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Nanoparticles Use for Delivering Ursolic Acid in Cancer Therapy: A Scoping Review

Andang Miatmoko* , Ester Adelia Mianing, Retno Sari and Esti Hendradi

Review, Front. Pharmacol. - Pharmacology of Anti-Cancer Drugs
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Research Topic: The Inaugural Monash International Health Science and Technology Conference: Pharmacology Perspectives
Keywords: Ursolic acid, Cancer, nanoparticle, Liposome, Nanosphere, Polymeric micelle, efficacy, Toxicity

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Date	Updates
29 Nov 2021	Article accepted for publication.
25 Nov 2021	Review of Review Editor 2 finalized.
21 Nov 2021	Review of Review Editor 1 finalized.
16 Nov 2021	Corresponding Author Andang Miatmoko submitted manuscript.
	Corresponding Author Andang Miatmoko re-submitted manuscript.
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27 Oct 2021	Interactive review forum activated.
30 Sep 2021	Corresponding Author Andang Miatmoko submitted manuscript.

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1 message

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I am pleased to inform you that your manuscript "Nanoparticles Use for Delivering Ursolic Acid in Cancer Therapy: A Scoping Review" has been approved for production and accepted for publication in Frontiers in Pharmacology, section Pharmacology of Anti-Cancer Drugs.
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Manuscript ID: 787226
Authors: Andang Miatmoko, Ester Adelia Mianing, Retno Sari and Esti Hendradi
Journal: Frontiers in Pharmacology, section Pharmacology of Anti-Cancer Drugs
Article type: Review
Submitted on: 30 Sep 2021

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Independent Review Report, Reviewer 1
EVALUATION
Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the review. If you have additional comments based on Q2 and Q3 you can add them as well.
The manuscript submitted by Andang Miatmoko and colleagues is good work submitted for publication. However it can not be accepted in its present form, authors require extensive revisions for this manuscript.
The Manuscript content is not giving good idea about the actual work the authors did, at some instances it seems like research (what the need to discuss characterization of formulation, Histopathology discussed like the authors are not writing review instead they are discussing their own findings).
The authors included figure, do they have copyright?
The discussion is not darfted properly, flow of the content is not good too.
In its present form readers will not have an idea about the concept of the review, authors must have straight forward approach for the readers (In results and discussion authors described their methodology of literature survey)
Referemces shloud be revised to match with given text. For example, Author reported the Ursolic Acid belong to BCS-IV but reference number 10 which not match with these text.

The authors should also refer some review/research articles published recently on novel nanotechnology based drug delivery systems, which will be more beneficial for their work.
✓ Novel nanotechnology approaches for diagnosis and therapy of breast, ovarian and cervical cancer in female: A review
✓ Nanomedicine in treatment of breast cancer – A challenge to conventional therapy
✓ Bioactive Apigenin loaded oral nano bilosomes: Formulation optimization to preclinical assessment
✓ Implications of Solid Lipid Nanoparticles of Ganoderic Acid for the Treatment and Management of Hepatocellular Carcinoma

✓ Nanocrystals: Characterization Overview, Applications in Drug Delivery, and Their Toxicity Concerns
✓ Anticancer effect of ursolic acid stearoyl glucoside in chemically induced hepatocellular carcinoma
Check List
a. Is the quality of the figures and tables satisfactory?
No
b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
No
c. Does this manuscript refer only to published data? (unpublished or original data is not allowed for this article type)
Yes
d. Does the review include a balanced, comprehensive, and critical view of the research area?
No
QUALITY ASSESSMENT:
Rigor
2
Quality of the writing
2
Overall quality of the content
2
Interest to a general audience
3

andang miatmoko <andang-m@ff.unair.ac.id>

Independent Review Report Submitted - 787226

1 message

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Journal: Frontiers in Pharmacology, section Pharmacology of Anti-Cancer Drugs
Article type: Review
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Independent Review Report, Reviewer 2
EVALUATION
Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the review. If you have additional comments based on Q2 and Q3 you can add them as well.
The authors are compiling the literature on the various nanoparticles that were formulated to encapsulate a potent anti-cancer agent, ursolic acid. The review comprises detail mechanism of data collection and secondary data from in vivo to clinical trials.
Strengths of the study:
- Comprehensive review supported with the flow of study selection and data collection
- Authors extracted comprehensive data and discussed extensively from efficacy, pharmacokinetics and toxicity in animal to human studies

Limitations:
- Half of the references are not updated (in recent 5 years)
- Lack of significant outcome: for example: comparison between different cancer or types of nanoparticles, the dose of UA in different studies that might contribute to discrepancy in data analysis.

Suggestions:
- It is suggested to include the period/date of data collection
- Explain why the clinical trial data is limited to “liposome nanoparticles”?
- For table 2, it us suggested to include the dose/concentration of UA being formulated, with the type of cancer tumours
- It is unclear that the In vivo anti-cancer efficacy was presented in both tumour tissue (only in liver cancer?) and tumour growth inhibition (without mentioning the type of cancer?).
- The authors are comparing the efficacy of different type of nanoparticles without mentioning the dose comparison

- It would be great if authors could derive some outcomes/impact of the research such as “which nanoparticles could enhance the efficacy, pharmacokinetic or reduce toxicity of UA”??
- It is suggested that authors may include the limitations of this comprehensive review.

Check List
a. Is the quality of the figures and tables satisfactory?
Yes
b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
Yes
c. Does this manuscript refer only to published data? (unpublished or original data is not allowed for this article type)
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d. Does the review include a balanced, comprehensive, and critical view of the research area?
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Nanoparticles Use for Delivering Ursolic Acid in Cancer Therapy: A Scoping Review

Andang Miatmoko[1*], Ester Adelia Mianing[2], Retno Sari[1], Esti Hendradi[1]

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Keywords: ursolic acid, cancer, nanoparticle, liposome, nanosphere, polymeric micelle, efficacy, toxicity.

Abstract

Ursolic acid is a natural pentacyclic triterpenoid that exerts a potent anticancer effect. Furthermore, it is classified as a BCS class IV compound possessing low permeability and water solubility, consequently demonstrating limited bioavailability in addition to low therapeutic effectiveness. Nanoparticles are developed to modify the physical characteristics of drug and can often be produced in the range of 30-200 nm, providing highly effective cancer therapy due to the Enhanced Permeation and Retention (EPR) Effect. This study aims to provide a review of the efficacy and safety of various types of Ursolic Acid-loading nanoparticles within the setting of preclinical and clinical anticancer studies. This literature study used scoping review method, where the extracted data must comply with the journal inclusion criteria of within years of 2010-2020. The identification stage produced 237 suitable articles. Duplicate screening was then conducted followed by the initial selection of 18 articles that had been reviewed and extracted for data analysis. Based on this review, the use of nanoparticles can be seen to increase the anticancer efficacy of Ursolic Acid in terms of several parameters including pharmacokinetic data, survival rates and inhibition rates, as well as the absence of serious toxicity in preclinical and clinical trials in terms of several parameters including body weight, blood clinical chemistry, and organ histipathology. Based on this review, the use of nanoparticles has been able to increase the anticancer efficacy of Ursolic Acid, as well as show the absence of serious toxicity in preclinical and clinical trials. Evenmore, the liposome carrier provides development data that has reached the clinical trial phase I. The use of nanoparticle provides high potential for Ursolic Acid delivery in cancer therapy.

1 Introduction

Cancer is a disease that can occur in almost any organ or tissue of the body when abnormal cells grow uncontrollably beyond their usual limits to invade adjacent parts of the body and/or spread to other organs (1). Data from the Global Burden of Cancer released by the World Health Organization (WHO) reported that the number of cases and deaths from cancer in 2018 totaled 18.1 million and 9.6 million respectively. Cancer-related deaths are predicted to increase to more than 13.1 million by

2030 (2). The most common types affecting men include lung, prostate, colorectal, and liver cancer, while in women, they comprise breast, colorectal, lung, cervical and thyroid cancers. In 2018, the incidence rate of liver cancer in Indonesia ranked eighth highest in Southeast Asia, while in Asia as a whole it occupied 23rd position with 348,809 cases (3).

The first-line options for cancer treatment include surgery, radiotherapy and the administering of biological and chemical drugs (chemotherapy, hormones and biological therapy). However, such forms of treatment fail to control metastatic tumors that have spread to other organs (4). Chemotherapeutic agents are predominantly toxic compounds that primarily inhibit the rapid proliferation of cancer cells, while also potentially restricting the growth of hair follicle, bone marrow and gastrointestinal cells culminating in severe undesired side effects (5). Consequently, the effect of certain natural compounds have been widely explored and it has been scientifically proven that Ursolic Acid (UA), an active agent, inhibits the proliferation of cancer cells (6).

UA is a natural pentacyclic triterpene of the cyclosqualenoid family present in many plants which can modulate cellular transcription factors, growth factor receptors, inflammatory cytokines, and numerous other molecular targets that regulate cell proliferation, metastasis, apoptosis, angiogenesis, and autophagy. The anticancer effects of UA have been reported for many types of cancer, one of which is liver cancer (7). The mechanisms of UA which produce such effects include nuclear-kappa B (NF-kB) factors and apoptosis signaling in cancer cells (8). The protease activity involving urokinase and cathepsin B, both of which are known to be associated with tumor invasion and metastasis, is also significantly inhibited by UA, interleukin-1 β (IL-1β), Tumor necrotic factor-α (TNF-α), and the expression of MMP-2 and MMP-9 (Mitochondria-Mediated Pathway) (6,9). Prolonged administration of excessive doses of UA, with an LD50 value of 9.26 g/kg in acute toxicity tests on mice (10), has the potential to cause liver cytotoxicity which is not classified as genetic toxicity. Within the Biopharmaceutical Classification System (BCS), UA is categorized as a BCS class IV compound demonstrating low permeability and solubility (10) which, consequently, requires a nanotechnology-based drug delivery system to reach the desired target (11). In particular, the development of drug delivery systems entails the use of nanoparticles targeted at cancer cells which significantly improve therapeutic and diagnostic efficacy as well as reducing unwanted side effects.

Nanotechnology represents a new therapeutic platform that employs nanoparticles (NPs) in the diagnosis and treatment of cancer. NPs are used in cancer therapy due to their relatively small size, 1-1000 nm, the fact that they frequently fall within the range of 10-200 nm, and the presence of the EPR effect (12–14). Nano-drug delivery systems have been lauded for their excellent biocompatibility properties, low toxicity, increased solubility in water, in addition to their ability to deliver targeted drugs to tissues which limits their accumulation in the kidneys, liver, spleen, and other non-targeted organs, while improving therapeutic efficacy (4,15). The delivery of nanoparticles to tumor tissues through systemic circulation can be executed through two targeting strategies, including passive targeting, when nanoparticles entering circulation will accumulate at the tumor site due to enhanced permeation and retention (EPR). In contrast, active targeting, generally employs ligand molecules such as antibodies and peptides to recognize specific antigens expressed in tumor cells or the microenvironment (4).

Many types of nanoparticles exist, including polymeric therapy (polymer-protein and polymer-drug conjugates) in which drugs are covalently bound or conjugated to polymer structures and nanoparticulate drugs. The drugs are physically trapped in assembled particles composed of different materials such as polymers (polymer micelles, dendrimers and polymer nanoparticles), lipids

(liposomes), or organometallic compounds (carbon nanotubes). The first generation of anticancer nanoparticles approved by the FDA include liposomal drugs and polymer conjugates (16,17). However, certain products can be subjected to in vivo and clinical trials, while others remain limited to in vitro studies. Therefore, the effectiveness and safety of the nanoparticle drug constitute important assessed parameters.

As for the development of drugs with nanoparticle carriers, one example is Doxil®, the first nano-drug using liposomes approved by the FDA, which demonstrates the clinical performance advantages of doxorubicin in a variety of neoplastic conditions due to pharmacokinetics and the unique EPR-related biodistribution of liposomal doxorubicin (17,18). Doxil® can reduce side effects, especially that of cardiac toxicity, and improve patients' adherence and quality of life (19). Cisplatin is an anticancer drug prepared with a polymeric micelle through the formation of a metal-polymer complex between cisplatin and poly-(ethylene glycol)-poly(glutamic acid) block copolymers (20,21). These micelles are 28 nm in size with a very narrow distribution, demonstrate extremely extended blood circulation, and accumulate effectively in solid tumor models of Lewis lung carcinoma cells. However, because they undergo chemical synthesis, toxicity and scale-up production become major issues (22–24). In addition, the development of Abraxane®, a paclitaxel-albumin-bound nanoparticle ~130 nm in size, was approved by the FDA in 2005 for the treatment of metastatic breast cancer (25,26). This formulation has been shown to have several advantages in terms of toxicity reduction compared to Taxol®. Moreover, the total dose can be given within 30 minutes without pre-treatment. However, the manner in which Abraxane® can improve survival rates and overcome P-GP-mediated drug resistance remains unclear (25).

Certain nanoparticles have been used in the delivery of UA as a cancer therapy including liposomes, polymeric nanoparticles, and polymeric micelles (27). However, at the time of writing, in contrast to other chemotherapy drugs such as Doxorubicin (Doxil®), Cisplatin, Paclitaxel (Taxol®), or Amphotericin B (Ambisome®), no review of the effectiveness and safety of several types of nanoparticles for the delivery of UA for cancer therapy has been conducted (27). This study aims to provide a literature review related to the anticancer effectiveness and safety of UA delivered with various types of nanoparticles based on pre-clinical and clinical trial results from existing research published between 2011 and 2021.

