I^2 of each pooled analysis remained between 98-99%, which suggested no significant change in heterogeneity (Table II; Fig. S1).

Subgroup analysis. According to the Cochrane Handbook for Systematic Reviews of Interventions (58), as certain interventions shared a control group, only the total number of participants were divided into subgroup analyses. The original mean and standard deviation were unchanged.

Subgroup analyses were conducted based on the following factors: i) Dose of OA, categorized as low (<50 mg/kg), medium (50 mg/kg) or high (>50 mg/kg); ii) route of administration, including oral gavage, intraperitoneal, intragastric and subcutaneous injections; iii) type of cancer, including hepatocellular carcinoma, melanoma, and colorectal, cervical, gastric, breast and lung cancer; and iv) strains of mice utilized, including Kunming, severe combined immunodeficiency and BALB/c nude or athymic mice.

The subgroup analysis of the effects on the tumour volume demonstrated statistically significant differences among the different treatment regimens, tumour types and mouse strains (Table III). It should be noted that oleanolic acid had no significant effect on tumor volume inhibition in melanoma and gastric cancer (P>0.05). The subgroup analysis of tumour weight indicated that the dose, administration mode, tumour type and mouse strain had significant effects on the outcome (Table IV).

Risk of bias analysis. Due to the limited number of included studies that assessed tumour weight (<10 publications), the evaluation of publication bias was limited to studies that evaluated tumour volume as an outcome. Egger's test was performed to quantify bias in this parameter and a funnel plot

Table III. Subgroup analysis of tumor volume.

Variable/subgroup	No. of studies	SMD (95% CI)	I^2, %	P-value
Oleanolic acid dosage				
Low	6	-2.51 (-3.35, -1.67)	44	<0.00001
Medium	2	-2.59 (-3.56, -1.62)	0	<0.00001
High	4	-3.41 (-5.71, -1.11)	86	0.00400
Route of administration				
Intraperitoneal	6	-2.22 (-3.22, -1.22)	64	<0.00001
Oral	1	-10.62 (-14.98, -6.27)	-	<0.00001
Intragastric	2	-2.75 (-3.75, -1.75)	0	<0.00001
Subcutaneous	1	-2.29 (-3.73, -0.86)	-	0.00200
Type of cancer				
Colorectal	4	-2.62 (-3.70, -1.54)	47	<0.00001
Cervical	1	-3.38 (-4.97, -1.79)	-	<0.0001
Gastric	2	-5.78 (-14.80, 3.25)	94	0.21000
Hepatocellular carcinoma	1	-2.84 (-4.15, -1.52)	-	<0.0001
Lung	1	-2.29 (-3.73, -0.86)	-	0.00200
Melanoma	1	-0.87 (-2.01, 0.26)	-	0.13000
Mouse species				
BALB/c nude	7	-3.14 (-4.60, -1.68)	80	<0.0001
BALB/c athymic (nude)	2	-2.23 (-3.05, -1.40)	0	<0.00001
Severe combined immunodeficiency disease	1	-2.64 (-4.17, -1.10)	-	0.00080

Low, dosages <50 mg/kg; Medium, dosages of 50 mg/kg; High, dosages >50 mg/kg; SMD, standardized mean difference; CI, confidence interval.

was generated to visualize the results (y-intercept, -4.97; 95% CI, 7.22-2.73; P=0.001; Fig. 3E and F). Additionally, the funnel plot for tumour volume exhibited asymmetry and skewness, with 3 out of 10 selected studies falling outside the 95% CI. These findings suggested the presence of publication bias in studies that reported the tumour volume.

Discussion

OA is an important pentacyclic triterpene that is widely found in plants, foods and medicines; it was initially used to treat chronic hepatitis and liver injury (18,67). Žiberna *et al* (68) reported that OA has potential in clinical adjuvant anticancer treatment, supported by its direct anticancer activity, synergistic effect with chemotherapy drugs, inhibition of transporters, enhancement of radiotherapy efficacy, low toxicity and lack of adverse reactions. These attributes suggest the promising potential for OA in clinical cancer therapy.