2 Methods

Article selection criteria

This study uses the scoping review method involving literature accessible through the PubMed, Sciencedirect, Scopus, and Google Scholar databases consisting of online research publications dating from 2011 to 2021. Clinical trial articles were sourced using the keywords"Clinical trial", "Ursolic Acid", "Cancer", and "OR Nanoparticle Liposome". As for the search for articles relating to in vivo studies, this employed the keywords "Pre-Clinical OR In Vivo OR Animal", Ursolic Acid", "Cancer", "Nanoparticle". Within this study, several inclusion and exclusion criteria were applied to select and screen articles for review as shown in Table 1.

123 **Table 1.** The inclusion and exclusion criteria for article screening and selection

Test Parameters	Inclusion Criteria	Exclusion Criteria
Type of research	**a)** Randomized or non-randomized phases 1, 2, or 3 clinical trials b) *In vivo* studies in animals	a) *In vitro* study b) *Ex vivo* study c) Review article
Intervention	a) Native UA as an active ingredient b) Nanoparticles (lipids, polymers, hybrid nanoparticles as carriers) c) Administration routes comprise oral route in addition to intravenous, intraperitoneal, and intratumoral injection d) Healthy patients and those suffering from all types of cancer (both individuals who have undergone surgery and those who have not)	a) Extracts containing UA and UA derivates b) Microparticles or other carrier systems more than 1000 nm in size c) Administration routes other than those meeting the inclusion criteria (topical, transdermal)
Comparison	a) No comparison with other drugs, only negative controls b) Comparison with other drugs	-
Outcome	a) Primary efficacy outcomes (improved lifespan, enhanced survival rate, tumor growth inhibition) b) Secondary efficacy outcomes (e.g., blood parameters, no complaints); improvement in physical condition (body weight, tissue histopathology); clinical and non-clinical improvements. c) Toxicity (body weight, blood parameters, clinical parameters, non-clinical parameters, adverse events, organ histopathology)	-
Types of Publications	a) Articles are written in English b) Not included as predatory journals.	The article is not written in English

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Evaluation of physical characteristics of UA nanoparticles

Data analysis involved comparing the physical characteristics of different types of nanoparticles identified in the selected articles.

Analysis of Particle size

Particle size and particle size distribution produce significant impacts on drug loading variation, drug release, bioavailability, and efficacy (28). In addition, particle sizes of 150 nm to 4.5 μm will be easily recognized by macrophages and dendritic cells during phagositosis (29). Instruments used in nanoparticle size and morphology determination include Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM).

Analysis of ζ-Potential

Zeta potential is used to predict the stability of colloidal dispersion systems during storage. Generally, ζ- potential values above +/-30 mV resulted in more stable particles because the repulsing force between particles can prevent aggregation. The ζ-potential is affected by surfactants, polymers, the surface active agent component of nanoparticles, the presence of absorbing compounds, dispersed phase media, ionic strength, and pH (28). The ζ-potential can be analyzed using the Electrophoretic Light Scattering (ELS) method (30,31).

Analysis of Encapsulation Efficiency

Entrapment Efficiency (EE%), or encapsulation efficiency, is defined as the portion of drugs encapsulated in nanoparticles. Free drugs that are not encapsulated in the drug delivery carriers or nanoparticles can be separated by means of centrifugation, dialysis, or gel chromatography. The concentration of entrapped and un-entrapped drugs can be determined through the use of instruments such as spectrophotometers or high-performance liquid chromatography (HPLC). The encapsulation efficiency percentage is calculated using the following equation:

$$EE(\%) = \frac{W_T - W_U}{W_T} \text{ x } 100\%$$

where, W_T is the total weight of AU and W_U is an un-entrapped UA weight (32).

Pharmacokinetic evaluation of UA nanoparticles

Plasma concentration versus time data was analyzed using non-compartmental methods. Peak plasma concentration (C_{max}) and time-to-peak plasma concentrations (T_{max}) were obtained through experimental observation. In elimination half-life ($t_{1/2}$) calculated as 0.693/λz, λz is the slope of the terminal phase. In areas under the curve (AUC_{0-t}) of plasma concentration versus time from zero to infinity, $AUC_{0-\infty}$ is equivalent to the total area from time = 0 to the last measurable concentration time. The value is calculated using the linear trapezoidal method up to C_{max}, log-trapezoidal method (until the last measured concentration), and extrapolated areas (33). In this study, the analysis was conducted by comparing pharmacokinetic profiles from various studies contained in the articles reviewed.

Evaluation of The Effectiveness Of Ursolic Acid Nanoparticles

The analysis was conducted by comparing the results of efficacy studies including survival rate, tumor growth inhibition, tumor weight, and tumor volume, as well as tumor tissue histopathology extracted from reviewed articles.

Cancerous Tissue Histopathology

The histological section of the liver was stained with haematoxylin and eosin (H&E) staining and subsequently compared to the histopathological appearance of each organ in the control and treatment groups (34). The microstructure and morphology of tissues were observed using a light microscope (31). Hematoxylin is a base dye that has an affinity for the acidic components of cells, primarily the nucleic acids contained in the nucleus, while eosin is an acidic dye that binds to the cell cytoplasm. As a result, H&E stained the core blue and cytoplasm orange-red (35).

Analysis of relative tumor volume

In the articles, the size of the tumor is determined by means of a calliper, while its volume is calculated using the following equation:

$$V = 0.5xy^2$$

where x is the longest and y the shortest diameter (30,36–39). In this study, the relative tumor volume is calculated using the following formulas:

$$relative\ tumor\ volume = \frac{V_T\ NC}{V_T\ AUNP} \quad \text{or} \quad relative\ tumor\ volume = \frac{V_T\ AU}{V_T\ AUNP}$$

where V_T NC is the volume of the negative control group tumor, V_T AU is the volume of the native UA treatment group tumor, and V_T AUNP is the volume of the UA nanoparticle treatment group tumor.

Analysis of relative tumor weight and growth inhibition rate

The antitumor activity of nanoparticles is assessed through the tumor growth inhibition rate (IR) or tumor growth inhibition (TGI) at the experimental endpoint, calculated using the following equations:

$$\text{IR (\%) or TGI (\%)} = \frac{W_T \text{ of negative control group} - W_T \text{ of treatment group}}{W_T \text{ of negative control group}}$$

where W_T is the weight of the tumor (31,40,41). In this study, the relative tumor weight and relative inhibition rate were each calculated using the following formulas:

$$\text{Relative tumor weight} = \frac{W_T\ NC}{W_T\ AUNP} \quad \text{or relative tumor weight} = \frac{W_T\ AU}{W_T\ AUNP}$$

where W_T NC is the weight of the negative control group tumor, W_T AU is the tumor weight of the native UA treatment group, and W_T AUNP is the volume of the UA nanoparticle treatment group tumor.

$$Relative\ inhibition\ rate = \frac{I_R\ AUNP}{I_R\ AU}$$

where I_R AUNP is the tumor inhibition rate of UA nanoparticle treatment group, and I_R AU represents the tumor inhibition rate of the native UA treatment group.

Analysis of relative survival rate

Survival rates can be used as a standard assessment of effective therapy. The survival period is usually calculated from the date of diagnosis or commencement of the treatment period. The survival curve of each group was estimated using the Kaplan-Meier method with the average survival time difference being assessed by means of a log-rank test (38). This method involves non-parametric estimation of the survival function commonly used to describe the survival of a single population or compare the survival of two populations. The Kaplan-Meier estimate is one of the most effective statistical methods of measuring the probability of a patient's survival observed during a post-treatment period (42). In this study, the relative survival rate was calculated using the following formula:

$$Relative\ survival\ rate = \frac{S_R\ AUNP}{S_R\ NC}$$

where S_R AUNP is the survival rate following the administration of UA nanoparticles and S_R NC is the survival rate of the negative controls.

Evaluation of toxicity of UA nanoparticles in pre-clinical trials

Weight analysis

Weight loss represents a significant parameter of biological safety analysis or drug safety. The weight of the mice subjects is measured on the day of tumor inoculation and continues daily, or at least several times per week, during treatment. Each treatment group mouse is quantitatively weighed with the result being compared to that of a normal mouse in order to identify any significant difference between the two groups (43). In this study, the weight of the mice was calculated using the following formulas:

$$\text{Relative body weights} = \frac{W_B\ NC}{W_B\ AUNP} \quad \text{or} \quad \text{Relative body weights} = \frac{W_B\ AU}{W_B\ AUNP}$$

where W_B NC is the mice body weight in the administration of negative control, W_B AU is the mice body weight in the administration of native UA, and W_B AUNP represents the mice body weight in the administration of UA nanoparticles.

Other toxicity analysis

Other toxicity data on the in vivo studies was derived by data recapitulation that included: tissue histopathology, increased levels of ALT and AST, and the amount of WBC as an indicator of hematological toxicity (36,44,45).

Evaluation of AU-NP Toxicity in clinical trials

Analysis of clinical chemistry data

Toxicity was evaluated in all subjects treated with at least one cycle of UA Liposome therapy. Hematological parameters (red blood cells, WBC, hemoglobin, ANC, and platelets), urinary routines (urine protein, glucose, erythrocytes, leukocytes, and urine bilirubin), and stool routines (stool erythrocytes and stool leukocytes) were evaluated. Blood biochemistry including ALT, AST, ALP, gamma-glutamyl transpeptidase (GGT) were further analyzed (46). In this study, the analysis was conducted by comparing clinical laboratory data (ALT, AST, GGT, DBIL, and TBIL) extracted from reviewed articles.

Analysis of Adverse Events

Adverse events are used to assess unintended events (AE) in healthy adult volunteers and patients with advanced solid tumor disease. In addition, the toxicity can be seen from the value of the maximum tolerated dose (MTD) used to determine the highest dose of the drug that can be administered without adverse events. The adverse events documented during the study were classified as mild, moderate, or severe based on the dosage (43). In this study, the analysis was conducted by comparing adverse events or side effects occurring in subjects who had received the treatment mentioned in reviewed articles.

3 Results and Discussion

This study provides a literature review focusing on the anticancer effectiveness and safety of UA delivered with various types of nanoparticles to increase its anticancer effects as confirmed by both pre-clinical and clinical trials. Literature searches of all four databases using pre-determined keywords identified 237 articles in the prescreening stage as can be seen in Figure 1. Of the total literature reviewed, duplication screening was conducted using the Mendeley application to produce a final body of 226 articles. Application of exclusion criteria resulting in a body of 196 selected articles which were subsequently subjected to inclusion criteria to produce a final total of 30. The initial selection process identified 24 articles which were subsequently reviewed, culminating in 18 which satisfied the inclusion criteria. The summary of reviewed articles is presented in Table 2.

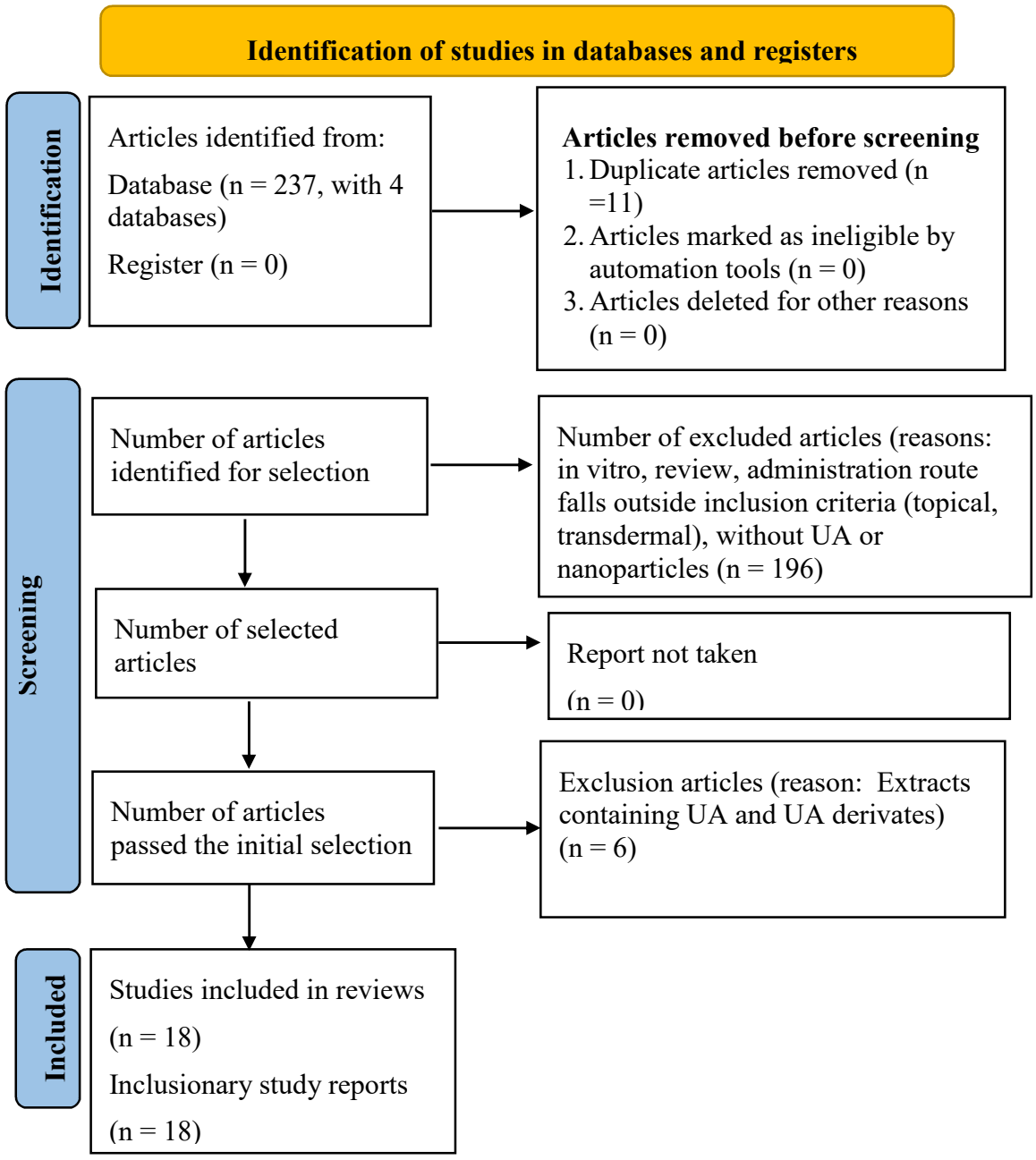

Figure 1. Flow chart of PRISMA method for article identification, screening, and selection

The data extraction of the literature used can be seen in Table 2.

Table 2. The summary of literature reviews for UA-loaded nanoparticles

No.	Code	Carrier Type	Formula-tion	Type of Research	Information Research	Administra-tion Route	Reference
1	Lipo A	Liposomes	Not Available	Phase I Clinical	Safety Evaluation of Double Dose and	Intravenous 4 hours	(46)

				Trials	Antitumor Activity of Ursolic Acid (UAL) Liposomes in Subjects with Advanced Solid Tumors including: Non-Hodgkin Lymphoma (24%), Hodgkin Lymphoma (24%), Renal Carcinoma (5%), Hepatocellular Carcinoma (5%), Breast Cancer (9%), Lung Cancer (9%), Other Cancers (19%)	infusion at doses equivalent to 54, 74, and 96 mg UA/m² for 14 consecutive days	
2	Lipo B	Liposomes	Not Available	Phase I Clinical Trials	Toxicity evaluation of a single dose of intravenous ursolic acid liposomes (UAL) in healthy adult volunteers and patients with advanced solid tumors including Non-Hodgkin Lymphoma, Hodgkin Lymphoma, Renal Carcinoma, and Hepatocellular Carcinoma	Intravenous (IV) route at doses equivalent to 11, 22, 37, 56, 74, and 96, and 130 mg UA/m² administered as a 4 hours infusion	(47)
3	Lipo C	Liposomes	Not Available	Phase I Clinical Trials	Toxicity evaluation of ursolic acid nanoliposomes (UANL) in healthy volunteers and patients with advanced solid tumors including: Non-Hodgkin Lymphoma (50%), Hodgkin Lymphoma (12.5%), Gut Cancer (12.5%), Hepatocellular Carcinoma (25%)	Intravenous (IV) route at doses equivalent to 74 mg/m² as a single dose, 98 mg/m² , and 74 mg/m² as double doses daily for 14 days via a 4 hours infusion	(33)
4	Lipo D	Liposomes	Hydrophobic compone	Preclinical or in vivo	Tumor inhibition activity and toxicity studies of UA-PLL-	Intravenous (IV) at a dose of	(32)

			nts (PC, Chl, and UA) at a weight ratio of 2:1:0.5; ethanol injection method.	study	HA in SCC-7 tumor-induced mice	equivalent to 20 mg UA/Kg mouse for 5 times every 4 days	
5	Lipo E	Liposomes	PEGylated UA Liposomes composed of SPC, CHOL, and UA at a weight ratio of 50:8:5, respectively; Ethanol injection method.	Preclinical or in vivo study	Tumor growth inhibition study and cytotoxicity of UA PEGylated liposomes in mice with U14 cervical carcinoma cells	Intragastric route at a dose of equivalent to 80 mg UA/kg mouse twice a day for a total of 14 days	(37)
6	Lipo F	Liposomes	Liposomes composed of hydrophobic components (SPC, CHOL and UA) at a weight ratio of 0:6:5; ethanol injection method.	Preclinical or in vivo study	Tumor growth inhibition and toxicity studies of CS-UA-L in mice with U14 cervical carcinoma cells	Intragastric route at a dose of equivalent to 80 mg UA/Kg mouse once a day for 14 days	(31)
7	Lipo G	Liposomes	Lipids-UA (HSPC/Kolesterol/ DSPE-	Preclinical or in vivo study	Tumor and growth inhibition study of UA-L in mice with 4T1 tumors (breast cancer)	Intravenous (IV) route at a dose of equivalent to 10 mg	(48)

			PEG2000 /UA = 90/0/5/5 and 90/0/5/10 , (molar ratio); thin film hydration method			UA/kg mouse for 5 times every other day	
8	Lipo H	Liposom es	Lipid compone nts of FA-UA-L: DOTAP/ CHOL/M PEG-DSPE200 0/FA-PEG-CHEMS at a molar ratio of 40:55:4,5 :0,5 (equal to weight ratio of 28; 21,3; 12,6, dan 2,1 mg). The ratio of UA to lipid is 1:20 (w/w); thin film hydration method	Preclinic al or in vivo study	Tumor growth inhibition and toxicity studies of FA-UA/siRNA-L in mice with human KB cells tumor	Intravenous (IV) injection with the dose of 4.5 mg/kg for UA and 170 µg/ kg for siRNA for 5 times every other day	(44)
9	Lipo I	Liposom es	Lipid compositi on: HSPC/C HOL/mP EG-DSPE200 0/FA-	Preclinic al or in vivo study	Efficacy study of FTL-UA for tumor inhbition in mice with human KB tumor cells	Intravenous (IV) at a dose of equivalent to 4.5 mg UA/kg mouse for 5 times	(38)

			PEG-CHEMS at molar ratio 63:32:4.5:0.5 (equal to weights amount of 48, 12, 13.4, and 5 mg), respectively. The ratio of UA to lipids is 1:20 (w/w); thin film hydration method			every other day, which is similar to 23 mg/kg or 98 mg/m^2 drug administration	
10	Nano A	Nanospheres	Not available	Preclinical or in vivo study	Tumor growth inhibition and toxicity studies of HCPT @F-Pt-PU NPs in mice with H22 subcutaneous tumors (liver cancer)	Intravenous (IV) injection at a dose of equivalent to 10 mg UA/kg mouse for 5 times every 2 days	(36)
11	Nano B	Nanospheres	NP composed of 32 mg chitosan, 10 mg UA, 30 mg EDC, and 8 mg NHS. The ratio of UA to lipids is 1:10 (w/w); overnight	Preclinical or in vivo study	Tumor inhibition study of CH-UA-NPs in mice with H22 subcutaneous tumors (liver cancer)	Oral administration at a dose of equivalent to 11 mg UA/Kg mouse once every 2 days for a total of 8 times	(49)

			magnetic stirring method				
12	Nano C	Nanospheres	NP composed of 32 mg chitosan, 10 mg UA, 30 mg EDC, and 8 mg NHS. The ratio of UA to lipids is 1:10 (w/w); overnight magnetic stirring method	Preclinical or in vivo study	FA-CS-UA-NPs tumor inhibiting activity study in MCF-7 xenograft bearing models (breast cancer)	Intraperito-nial (IP) injection at a dose of equivalent to 12.5 mg UA/kg mouse once a day for 9 times	(50)
13	Nano D	Nanospheres	Not available	Preclinical or in vivo study	Tumor growth inhibition efficacy and toxicity studies of UA-LA-ICG NPs in tumor bearing mice by murine H22-hepatocarcinoma cells induced tumor xenograft models.	Intravenous (IV) injection at a dose of 10 mg/kg of UA and 2.5 mg/kg of ICG with 5 minutes irradiation at 24 h post injection	(45)
14	Nano E	Nanospheres	Prepared by making 3 mg UA solution in ethanol (1 mL, 6,569 mM) in 10 mL of water. The ratio of UA	Preclinical or in vivo study	Tumor inhibition efficacy and toxicity studies of UA NPs in mice bearing A549 xenograft models (lung cancer)	Intravenous (IV) injection at a dose of 8 mg/kg of UA for 21 days	(51)

			and NPs was 1:10, respectively; solvent exchange preparation method.				
15	Nano F	Nanospheres	Not available	Preclinical or in vivo study	Tumor inhibiting activity and toxicity studies of UA NPs in H22-induced mice (Hepatocellular carcinoma)	Intraperitoneal (IP) injection at a daily dose of 50 mg/kg of UA for ten days	(52)
16	Nano G	Nanospheres	Self-assembly method of polymer deposition	Preclinical or in vivo study	Antitumor activity and toxicity studies of Pec-8PUH NPs in mice with 4T1 tumors (breast cancer)	Intravenous (IV) injection at a dose of 10 mg/kg of UA once every 2 days for 5 times	(53)
17	Poli A	Polymeric Micelles	PM composed of UA (4 mg) and mPEG2000-PLA2000 (40 mg) at a weight ratio of 1:10; thin film dispersion method	Preclinical or in vivo study	Antitumor activity and toxicity studies of UA-PMs in H22-induced mice (Hepatocellular carcinoma)	Intraperitoneal (IP) injection at a dose of 50 mg/kg of UA every 2 days for 6 times	(54)
18	Poli B	Polymeric Micelles	Solvent evaporation method	Preclinical or in vivo study	Antitumor activity and toxicity studies of U-SS-M in tumor bearing MG-63/Osteosarcoma (OS)	Intravenous (IV) injection at a dose of 11 mg/kg of UA	(41)

						every 3 days for 5 times	

Notes:

UAL	:	*Ursolic Acid Liposome*
UANL	:	*Ursolic Acid Nanoliposome*
UA-PLL-HA	:	*Ursolic Acid-Poly-L-Lysine-Hyaluronic Acid*
UA-PEGylated	:	*Ursolic Acid-Polietilenglikolisasi*
CH-UA-NPs	:	*Chitosan-Ursolic Acid-Nanoparticles*
CS-UA-L	:	*Chitosan- Ursolic Acid-Liposome*
CHOL/Chl	:	*Cholesterol*
DSPE-PEG2000	:	*1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N [methoxy (poly- ethylene glycol) -2000]*
DOTAP	:	*1, 2-dioleoyl-3-trimethylammonium-propane*;
EDC	:	*Ethyl-(3-3-dimethylaminopropyl) carbondiimide hydrochloride*
FA-CS-UA-NPs	:	*Folate- Chitosan-Ursolic Acid-Nanoparticles*
FA-PEG-CHEMS	:	*Folate Polyethylene Glycol Cholesteryl hemisuccinate*
FA-UA/siRNA-L	:	*Folate- Ursolic Acid/Small Interfering RNA-Liposome*
F-Pt-PU	:	*Folic Acid-Pectin-Eight-Arm PEG-UA conjugate*
FTL-UA	:	*Folate Receptor Targeted Liposome-Ursolic Acid*
HCPT @F-Pt-PU NPs	:	*Hydroxycamptothecin @folic acid-pectin-eight-arm PEG-UA nanoparticle*
HSPC	:	*Hydrogenated Soybean Phosphatidyl Choline*
mPEG2000-PLA2000	:	*Monomethoxy Polyethylene Glycol 2000 Poly Lactic Acid 2000*
MPEG-DSPE2000	:	*Monomethoxy Polyethylene Glycol 2000-Distearoyl Phosphatidylethanolamine*
NHS	:	*N-Hydroxy-Succinimide*
PC	:	*Phosphatidylcholine*
Pec-8PUH NPs	:	*pectin-eight-arm polyethylene glycol-ursolic acid/hydrooxycampothecin nanoparticle*
SPC	:	*Soybean Phosphatidyl Choline*
UA-NPs	:	*Ursolic Acid- Nanoparticles*
UA-LA-ICG NPs	:	*Ursolic Acid- Lactobionic Acid -Indocyanine Green*
UA-PMs	:	*Ursolic Acid-Polymer Micelles*
U-SS-M	:	*Micelles assembled by PEG-SS-UA (polyethylene glycol using a disulfide bond)*

Nanoparticle characterization results

From the review of the 18 articles, it was clear that three types of drug represent the most frequent delivery carriers of UA as an anticancer agent, namely; Liposome (50%), Nanosphere (39%) and Polymeric Misel (11%), (see Figure 2A).

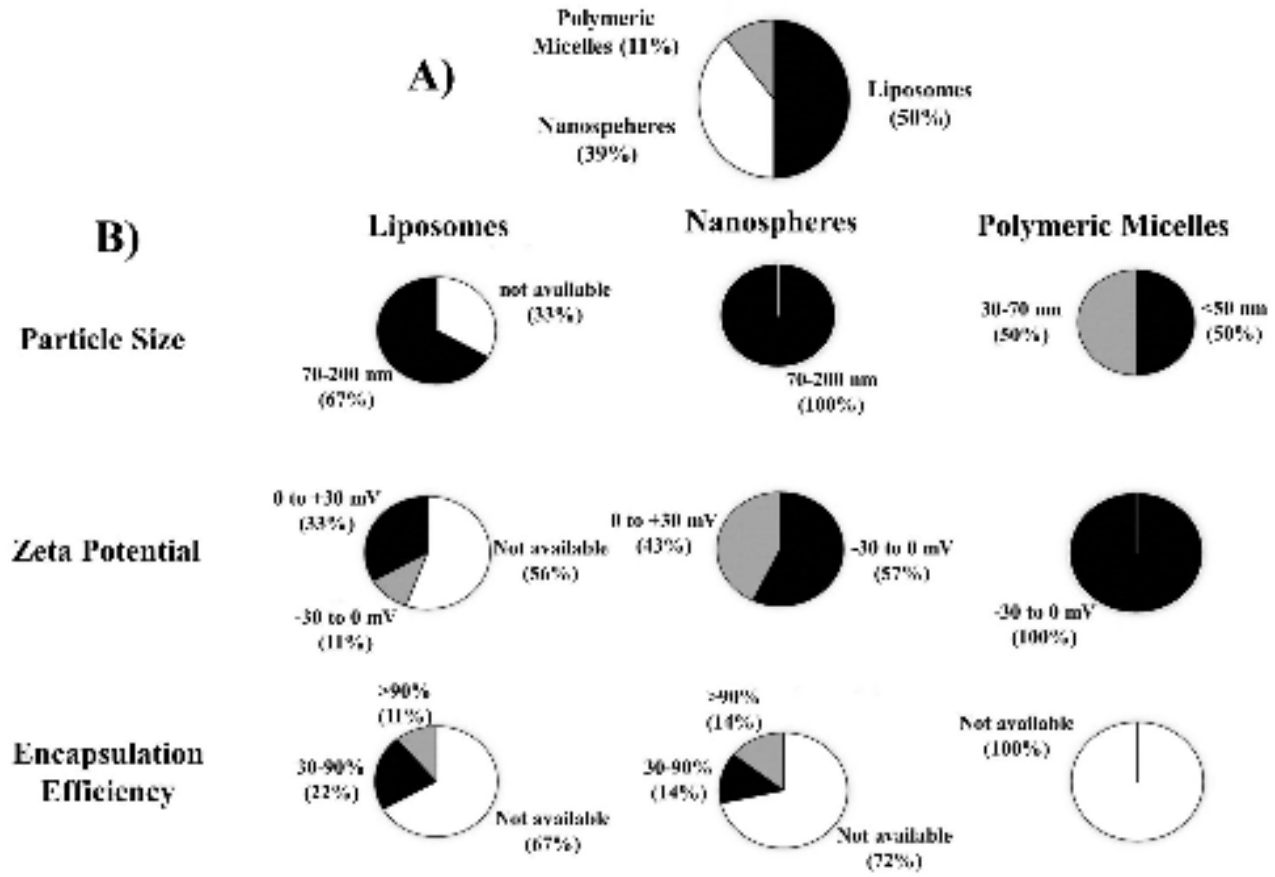

Figure 2. (A) Types of drug carrier extracted from the article review regarding the preclinical and clinical studies of nanoparticle use for UA delivery within cancer therapy, (B) the physical characteristics of UA-loaded nanoparticles including particle size, zeta potential, and efficiency of encapsulation