However, the therapeutic efficacy of oleanolic acid is constrained by its limited water solubility, low bioavailability (69). Structural modification and dosage form optimization could notably increase the water solubility and bioavailability of OA (70). At present, OA derivatives are primarily modified on the C-3 hydroxyl group, C-12/C-13 double bond and C-28 carboxyl group (71). Novel OA dosage forms consist of nanoparticles, liposomes, solid dispersions and phospholipid complexes (72-75).

Research has indicated that OA demonstrates antitumour activity across a number of *in vitro* and *in vivo* models, exerting its effects on multiple targets. These targets include, but are not limited to, aldoketo reductase family member 1B10, protein tyrosine phosphatase 1B and cell division cycle 25 phosphatase (76-78). To the best of our knowledge, no systematic review has evaluated the antitumour effects of OA in animal models. Therefore, the present meta-analysis holds particular significance to address this limitation in the field and lays the groundwork for future clinical explorations of OA.

The present study systematically evaluated the antitumour effects of OA by analysing the tumour volume and weight in experimental mice. Studies have demonstrated that oleanolic acid can significantly reduce tumor weight and inhibit the volumetric growth of most tumors. However, its anti-tumor efficacy appears suboptimal for certain malignancies, such as gastric cancer and melanoma (P<0.05). This limitation may be attributed to the small sample size, which compromises the reliability of the findings. Future research should aim to increase the sample size and employ robust statistical methodologies to enhance the reliability and clinical applicability of the results. At the same time, further research on the anti-tumor mechanism of oleanolic acid and development of water-soluble and bioavailable oleanolic acid derivatives will help improve its clinical significance in tumor therapy. OA exerts its antitumour effects through the modulation of various signalling pathways. OA can induce HCT116 colon cancer cell death through the

Table IV. Subgroup analysis of tumor weight.

Variable/subgroup	No. of studies	SMD (95% CI)	I^2, %	P-value
Oleanolic acid dosage				
Low	7	-3.77 (4.88, -2.66)	54	<0.00001
Medium	1	-1.63 (2.90, -0.36)	-	0.01000
High	4	-3.04 (4.81, -1.27)	81	0.00080
Route of administration				
Intraperitoneal	5	-2.65 (3.35, -1.95)	0	<0.00001
Oral	1	-7.06 (9.60, -4.52)	-	<0.00001
Intragastric	2	-5.07 (9.14, -1.01)	66	0.00100
Subcutaneous	1	-1.63 (2.90, -0.36)	-	0.00100
Type of cancer				
Colorectal	3	-3.36 (4.37, -2.35)	0	<0.00001
Cervical	2	-4.86 (8.90, -0.81)	87	0.02000
Breast	1	-7.74 (12.27, -3.21)	-	0.00080
Gastric	1	-2.53 (4.43, -0.64)	-	0.00900
Lung	1	-1.63 (2.90, -0.36)	-	0.01000
Melanoma	1	-1.74 (3.03, -0.44)	-	0.00900
Mouse strain				
BALB/c	1	-7.74 (12.27, -3.21)	-	0.00080
BALB/c nude	5	-2.18 (2.85, -1.51)	0	<0.00001
BALB/c athymic (nude)	1	-3.61 (5.14, -2.09)	-	<0.00001
Kunming	1	-7.06 (9.60, -4.52)	-	<0.00001
Severe combined immunodeficiency disease	1	-3.46 (5.26, -1.66)	-	0.00020

Low, dosages <50 mg/kg; Medium, dosages of 50 mg/kg; High, dosages >50 mg/kg; SMD, standardized mean difference; CI, confidence interval.

p38/FOXO3a/Sirt6 pathway (27), and induce apoptosis and autophagy in AGS human gastric cancer cells through the PI3K/AKT/mTOR pathway (63). Additionally, OA inhibits the migration and invasion of glioma cells by inactivating the MAPK/ERK signalling pathway (29); it also induces apoptosis in A375SM and A375P melanoma cells through the NF-κB pathway (65). Furthermore, OA suppresses the proliferation of human bladder cancer cells by targeting the Akt/mTOR/S6K and ERK1/2 signalling pathways (26).