According to the review results, several characterization parameters of liposomes, nanospheres, and polymeric micelles exist, including particle size, ζ- potential, and encapsulation efficiency (EE). From the data analysis of the 18 articles, the size of liposome particles was found to range from 70 nm to 200 nm (67%); nanosphere particle size to be between 70nm and 200 nm (100%); and micelle polymeric particle size to be between 30 nm and 70 nm (50%). The ζ-potential of liposomes ranged from (-)30 to 0 mV (11%) and 0 to (+)30 mV (33%), while the nanosphere ζ-potentials were between (-)30 to 0 mV (57%), 0 to (+)30 mV (43%); and ζ liposomes of (-)30 to 0 mV (100%). For the EE of liposomes ≥90% (11%) and 30-90% (22%); EE nanospheres ≥90% (14%) and 30-90% (14%); as for EE polymeric micelles, these are not mentioned in the article, as presented in Figure 2B.

Characterization of liposome particle size is important because it affects the interaction of liposomes with target cells as well as the elimination, penetration and retention of drugs in the target sites (55). Phospholipids represent the main constituents of liposomal membranes and the use of lipid types and ratios within different preparation methods can affect the size of liposomes (55,56). From Figure 3A it can be seen that liposomes prepared with ethanol injection and thin-film hydration methods have particle sizes ranging from 70 nm to 200 nm. This finding is in accordance with that of previous research arguing that, with the ethanol injection method, liposome could be generated as SUVs with diameters of 30-110 nm (55,57), while with the adoption of thin film hydration methods, continued use of sonication or extrusion processes can produce liposomes as 25 nm to 1μm-sized ULVs (55).

Liposome size depends on that of the phospholipid molecule assembly whose average dimensions depend on their lipid composition, while it is supposed that the size of liposome particles increases slightly with a reduction in the molar ratio of HSPC/SPC in the range of 119-143 nm (58). On the other hand, liposomes made from DMPC, DSPC and HSPC (at a weight ratio of 2:1 to cholesterol) experienced different increases in particle size, e.g., DMPC:Chol liposomes increased in size from 149 to 190 nm, DSPC:Chol expanded from 83 to 104 nm, and HSPC:Chol liposomes from 88 to 122 nm (59).

For nanospheres, particle size, which is greatly affected by lipid type, ranges from 70nm to 200 nm. This is in accordance with a previous report stating that nanospheres have a diameter of 10-200 nm (60). With regard to polymeric micelles, studies show that particle sizes ranging from 30-70 nm are affected by polymer types based on the characteristics of hydrophilic and hydrophobic block copolymers. This finding is in keeping with that of earlier research which reported that the size of polymeric micelle particles is determined by the ratio of hydrophobic and hydrophilic block chains and can produce particle sizes of ≤50 nm (61). Increased targeting of drugs to cancer cells within the tumor tissues with the use of long-circulating polymeric micelles depends on the size of the micelle and the vascular permeability of the tumor tissues. In hypervascular tumors with highly permeable vascular structures, sub-100 nm polymeric micelles show no limits for drug extravasation and tumor penetration. In contrast, only micelles smaller than 50 nm can penetrate hipovascular tumors whose vascular permeability is poor (62).

The zeta potentials which reflect the liposome surface charges were evaluated (63). Figure 2B shows that liposomes and nanospheres had zeta potentials ranging from (-30) to 0 mV and 0 to (+)30 mV, while those of polymeric micelles varied from (-30) to 0 mV. If the system has a strong negative or positive zeta potential the particles will tend to repulse each other and no aggregation occurs. Therefore, if the system has zeta potential >+30 mV or <-30 mV, then it can be considered stable (64,65). The positive or negative charges measured in nanoparticles are highly dependent on lipid components. Analysis of the composition and intracellular delivery mechanisms confirmed that conventional liposomes had a relative neutral charge due to their neutral phospholipid composition such as HSPC and became negatively charged when added to cholesterol. pH sensitive liposomes contained a DOPE-like phospholipid component with CHEMS causing their negative charges; cationic liposomes had a cationic lipid composition such as DDAB, DOGS, DOTAP, DOTMA, DMRIE, DORIE with DOPE; Long-circulating liposomes (LCL) had a high T_C neutral lipid composition, cholesterol, added to approximately 5-10 mole % of PEG-DSPE rendering these liposomes stable when under protein opsonization (66,67).

The tendency of a drug to interact with polar or non-polar bonds and/or electrostatic interactions with lipid bilayer will determine whether it will be encapsulated into inner aqueous compartments or the lipid bilayer membrane, or whether it will be closely related to the polar head group of the bilayer membrane through electrostatic interactions. It will correlate to encapsulation efficiency (EE) or loading capacity, which is usually defined as a fraction of the percentage of the total encapsulated drug, in the bilayer membrane or aqueous intravesicular compartments or the matrix of nanoparticles (68). UA has poor permeability and low water (10), thus causing possibly encapsulated within membrane bilayer of lipid vesicles. As can be seen in Figure 2B, the EE of liposomes and nanospheres ranges from 30% to ≥90%, while the EE of polymeric micelles is not mentioned. This suggests that drugs are successfully encapsulated in nanoparticles in order to increase the amount of drugs delivered to the target sites.

Pharmacokinetic data in clinical trials

From the review of pharmacokinetic data relating to clinical trials, it was found that in Lipo A the average $t_{1/2}$ of UAL was 4.00–4.58 hours, a low value of $t_{1/2}$ resulting in its rapid elimination from the blood circulation as shown by the contents of Table 3. This suggests that UAL does not accumulate in the body but must be infused repeatedly to ensure the steady plasma concentration of UA and further enhance its antitumor effect (46). In Lipo B, a linear relationship exists between C_{max} or $AUC_{0\rightarrow 24h}$ or $AUC_{0\rightarrow\infty}$ and increased doses of UAL, signifying that UAL has a linear pharmacokinetic profile (47). In Lipo C, after administration of a single IV dose, the total concentration of UA in all subjects experienced a two-fold decrease. On completion of IV infusion, the plasma concentration of UA rapidly decreases to one approximately ten times lower than the peak concentration after two hours. The pharmacokinetics profiles of UAL are linear and dosage proportional at a range of 37 mg/m² to 98 mg/m². No accumulation of UA was observed following repeated doses of UAL in eight patients after receiving continuous IV infusion 74 mg/m² over a 14-day period (33).

Table 3. Pharmacokinetic data from clinical trials of UA-loading nanoparticles

Parameter	**Lipo A**	**Lipo B**			**Lipo C**			
Administration Route	**Intravenous**	**Intravenous**			**Intravenous**			
Dose (mg/m²)	74 (double dose)	37	74	98	37	74 (single dose)	98	74 (double dose)
$t_{1/2}$ T1/2(hours)	4.58 ± 2.04	4.59 ± 2.44	4.46 ± 1.41	3.90 ± 2.08	4.59 ± 2.44	4.46 ± 1.41	3.90 ± 2.08	4.58 ± 2.04
V_d (L/m²)	NA	NA	NA	NA	58.7 ± 33.0	64.3 ± 17.9	55.4 ± 28.1	88.6 ± 31.8
CL (L/h/m²)	NA	8.65 ± 1.09	10.2 ± 1.46	9.94 ± 1.13	8.67 ± 1.07	10.20 ± 1.46	9.94 ± 1.13	14.40 ± 3.94
AUC_{0-t} (ng·h/mL)	5172 ± 1136	4213 ± 606	7175 ± 999	9696 ± 1134	4203 ± 588	7175 ± 999	9696 ± 1134	5172 ± 1136
$AUC_{0-\infty}$ (ng·h/mL)	5498 ± 1525	4339 ± 574	7418 ±1057	9971 ± 1144	4329 ± 556	7418 ± 1057	9971 ± 1144	5498 ± 1525
MRT_{0-t} (hour)	NA	3.69 ± 0.36	3.93 ± 0.37	3.84 ± 0.34	3.69 ± 0.36	3.93 ± 0.37	3.84 ± 0.34	3.34 ± 0.55
$MRT_{0-\infty}$ (hour)	NA	4.28 ± 0.91	4.56 ± 0.88	4.41 ± 0.95	4.29 ± 0.90	4.56 ± 0.88	4.41 ± 0.95	4.31 ± 1.89
C_{max} (ng/mL)	1589 ± 635	1835 ± 438	2865 ± 868	3457 ± 856	1835 ± 438	2865 ± 868	3457 ± 856	1589 ± 635
T_{max} (hour)	NA	4.03 ± 0.04	4.02 ± 0.04	4.0 ± 0.00	4.03 ± 0.04	4.02 ± 0.04	4.00 ± 0.00	3.00 ± 1.41

Notes:

$t_{1/2}$ = half-life time; V_d = distribution volume; CL = clearance; AUC = area under curve of concentration vs time; MRT = mean retention time; C_{max} = maximum plasma concentration; T_{max} = time required to reach maximum plasma concentration

Pharmacokinetic data review on *in vivo* studies

The pharmacokinetic data on *in vivo* studies of Lipo E shows that the highest plasma UA concentration in the PEGylated liposome treatment group was 19.87 ug/mL, which exceeded that of both the Ursolic Acid Liposomes (UAL) and Ursolic Acid (UA) solution groups. In addition, as seen from Table 4, PEG-modified liposomes have the longest $t_{1/2}$, while C_{max} and AUC in the bloodstream have similar trends. This suggests that PEGylated UA liposomes may extend the time required for the drug to circulate in the circulatory system and produce a slow release effect (37).

In Nano A, after administration of a hydrophobic drug, i.e., hydroxycamptothecin (HCPT), conjugated to folic acid-pectin-eight-arm PEG-UA (F-Pt-PU) the concentration of UA and HCPT in plasma decreases slowly, resulting in the longer circulation period of native UA, which may be due to the breaking of ester bonds between 8 arm-PEG and UA. The concentration of NP HCPT@F-Pt-PU in the bloodstream, still detectable at 80 hours, is higher than that of native UA (7 hours) and HCPT (8 hours). The concentration of NP HCPT @F-Pt-PU in plasma is higher than that of np F-Pt-PU, possibly because the conjugation of HCPT into polymers increases the strength of the hydrophobic bonds in the particle cores, thereby reducing the hydrolysis rate of nanoparticles (69).

In Nano G, UA blood circulation in pectin-eight-arm polyethylene glycol-ursolic acid/hydrooxycampothecin nanoparticles (NP Pec-8PUH) at 80 hours can be maintained at a higher concentration in plasma, while native UA and HCPT rapidly disappear from plasma. The half-life of UA blood circulation in NP Pec-8PUH is 8.7 hours which is 7.3 times longer than in native UA (53).

In Poly B, polymeric drug conjugates are synthesized by conjugating UA into polyethylene glycol using disulfide bonds (U-SS-M), while UA is eliminated relatively slowly and maintained at high concentrations in plasma for up to 48 hours after administration. U-SS-M exhibits a similar pattern of biodistribution and accumulates mainly in the liver and kidneys before being subsequently eliminated by these organs. In tumor tissue, the concentration of UA decreases over time, although the amount delivered by the polymer-drug conjugate gradually increases. The concentration of U-SS-M in tumor tissue is significantly higher than that of native UA at both 6 hours and 12 hours after administration (41).

Table 4. Pharmacokinetic data summary of preclinical studies of nanoparticles containing UA

Parameter	Lipo E	Nano A	Nano G	Poli B
Administration route	intragastric	intravenous	intravenous	intravenous
UA dose (mg/kg)	80	10	10	11

$T_{1/2}$ (hour)	8.6	8.3 and 10	8.7	4.9 and 5.2
AUC (µg.h/mL)	134.061	NA	NA	NA
C_{max} (µg/mL)	19.87	NA	NA	NA
T_{max} (hour)	NA	80	80	48

Notes:
$T_{1/2}$ = half-life time; AUC = area under curve of concentration versus time;C_{max} = maximum plasma concentration; T_{max} = time required to reach maximum plasma concentration

Recapitulation of pre-clinical and clinical research relating to UA nanoparticles

The results indicate that the available articles which discuss pre-clinical/in vivo trials amounted of 83%, including the use of nanoparticle carrier types of nanospheres (47%), liposomes (40%), and polymeric micelles (13%). As for those that discussed clinical trials (17%), as seen in Figure 5, these featured only the use of liposomes (100%). Clinical trials are still being conducted in phase 1, indicating that they remain at the stage of evaluating dose levels, acute toxicity, and drug distribution in humans (43).

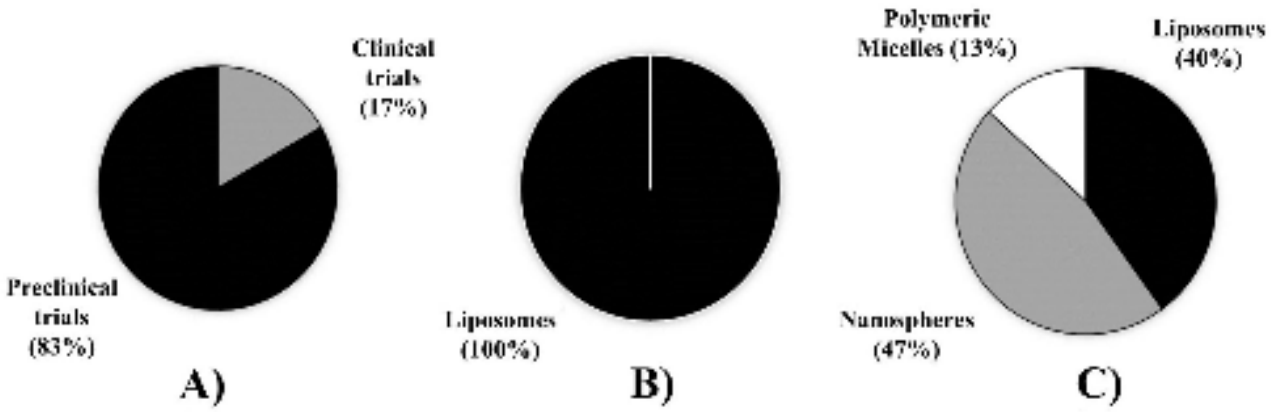

Figure 3. Research recapitulation of (A) clincial and preclinical studies, (B) types of nanoparticle use in clinical trials, (C) and pre-clinical trials

In vivo anti-cancer efficacy of nanoparticles containing UA

Analysis of tumor tissue histopathology

The anticancer effectiveness of nanoparticles containing UA compared to negative control and free UA are shown to have a significant effect on tumor growth inhibition as shown in Table 5.

Table 5. Tissue histopathology of liver cancer after administration of negative control, native UA and nanoparticles containing UA

Code	Tissue histopathology		
	Negative control	**Free UA**	**Nanoparticles containing UA**
Lipo E	It features no hemorrhagic or necrosis phenomena and the cell is round or polygonal	Tumor cells and angiogenesis occur in native UA solution and conventional UA liposomes treatment groups, which become rare with slight necrosis.	The tumor cells of the UA liposome with polyethylene glycols (PEGylated UA Liposome) group undergo severe necrosis, the nucleus/pulp ratio is significantly reduced, and apoptosis occurs due to a large number of scattered single tumor cells
Lipo F	The nucleus size and tumor cell shape are irregular. The tumor cells have clear cellular morphology and chromatin indicating that the tumor cells are growing quickly.	A limited shrinkage and fragmentation of the nucleus indicates a low rate of tumor cell necrosis.	Most tumor tissue cells in the group treated with Chitosan-Ursolic Acid-Liposomes (CS-UA-L) undergo apoptosis or necrosis, indicating good potential for killing cancer cells.
Nano B	There are numerous sinusoids and small blood vessels filled with blood (indicated by the arrow) spreading through the hepatocellular carcinoma trabeculae.	Not available	Several sinusoid liver or blood vessels can be observed in Chitosan-Ursolic Acid-Nanoparticle (CH-UA-NP) group with the exception of liver sinusoid dysplasia. Massive necrotic tissue can still be observed in hepatocellular carcinoma
Poli A	Tumor necrosis is undefined in the saline treatment group	Tumor cells and angiogenesis become rare with little necrosis	Most cancer cells in the high-dose Ursolic Acid-Polymer Micelles (UA-PMs) group at 100 mg/kg showed a high degree of H22 cell necrosis.

Comparative analysis of tumor growth inhibition

The results indicate that the normal tumor volume when compared to administration of UA-loaded liposomes (Lipo D, E, F, G, H, I), nanospheres (Nano A, B, D, E, F, G) and polymeric micelles (Poly A) decreased in relative tumor volume by approximately 2.0-21.2 times. The tumor volume of native UA compared to the administration of UA liposomes (Lipo D, E, F, G, H, I), nanospheres (Nano A, D, E, F, G) and polymeric micelle (Poly A) showed a relative reduction in tumor volume of about 1.6-15.9 times lower than that of the native UA group, as presented in Figure 4A. This suggests that nanoparticles can improve UA effectiveness in inhibiting expansions in tumor volume.

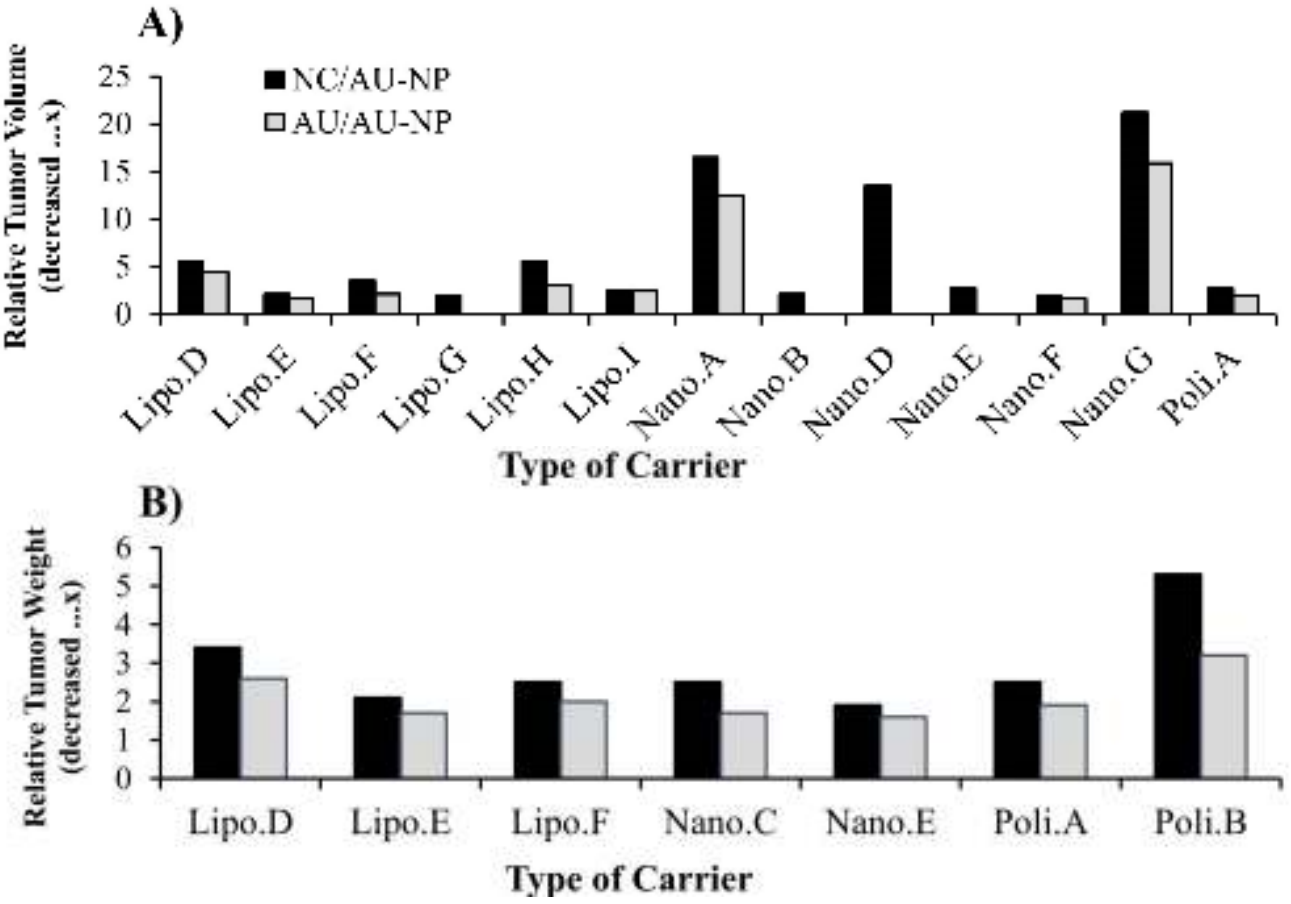

Figure 4. (A) Relative tumor volume in animal models treated with UA-loaded nanoparticles compared to negative control (black bars) and UA-free treatment groups (grey bars), (B) relative tumor tissue weight of animal models treated with UA-loaded nanoparticles compared to negative control (black bars) and native UA-treatment groups (grey bars).

The relative tumor weight analysis results relating to groups treated with UA liposomes (Lipo D, E, F), nanospheres (Nano C, E) and polymeric micelles (Poly A,B) indicated a relative reduction in tumor weight approximately 1.9-5.3 times that of the negative control group. Tumor weight in the native UA group compared to that of groups administered with UA liposomes (Lipo D, E, F), nanospheres (Nano C,E) and polymeric micelles (Poly A,B) showed a relative reduction of about 1.6-3.2x, as shown in Figure 4B. This suggests that nanoparticles may increase the effectiveness of UA in inhibiting tumor growth resulted in reduction of tumor weight.

The relative inhibition rate analysis results indicate that the administration of UA liposomes (Lipo E, F), nanospheres (Nano A) and polymeric micelles (Poly B) produces an increase in the relative tumor inhibition rate of approximately 1.9-3.4x compared to the native UA group. Of the three types of drug carriers, liposomes (Lipo F) experienced the highest relative inhibition rate increase of 3.4x the native UA group, as seen in Figure 5A. This suggests that nanoparticles may increase the effectiveness of UA in inhibiting tumor growth.

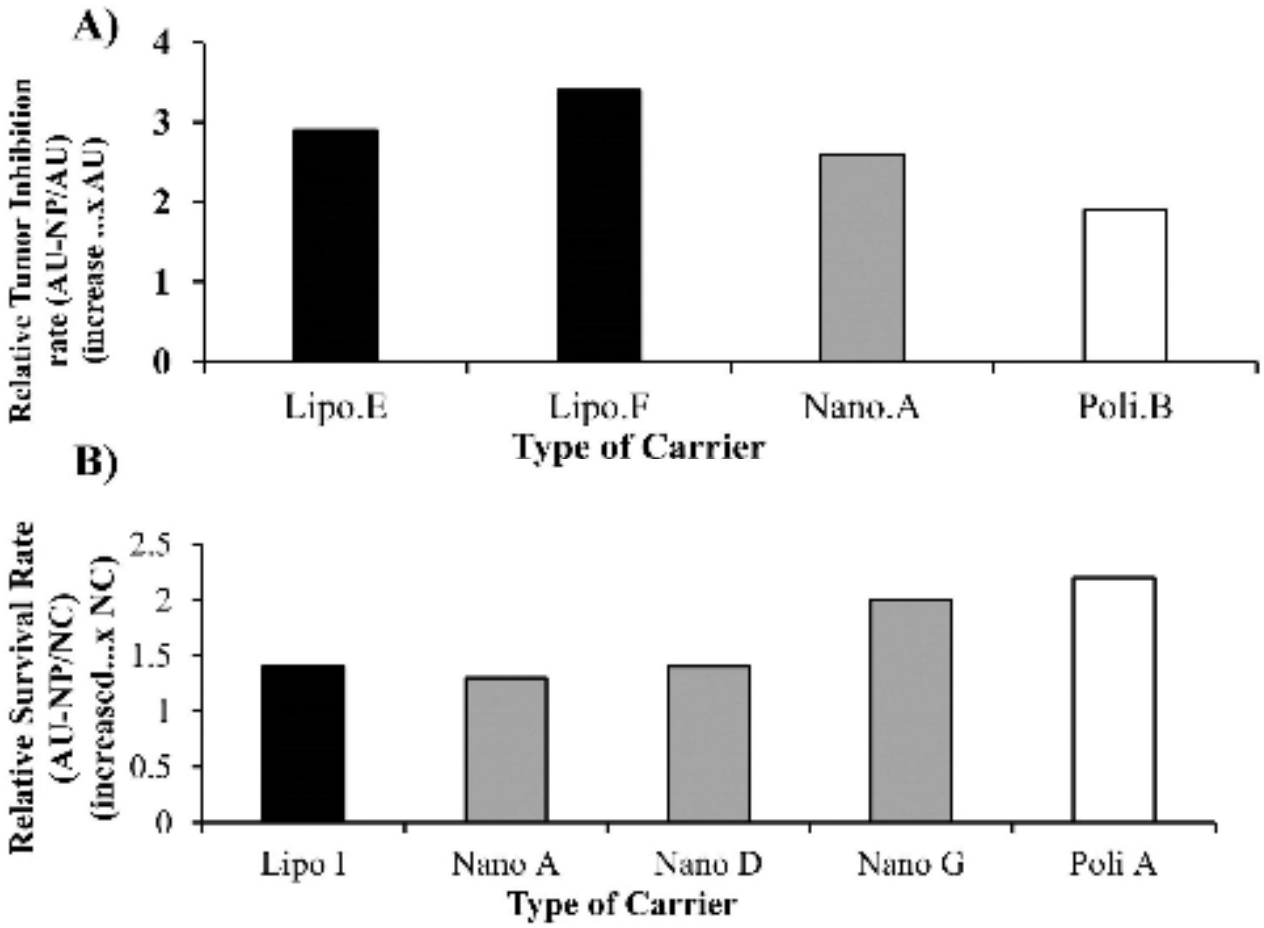

Figure 5. (A) Relative tumor growth inhibition rate of animal models treated with nanoparticles loading UA compared to native UA treatment groups, (B) Relative survival rate of animal models treated with UA-loaded nanoparticles compared to the negative control

Analysis of Survival rate

Based on the results, the administration of liposomes (Lipo E, F), nanospheres (Nano A) and polymeric micelles (Poly B) produced an increase in the relative survival rate about 1.3-2.2x higher when compared to that of the negative control group, as seen in Figure 5B. This suggests that nanoparticles may increase the effectiveness of UA associated with improved survival rate.