The present meta-analysis revealed substantial heterogeneity, with I^2 values of 99% for both the tumour volume and weight. A subgroup analysis of the included studies was performed to identify sources of heterogeneity in the data. This indicated that the mode of administration, tumour type and mouse strain were potential sources of heterogeneity. Despite a comprehensive search, the limited number of studies included in the present analysis may have introduced bias. Given the lack of standardization of animal experiments, meta-analyses of preclinical studies are more heterogeneous compared with clinical studies (57,79). Maganti *et al* (80) conducted a systematic review of preclinical animal studies on the efficacy of CRISPR/Cas9 gene-edited chimeric antigen receptor T cells against malignant tumours, demonstrating significant heterogeneity (I^2=96%), which may be attributed to the range of cancer types studied. Similarly, a meta-analysis by Singh *et al* (81) on the anticancer effects of apigenin on animal models of cancer also demonstrated high heterogeneity. Subgroup analyses demonstrated that the dose of apigenin, tumour model, route of administration and duration of treatment were significant factors that influenced heterogeneity. Furthermore, a sensitivity analysis was performed on the included studies using the leave-one-out method in the present review. After excluding one study at a time, the heterogeneity did not change significantly and the I^2-value of tumour growth inhibition and tumour weight remained between 98-99%, which indicated the stability of the results. The high heterogeneity in the present study may be attributed to variations in the animal models and experimental conditions used. Since the tumour weight index was included in <10 studies, publication bias was assessed only for the tumour volume, and Egger's test and funnel plot results both indicated publication bias.

The antitumour effect of OA is suggested to be closely related to the immune system. Programmed cell death protein 1/programmed death-ligand 1 (PD-L1) blockade therapy is a promising cancer treatment strategy that is considered to have revolutionized the treatment landscape for malignant tumours (82). Lu *et al* (41) reported that OA can restore the effect of T cells on killing gastric cancer cells, achieve DNA hypomethylation and downregulate PD-L1 by inhibiting the IL-1β/NF-κB/TET3 signalling pathway, thus serving

an antitumour role. In addition, Luo *et al* (83) reported that OA nanomicelles showed significant anticancer potential in a tumour-bearing mouse model and stimulated immune cell infiltration. As the main component of the tumour immune microenvironment, immune-infiltrating cells can effectively control tumour suppression and immune escape (84). However, through an analysis of the available literature, it was found that the quantification of the antitumour effects of OA in animal experiments has focused mainly on the tumour volume and weight, and has almost never involved an evaluation of immunity. Further studies are needed to clarify the relationship between the antitumour effects of OA and immune regulation.

The present study had several limitations, including the small number of included references and potential publication bias. But it still demonstrates significant strengths and research value. Extensive searches of major medical databases were conducted, and standard search terms were used to retrieve the relevant literature as comprehensively as possible and to reduce bias in selective reporting. An assessment of the risk of bias in the included studies increased the reliability of the results. Through subgroup analyses, the antitumour effects of OA under different conditions were further investigated, which provided guidance for further research and clinical application. In addition, this study also explored the possibility of OA enhancing the anti-tumor effect by modulating the immune system, opening up a new perspective for understanding its mechanism of action. In summary, this study provides solid theoretical support for the anti-tumor potential of oleanolic acid and promotes its transformation from laboratory research to clinical application.

The present review highlighted the antitumour potential of OA in animal models. Future studies are necessary to expand the sample size, unify the experimental design and further explore how OA serves an antitumour role by regulating the immune system. Future research could include determining the optimal dose and route of administration of OA, and developing derivatives to improve their solubility and bioavailability, while evaluating their long-term safety and toxicity. In addition, combination therapy, the standardization of preclinical models, molecular mechanism analyses, personalized medicine strategies, drug delivery system innovation and clinical trial design are key directions for promoting the clinical application of OA. Ultimately, the discovery and validation of biomarkers that predict OA responses will improve the development of precision medicine and provide novel strategies for cancer treatment.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YZ and ZW were responsible for screening the data and writing the draft paper. JZ and WJ completed the data extraction and analysis of the results. QF was responsible for a thorough review of the manuscript prior to submission. QF and YZ jointly planned the overall structural design of the paper. YZ and ZW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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