The increased anti-tumor activity observed from the volume and weight of the tumor was associated with necrosis in the tumor tissues caused by large dose exposures of UA reaching cancer cells due to the increased permeability of small nanoparticles with high drug loading due to the EPR effect.

Furthermore, the drug will be released into the extracellular and/or intracellular matrix. In the extracellular fluids, the drug will leak from nanoparticles and subsequently diffuse into cancer cells, while in intracellular fluids nanoparticles will experience endocytosis and the matrix will be destroyed in the endosome and release free drugs which then diffuse into the cytoplasm and nucleus subsequently causing cell necrosis. These results show that the use of nanoparticles as carriers within anticancer drug delivery can increase the in vivo survival rate.

Other studies have suggested that when nanoparticles such as liposomes interact with cells, drug delivery and diffusion into target cells can occur in several ways. Liposomes can penetrate the tumor tissue matrix resulting in degradation of carrier lipids by enzymes, such as lipase, or by mechanical strain inducing release of active substances into the extracellular fluid. This process induces drug diffusion into cell membranes culminating in cytoplasm and nucleus delivery. However, the latter process cannot easily be achieved by the use of hydrophilic drugs. Secondly, liposome membranes will fuse with those of the target cell leading to the release of liposomes directly into the cytoplasm. The third and most frequent method is that of receptor-mediated endocytosis. This process involves only vesicles with a maximum diameter of 150 nm and active substances demonstrating significant stability in such an acidic lysosome environment where liposomes are metabolized enzymatically. Phagocytosis may also ensue but involving large size nanoparticles affected by specialized immune system cells, such as macrophages, monocytes, and Kupffer cells. This process may eliminate the nanoparticles from the circulatory system (70).

The survival rate of liposomes is higher than that of other nanoparticles indicating the stability of the system in the blood circulation which ensures that the trapped drug is carried by the nanoparticles for further release into the cancer cells. In addition, because of the biomimetic property of liposome components that resemble phospholipid cell membranes it is easier for them to be absorbed by the cell.

In vivo toxicity studies of nanoparticles containing UA

Pre-clinical toxicity based on the analysis of relative body weight

From the results of relative body weight calculations contained in Table 6, no significant differences existed in the weight of the mice, proving that nanoparticle administration neither caused side effects nor produced symptoms of toxicity (32,36,41). This result is also supported by other toxicity data presented in Table 7, which shows that there was no clear cell damage and no morphological changes in the major organs i.e. heart, liver, spleen, lungs, and kidneys. However, ALT, AST and WBC levels all decreased after administration of UA nanoparticles when compared to native UA (45,52). This suggested that UA nanoparticles do not cause serious toxicity, indeed, do not even produce toxicity. Rather, the effectt is mild and of short duration (44).

Table 6. The relative body weight of animal models treated with UA-loaded nanoparticles compared to negative control and native UA-treatment groups.

Code	Toxicity

Lipo H	ALT and AST levels were significantly higher following an injection of FA-UA/siRNA-L compared to that of saline solution. The AST/ALT ratio of the FA-UA/siRNA-L group was significantly lower than that of the saline group. These results suggest that liver toxicity caused by liposomes produces mild, temporary liver toxicity.
Nano A	The number of rat WBCs in the NP HCPT@F-Pt-PU treatment group increased more rapidly than in the native UA group which suggests that folate-targeted pectin delivery systems may prevent serious hematological toxicity.
Nano D	There was no obvious cell damage or morphological changes in the major organs i.e., heart, liver, spleen, lungs, and kidneys in the NP UA-LA-ICG treatment group members compared to those of the negative control group.
Nano E	ALT levels in mice treated with UA-NP were significantly lower than in the CCl4 group members, but there were no changes in the native UA- treatment group. In addition, AST levels in the UA-NP treatment group were also significantly lower compared to the CCl4 group and the native UA-treatment groups.
Nano F	The native UA group experienced necropsy in the central section of the tumor tissue. These results partly suggest that native UA causes more toxicity than UA-NP. Meanwhile, H&E staining indicated that there were no obvious abnormalities or inflammatory lesions in any of the five organs, i.e., heart, liver, spleen, lungs, kidneys for the UA-NP treatment group when compared to their negative control and native UA counterparts.
Nano G	Rats treated with the Pec-8PUH-NPs group did not experience any significant reduction in WBC counts as an indicator of hematotoxicity suggesting that the use of nanoparticles might prevent hematological toxicity.

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Table 7. Recapitulation of other preclinical toxicities

Relative Body Weight		
Code	**Results**	
	NC/AU-NP	**AU/AU-NP**
Lipo D	Decreased by 1.2 x normal value (Not significant)	Increased by 1.0x AU value (Not significant)
Lipo E	Decreased by 1.1x normal value (Not significant)	Increased by 1.0x AU value (Not significant)
Lipo F	Decreased by 1.2x normal value (Not significant)	Decreased by 1.0x AU value (Not significant)
Lipo G	There is no obvious difference	Not available
Lipo H	Decreased by 1.0x normal value (Not significant)	There is no obvious difference
Nano A	There is no obvious difference	There is no obvious difference
Nano D	There is no obvious difference	There is no obvious difference
Nano E	There is no obvious difference	There is no obvious difference
Nano F	Decreased by 1.0x normal value (Not significant)	Increased by 0.9x AU value (Not significant)
Nano G	There is no obvious difference	There is no obvious difference
Poli A	Decreased by 1.1x normal value (Not significant)	Decreased by 1.0x AU value (Not significant)
Poli B	There is no obvious difference.	There is no obvious difference.

Safety aspects of the use of nanoparticles containing UA based on the clinical trials

Toxicity based on clinical laboratory parameters

The content of the graphs contained in Figure 6A, confirm an increase in levels of AST, ALT, GGT, TG, DBIL, and TBIL after UA liposome (Lipo A, B, C) administration occurred. It can also be seen that Dose Limiting Toxicity (DLT) related to hepatotoxicity, which was monitored for substantial side effect parameters, was at a moderate level (43).

In Lipo A, an increase in levels of AST (5%), ALT (5%), GGT (14%), TG (5%) was observed in 21 subjects who received doses of 56, 74, and 98 mg/m². In Lipo B, elevated levels of AST (13%), ALT (13%), GGT (15%), TG (8%), DBIL (5%) and TBIL (8%) were recorded in 39 subjects receiving doses of 11, 22, 37, 56, 74, 98, and 130 mg/m². In Lipo C, there was an increase in AST (4%), ALT (4%), GGT (2%), TG (9%), and TBIL (4%) levels observed in 24 subjects who received doses of 74 mg/m² as a single dose and 98 mg/m² and 74 mg/m² as multiple doses.

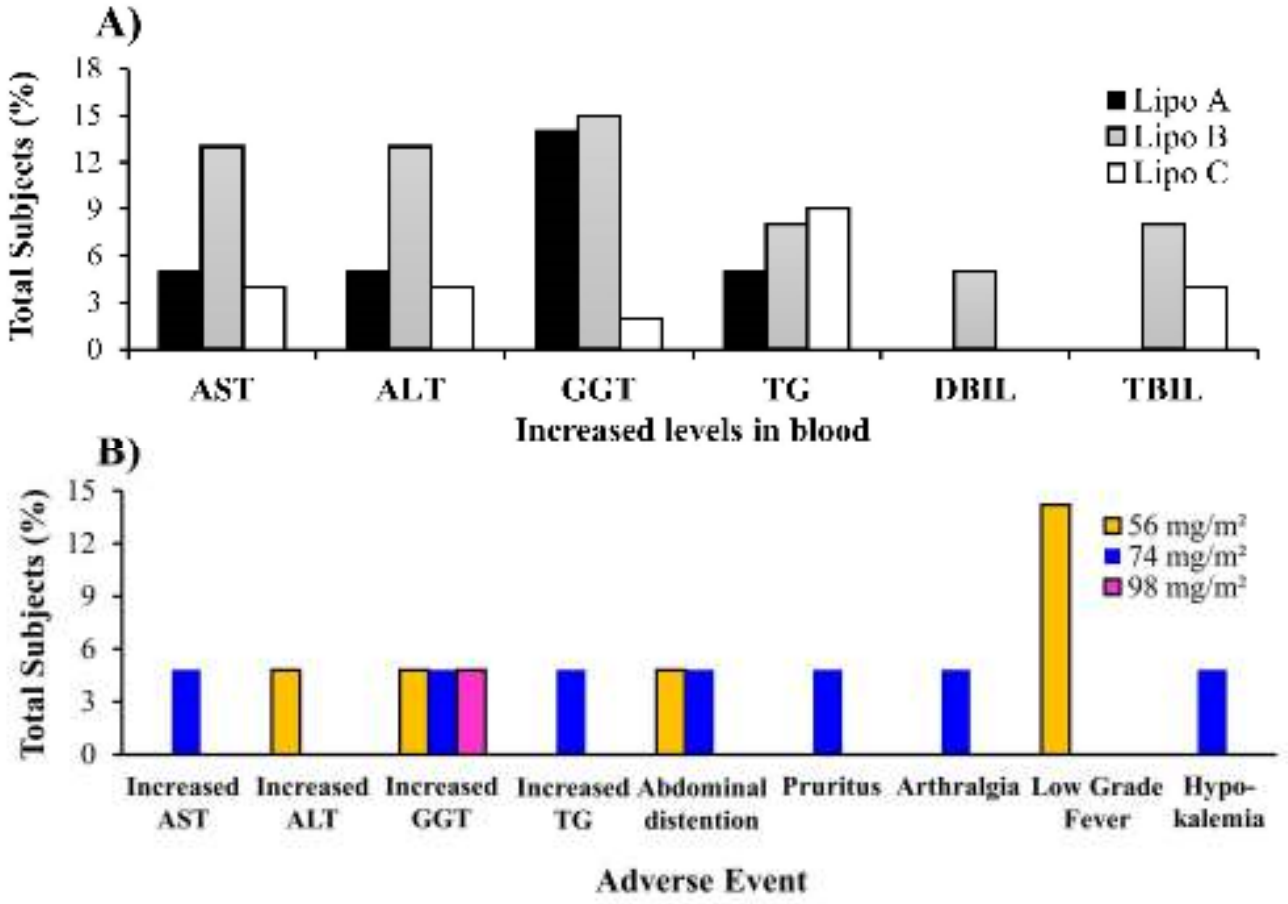

Figure 6. (A) Clinical laboratory data on clinical trials of Lipo A, Lipo B, and Lipo C, (B) Adverse events of Lipo A during phase I clinical trials

Notes:
AST : *Alanine Aminotransferase* / SGPT (*serum glutamic pyruvic transaminase)*
ALT : *Aspartate Aminotransferase* / SGOT *(serum glutamic oxaloacetic transaminase)*
GGT : *Gamma Glutamyl Transpeptidase*
TG : *Triglycerides*
DBIL : *Direct Bilirubin*
TBIL : *Total Bilirubin*

Clinical toxicity based on the occurences of adverse events

According to the data contained in Figure 6B, three subjects (14%) treated with a dose of 56mg/m² of Lipo A experienced a mild fever but recovered after two hours without receiving treatment. Moreover, three subjects (14%) treated with sequential doses of 56, 74, and 98mg/m² of Lipo A experienced an increase in GGT, two subjects (10%) administered with doses of 56 and 74mg/m² of Lipo A experienced abdominal distension, and one patient (5%) experienced a rise in ALT levels. Other mild symptoms included increased AST and TG, pruritus, arthralgia, and hypokalemia. The most common adverse conditions included fever, increased GGT, and flatulence. These results indicated that a 4-hour intravenous administration of Lipo A was tolerable and safe if a timetable of three doses per day for 14 consecutive days followed by a break lasting seven days within each 21-

day cycle was adhered to. Therefore, a 98 mg/m² dose of Lipo A is the recommended dose for phase II trials (46).

In addition, from the contents of Figure 7A, it can be seen that one patient treated with a 11 mg/m² dose experienced a first degree skin rash which healed untreated after three days. In addition, two patients who had been administered with a 98 mg/m² dose experienced vascular stimulation. First degree microscopic hematuria was observed in three subjects (7.7%) suffering from hepatoma malignancy who had received 11 doses of 11, 74, and 130 mg/m² respectively. However, these side effects disappeared after three days without any treatment being administered. Elevated levels of AST, ALT, GGT, DBIL, and TBIL were observed in several subjects receiving doses of 74, 98, and 130 mg/m². Dose Limiting Toxicity (DLT) resulted in hepatotoxicity: two subjects (5.1%) experienced an increase in AST, four subjects (10.3%) an increase in ALT, one subject (2.6%) an increase in GGT, and one subject (2.6%) an increase in DBIL. Diarrhea (2.6%) constitutes another DLT. Other drug-related side effects included nausea reported by one subject (2.6%), abdominal distension observed in another (2.6%), vascular stimulation occurred in two subjects (5.1%), while elevated TG was reported in three subjects (7.7%). Other reported adverse events included one subject (2.6%) suffering a skin rash and another (2.6%) experiencing higher serum sodium levels. At a dose of 74 mg/m², one of six subjects experienced DLT, which is a form of non-hematological toxicity, including increased AST/ALT and diarrhea. At a dose of 98 mg/m², one of the eleven subjects experienced DLT, i.e., non-hematological toxicity including increased ALT/GGT). At a dose of 130 mg / m², two thirds of the subjects experienced DLT (increased ALT, AST, and DBIL). Therefore, the increased dosage was suspended and MTD was confirmed to be 98 mg/m². Double administration of trial doses of UAL at recommended levels of 56, 74, and 98 mg/m² was completed (47).

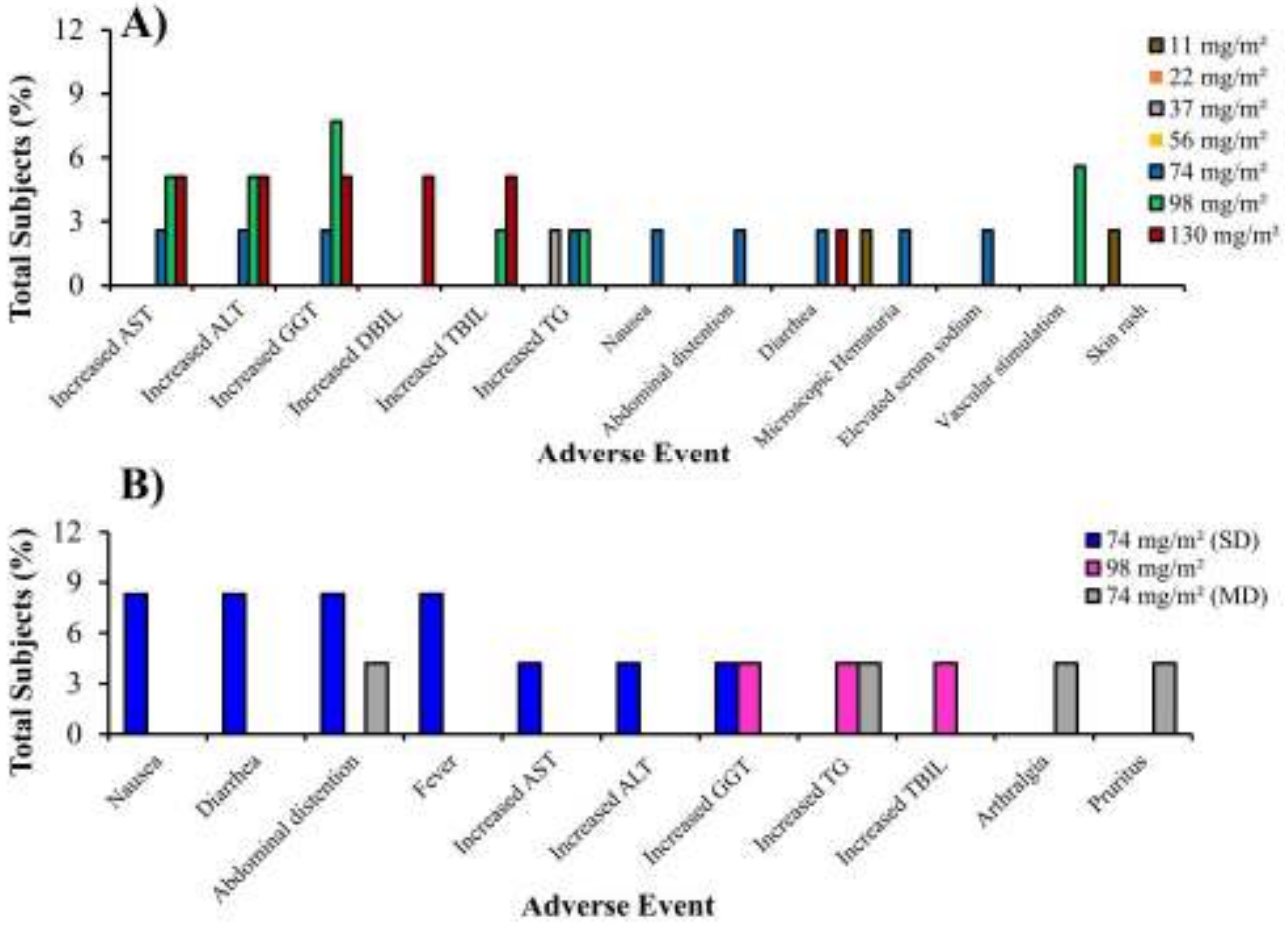

Figure 7. Adverse events of (A) Lipo B, and (B) Lipo C in phase I clinical trials

AST : *Alanine Aminotransferase* / SGPT (*serum glutamic pyruvic transaminase)*
ALT : *Aspartate Aminotransferase* / SGOT *(serum glutamic oxaloacetic transaminase)*
GGT : *Gamma Glutamyl Transpeptidase*
DBIL : *Direct Bilirubin*
TBIL : *Total Bilirubin*
TG : *Triglycerides*

From the graph in Figure 7B, it is clear that all subjects in the study tolerated the Lipo C treatment. Most adverse events varying from mild to moderate related to Lipo C, which is Ursolic Acid Nanoliposome (UANL), were non-dose dependent. The most commonly observed adverse events included abdominal distension, nausea, and diarrhea. The adverse events after a 14-day continuous infusion of UANL comprised skin pruritus, arthrisgia, and increased triglycerides levels. UANL has minimal toxic effects. The limiting toxicity of UANL dose is hepatotoxicity. In this study, intravenous UANL infusions were well tolerated both by healthy volunteers and patients with advanced tumors (33).

Based on the review analysis, only three articles which focused on liposomes as the drug carrier discussed clinical trials of UA. Although UA is classified as a BCS class IV drug, its permeability

and solubility can be enhanced with the use of liposomes. It is related to the natural phase properties of the liposomal membrane that significantly affect permeability, aggregation, protein binding and liposome fusion. Membrane permeability largely depends on lipid components. Lipids that contain saturated chains or do not have carbon double bonds are more stable because they demonstrate greater resistance to oxidation. Lipid bilayer and liposome membranes possess a good lipid-packing order or gel phase below the lipid phase transition temperature (Tc), where the temperature is in a balanced proportion in the two phases. The fluidity of the lipid bilayer can be controlled by the selection and combined use of lipids, as the various Tcs depend on the length and origin sources (saturated or unsaturated) of fatty acid chains. For example, the incorporation of cholesterol at low concentrations into the lipid bilayer leads to increased trans-membrane permeability, where the incorporation of large amounts (>30 moles%) of cholesterol can reduce the transition phase and decrease membrane permeability at higher Tc temperatures (71). Liposome permeability is related to the rate of solute diffusion through the lipid bilayer. The liposome membrane will achieve the highest permeability in the transition temperature phase, while its permeability is lower in gel form than in the fluid phase. The temperature of the bilayer phase transition is determined by the composition of the liposome. In the transition temperature phase, the permeability of liposomes to molecules such as protons and water increases (72–74). In addition, the in vivo biodistribution and disposition of liposomes varies depending on the composition of the lipids, particle size, potential charge and degree of steric surface/hydration. In addition, the administration route may affect the in vivo disposition of liposomes. During intravenous administration, liposomes usually interact with serum proteins and are absorbed by RES cells, thus accumulating in the liver or spleen (75).

The development of nanoparticles for drug delivery, one of which is Doxil® (Doxorubicin HCl liposome injection), the first nanoliposomal drug approved by FDA in 1995, was based on three principles: (i) prolonging drug circulation time and RES avoidance due to the PEGylation of nanoliposomes; (ii) higher stable loading of doxorubicin driven by the transmembrane ammonium sulfate gradient which also allows the re-release of the drug in tumors; and (iii) having lipid bilayer liposomes in a ″liquid ordered″ phase consisting of phosphatidylcholine with a high melting temperature (T_m= 53 °C), and which largely use cholesterol as a membrane stabilisator (19). In addition, various drug formulas in liposomes have received approval to be marketed and are widely used in clinical settings including Myocet® (Elan Pharmaceuticals Inc., Princeton, NJ, USA). This is an encapsulation of doxorubicin in liposomes (76,77); Daunoxome® (Gilead Sciences), daunorubicin formulated into liposomes (78,79); Marqibo® non-PEGylated liposomal vincristine developed in 2012 as a therapy for various cancers including lymphoma, brain, leukemia, or melanoma (80); Onivyde® MM-398, which is a PEGylated liposomal irinotecan developed in 2015 as a drug to treat metastatic pancreatic cancer (81), and many other forms of cancer (82,83). Various developments of the liposome delivery system indicated that liposomes possess non-toxic, flexible, biocompatible, and biodegradable properties that can enhance the therapeutic effects, safety, and efficacy of various anticancer drugs (57).

As for the development of cisplatin therapy, which incorporates the use of an anticancer drug, this involves a polymeric micelle delivery system. Polymeric micelles were prepared through the formation of a metal-polymer complex between cisplatin (CDDP) and poly-(ethylene glycol)-poly(glutamic acid) block copolymers. Cisplatin polymeric micelles (CDDP/m) are 28 nm in size with the ability to distribute themselves through narrow spaces such as blood vessels in pancreatic tissue. These micelles undergo lengthy blood circulation and accumulate effectively in solid tumors of Lewis lung carcinoma cells. However, because they are produced synthetically, the toxicity and safety aspects as well as manufacturing production scale constitute extremely important issues (84).

Abraxane®, a paclitaxel albumin-bound nanoparticle with a particle size of ~130 nm which received FDA approval in 2005 for the treatment of metastatic breast cancer succesfully reduces toxicities in comparison to Taxol®. Moreover, it enables a complete dose to be administered within 30 minutes without the need for any pre-treatment. Nevertheless, the mechanism of Abraxane® in improving survival rate and overcoming P-GP-mediated drug resistance remains unclear (25).

The findings of this scoping review suggest that liposomes provide more comprehensive data than other forms of nanoparticles. This is demonstrated by the existence of in vivo studies of anticancer effectiveness assessed using several parameters such as increasing relative survival rate; more robust tumor growth inhibition (increasing relative inhibition rate, decreasing relative tumor weight, and reducing tumor volume); and improvements in tumor tissue histopathology. In addition, in vivo studies related to safety were also evaluated employing several parameters, i.e., weight loss, and other toxicity (lowering AST, ALT, and WBC), and well-tolerated toxicity by healthy volunteers and patients with advanced tumors.

There needs multi-faceted views of the use of nanoparticles for reviewing drug delivery. The components of the nanoparticle formulation would greatly affect the characteristics of the nanoparticles including particle size, potential charges, stealth and biomimetic properties, and others, which are closely related to drug delivery to cancer tissue, due to the Enhanced Permeation and Retention (EPR) effects. In addition, *in vivo* analysis of different types of cancer, where each type of cancer cell has different biological properties, also requires an in-depth study to provide data on supporting the effectiveness of drug delivery to target cancerous tissues. Moreover, the route of administration, dose, and frequency of drug administration related to the physicochemical properties and pharmacokinetic profile of the drug also greatly affect the systemic bioavailability and effective drug amount capable of reaching cancer tissue as the target of drug delivery. All these aspects provide important views for comprehensive study of the drug delivery system in cancer therapy.

4 Conclusions

Based on the scoping review of the relevant literature, it can be concluded that UA loaded into nanoparticles is effective as a form of anticancer therapy. Pre-clinical trials confirm that it increases the relative survival rate*;* tumor resistance (increasing the relative inhibition rate, lowering the relative tumor weight, and decreasing tumor volume); and improves tumor tissue histopathology. In addition, UA-loaded nanoparticles have been proven safe for anticancer therapy based on the evaluation of weight loss and other toxicity (decreased AST/ALT). The results from the last 10-year analysis have indicated that, compared to nanospheres and polymeric micelles, liposomes have been assessed as more effective and safer during more comprehensive pre-clinical and clinical trials. This finding highlights the potential for liposomes to be further developed as a means of delivering UA as an anticancer therapy.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

Andang Miatmoko: 1) conception and design of the work, data acquisition, data analysis and interpretation; 2) critically revising the article for important intellectual content; 3) Final approval of the version to be published; 4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Ester Adelia Mianing: 1) data acquisition; 2) Drafting the article; 3) Final approval of the version to be published; 4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Retno Sari: 1) data analysis and interpretation; 2) Final approval of the version to be published; 3) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Esti Hendradi: 1) data analysis and interpretation; 2) Final approval of the version to be published; 3) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated

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9 Reference styles

1. Kaminska K, Szczylik C, Bielecka ZF, Bartnik E, Porta C, Lian F, et al. The role of the cell-cell interactions in cancer progression. J Cell Mol Med. 2015;19(2):283–96.

2. Internatioanl Agency for Research on Cancer. Latest global cancer data : Cancer burden rises to 18.1 million new cases and 9 . 6 million cancer deaths in 2018. 2018.

3. Globocan. Indonesia - Global Cancer Observatory. WHO; International Agency for Research on Cancer, 2018. Https://GcoIarcFr/Today/Data/Factsheets/Populations/360-Indonesia-Fact-SheetsPdf. 2020;256:1–2.

4. Kumari P, Ghosh B, Biswas S. Nanocarriers for cancer-targeted drug delivery. J Drug Target. 2016;24(3):179–91.

5. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm [Internet]. 2015;93:52–79. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0939641115001514

6. Kashyap D, Tuli HS, Sharma AK. Ursolic acid (UA): A metabolite with promising therapeutic potential. Life Sci. 2016;146:201–13.

7. Khwaza V, Oyedeji OO, Aderibigbe BA. Ursolic acid-based derivatives as potential anti-cancer agents : an update. 2020;

8. Seo DY, Lee SR, Heo JW, No MH, Rhee BD, Ko KS, et al. Ursolic acid in health and disease. Korean J Physiol Pharmacol. 2018;22(3):235–48.

9. Ali SA, Ibrahim NA, Mohammed MMD, El-Hawary S, Refaat EA. The potential chemo preventive effect of ursolic acid isolated from Paulownia tomentosa, against N-diethylnitrosamine: initiated and promoted hepatocarcinogenesis. Heliyon. 2019;5(5):e01769.

10. Sun Q, He M, Zhang M, Zeng S, Chen L, Zhou L, et al. Ursolic acid: A systematic review of its pharmacology, toxicity and rethink on its pharmacokinetics based on PK-PD model. Fitoterapia. 2020;147:104735.

11. Bethlehem. Biopharmaceutical Classification System and Formulation Development. Part Sci. 2011;9:1–4.

12. Rajesh S, Jr. JWL. Nanoparticle-based targeted drug delivery. Exp Mol Pathol. 2000;86(3):215–23.

13. Reddy LH. Drug delivery to tumours: recent strategies. J Pharm Pharmacol [Internet]. 2005;57(10):1231–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16259751

14. Fang J, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev [Internet]. 2011;63(3):136–51. Available from: http://dx.doi.org/10.1016/j.addr.2010.04.009

15. Li Z, Tan S, Li S, Shen Q, Wang K. Cancer drug delivery in the nano era: An overview and perspectives (Review). Oncol Rep. 2017;38(2):611–24.

16. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm. 2015;93:52–79.

17. Barenholz Y. Doxil® - The first FDA-approved nano-drug: Lessons learned. J Control Release [Internet]. 2012;160(2):117–34. Available from: http://dx.doi.org/10.1016/j.jconrel.2012.03.020

18. Wei X, Cohen R, Barenholz Y. Insights into composition/structure/function relationships of Doxil® gained from “high-sensitivity” differential scanning calorimetry. Eur J Pharm Biopharm [Internet]. 2016;104:260–70. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0939641116301357

19. Barenholz Y. Doxil® - The first FDA-approved nano-drug: Lessons learned. J Control Release. 2012;160(2):117–34.

20. Nishiyama N, Okazaki S, Cabral H, Miyamoto M, Kato Y, Sugiyama Y, et al. Novel cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice. Cancer Res [Internet]. 2003;63(24):8977–83.

21. Uchino H, Matsumura Y, Negishi T, Koizumi F, Hayashi T, Honda T, et al. Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. Br J Cancer. 2005;93(6):678–87.

22. Plummer R, Wilson RH, Calvert H, Boddy a V, Griffin M, Sludden J, et al. A Phase I clinical study of cisplatin-incorporated polymeric micelles (NC-6004) in patients with solid tumours. Br J Cancer. 2011;104(4):593–8.

23. Mitchell MJ, Billingsley MM, Haley RM, Langer R, Wechsler ME, Peppas NA. Engineering precision nanoparticles. Nat Rev Drug Discov. 2021;20:101–24.

24. Mizumura Y, Matsumura Y, Hamaguchi T, Nishiyama N, Kataoka K, Kawaguchi T, et al. Cisplatin-incorporated Polymeric Micelles Eliminate Nephrotoxicity , While Maintaining Antitumor Activity. Jpn J Cancer Res. 2001;(March):328–36.

25. Ma P, Mumper RJ. Paclitaxel nano-delivery systems: A comprehensive review. J Nanomedicine Nanotechnol. 2013;4(2):6.

26. Miele E, Spinelli GP, Miele E, Tomao F, Tomao S. Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer. Int J Nanomedicine. 2009/04/20. 2009;4:99–105.

27. Zaimy MA, Saffarzadeh N, Mohammadi A, Pourghadamyari H, Izadi P, Sarli A, et al. New methods in the diagnosis of cancer and gene therapy of cancer based on nanoparticles. Cancer Gene Ther. 2017;24(6):233–43.

28. Kharia AA, Singhai AK, Verma R. Formulation and Evaluation of Polymeric Nanoparticles of an Antiviral Drug for Gastroretention. Int J Pharm Sci Nanotechnol. 2012;4(4):1557–62.

29. Koppolu B, Zaharoff DA. The effect of antigen encapsulation in chitosan particles on uptake, activation and presentation by antigen presenting cells. Biomaterials. 2013 Mar;34(9):2359–69.

30. Miatmoko A, Kawano K, Yoda H, Yonemochi E, Hattori Y. Tumor delivery of liposomal doxorubicin prepared with poly-L-glutamic acid as a drug-trapping agent. J Liposome Res. 2017;27(2):99–107.

31. Wang M, Zhao T, Liu Y, Wang Q, Xing S, Li L, et al. Ursolic acid liposomes with chitosan modification: Promising antitumor drug delivery and efficacy. Mater Sci Eng C. 2017;71:1231–40.

32. Poudel K, Gautam M, Maharjan S, Jeong JH, Choi HG, Khan GM, et al. Dual stimuli-responsive ursolic acid-embedded nanophytoliposome for targeted antitumor therapy. Int J

768 Pharm. 2020;582(December 2019):119330.

769 33. Zhu Z, Qian Z, Yan Z, Zhao C, Wang H, Ying G. A phase I pharmacokinetic study of ursolic
770 acid nanoliposomes in healthy volunteers and patients with advanced solid tumors. Int J
771 Nanomedicine. 2013;8:129–36.

772 34. Melo RCN, Raas MWD, Palazzi C, Neves VH. Whole Slide Imaging and Its Applications to
773 Histopathological Studies of Liver Disorders. Front Med. 2020;6(January):1–13.

774 35. Slaoui M, Fiette L. Histopathology procedures: from tissue sampling to histopathological
775 evaluation. Methods Mol Biol. 2011;691(December 2014):69–82.

776 36. Liu Y, Kong T, Yang Z, Zhang Y, Lei J, Zhao P. Self-Assembled Folic Acid-Targeted Pectin-
777 Multi-Arm Polyethylene Glycol Nanoparticles for Tumor Intracellular Chemotherapy. ACS
778 omega. 2021 Jan;6(2):1223–34.

779 37. Wang Q, Zhao T, Liu Y, Xing S, Li L, Gao D. An evaluation of anti-tumor effect and toxicity
780 of PEGylated ursolic acid liposomes. J Nanoparticle Res. 2016;18(2):1–13.

781 38. Yang G, Yang T, Zhang W, Lu M, Xiang G. In Vitro and in Vivo Antitumor E ff ects of
782 Folate-Targeted Ursolic Acid Stealth Liposome. 2014;62(10):2207-2215.

783 39. Hattori Y, Yamashita J, Sakaida C, Kawano K, Yonemochi E. Evaluation of antitumor effect
784 of zoledronic acid entrapped in folate-linked liposome for targeting to tumor-associated
785 macrophages. J Liposome Res. 2014;25(2):1–10.

786 40. Shen Z, Li B, Liu Y, Zheng G, Guo Y, Zhao R, et al. A self-assembly nanodrug delivery
787 system based on amphiphilic low generations of PAMAM dendrimers-ursolic acid conjugate
788 modified by lactobionic acid for HCC targeting therapy. Nanomedicine Nanotechnology, Biol
789 Med. 2018;14(2):227–36.

790 41. Fu D, Ni Z, Wu K, Cheng P, Ji X, Li G, et al. A novel redox-responsive ursolic acid polymeric
791 prodrug delivery system for osteosarcoma therapy. Drug Deliv. 2021;28(1):195–205.

792 42. Etikan I. The Kaplan Meier Estimate in Survival Analysis. Biometrics Biostat Int J.
793 2017;5(2):55–9.

794 43. Valcourt DM, Kapadia CH, Scully MA, Dang MN, Day ES. Best Practices for Preclinical In
795 Vivo Testing of Cancer Nanomedicines. Adv Healthc Mater. 2020;9(12):1–15.

796 44. Li W, Yan R, Liu Y, He C, Zhang X, Lu Y, et al. Co-delivery of Bmi1 small interfering RNA
797 with ursolic acid by folate receptor-targeted cationic liposomes enhances anti-tumor activity of
798 ursolic acid in vitro and in vivo. Drug Deliv. 2019;26(1):794–802.

799 45. Zhao R, Zheng G, Fan L, Shen Z, Jiang K, Guo Y, et al. Carrier-free nanodrug by co-assembly
800 of chemotherapeutic agent and photosensitizer for cancer imaging and chemo-photo
801 combination therapy. Acta Biomater. 2018;70:197–210.

802 46. Qian Z, Wang X, Song Z, Zhang H, Zhou S, Zhao J, et al. A phase I trial to evaluate the
803 multiple-dose safety and antitumor activity of ursolic acid liposomes in subjects with advanced

804 solid tumors. Biomed Res Int. 2015;2015:1–7.

805 47. Wang XH, Zhou SY, Qian ZZ, Zhang HL, Qiu LH, Song Z, et al. Evaluation of toxicity and
806 single-dose pharmacokinetics of intravenous ursolic acid liposomes in healthy adult volunteers
807 and patients with advanced solid tumors. Expert Opin Drug Metab Toxicol. 2013;9(2):117–25.

808 48. Zhang N, Liu S, Shi S, Chen Y, Xu F, Wei X, et al. Solubilization and delivery of Ursolic-acid
809 for modulating tumor microenvironment and regulatory T cell activities in cancer
810 immunotherapy. J Control Release. 2020;320(January):168–78.

811 49. Jin H, Pi J, Yang F, Wu C, Cheng X, Bai H. Ursolic acid-loaded chitosan nanoparticles induce
812 potent anti-angiogenesis in tumor. Appl Microbiol Biotechnol. 2016;6643–52.

813 50. Jin H, Pi J, Yang F, Jiang J, Wang X, Bai H, et al. Folate-Chitosan Nanoparticles Loaded with
814 Ursolic Acid Confer Anti-Breast Cancer Activities in vitro and in vivo. Sci Rep. 2016;6(July
815 2016):1–11.

816 51. Fan L, Zhang B, Xu A, Shen Z, Guo Y, Zhao R, et al. Carrier-Free, Pure Nanodrug Formed by
817 the Self-Assembly of an Anticancer Drug for Cancer Immune Therapy. Mol Pharm. 2018
818 Jun;15(6):2466–78.

819 52. Zhang H, Zheng D, Ding J, Xu H, Li X, Sun W. Efficient delivery of ursolic acid by poly (N-
820 nanoparticles for inhibiting the growth of hepatocellular carcinoma in vitro and in vivo. Int J
821 Nanomedicine. 2015;10:1909–20.

822 53. Liu Y, Liu K, Li X, Xiao S, Zheng D, Zhu P, et al. A novel self-assembled nanoparticle
823 platform based on pectin-eight-arm polyethylene glycol-drug conjugates for co-delivery of
824 anticancer drugs. Mater Sci Eng C. 2018;86:28–41.

825 54. Zhou M, Yi Y, Liu L, Lin Y, Li J, Ruan J, et al. Polymeric micelles loading with ursolic acid
826 enhancing anti-tumor effect on hepatocellular carcinoma. J Cancer. 2019;10(23):5820–31.

827 55. Monteiro N, Martins A, Reis RL, Neves NM. Liposomes in tissue engineering and
828 regenerative medicine. J R Soc Interface. 2014;11(101):20140459.

829 56. Shaker S, Gardouh A, Ghorab M. Factors affecting liposomes particle size prepared by ethanol
830 injection method. Res Pharm Sci. 2017;12(5):346–52.

831 57. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al.
832 Liposome: Classification, preparation, and applications. Nanoscale Res Lett. 2013;8(1):1.

833 58. Chen J, Ping QN, Guo JX, Chu XZ, Song MM. Effect of phospholipid composition on
834 characterization of liposomes containing 9-nitrocamptothecin. Drug Dev Ind Pharm. 2006
835 Jul;32(6):719–26.

836 59. Webb C, Khadke S, Schmidt ST, Roces CB, Forbes N, Berrie G, et al. The Impact of Solvent
837 Selection : Strategies to Guide the Manufacturing of Liposomes Using Microfluidics.
838 Pharmaceutics. 2019;11(12):653.

839 60. Baldim I, Oliveira WP, Kadian V, Rao R, Yadav N, Mahant S, et al. Natural ergot alkaloids in

ocular pharmacotherapy: Known molecules for novel nanoparticle-based delivery systems. Biomolecules. 2020;10(7):1–21.

61. Cabral H, Matsumoto Y, Mizuno K, Chen Q, Murakami M, Kimura M, et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. Nat Nanotechnol. 2011;6(12):815–23.

62. Cabral H, Matsumoto Y, Mizuno K, Chen Q, Murakami M, Kimura M, et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. Nat Nanotechnol. 2011;6(12):815–23.

63. Alves RF, Favaro MTP, Balbino TA, de Toledo MAS, de la Torre LG, Azzoni AR. Recombinant protein-based nanocarriers and their association with cationic liposomes: Characterization and in vitro evaluation. Colloids Surfaces A Physicochem Eng Asp. 2017;513:1–10.

64. Laouini A, Bernard C, Jaafar-maalej C, Limayem-Blouza I, Sfar S, Charcosset C, et al. Preparation , Characterization and Applications of Liposomes : State of the Art. J Colloid Sci Biotechnol. 2012;1:147–68.

65. Lunardi CN, Gomes AJ, Rocha FS, De Tommaso J, Patience GS. Experimental methods in chemical engineering: Zeta potential. Can J Chem Eng. 2021 Mar 1;99(3):627–39.

66. Sharma A, Sharma US. Liposomes in drug delivery: Progress and limitations. Int J Pharm. 1997;154(2):123–40.

67. Gabizon A, Shmeeda H, Grenader T. Pharmacological basis of pegylated liposomal doxorubicin: Impact on cancer therapy. Eur J Pharm Sci. 2012;45(4):388–98.

68. Kulkarni SB, Betageri G V., Singh M. Factors affecting microencapsulation of drugs in liposomes. J Microencapsul. 1995;12(3):229–46.

69. Liu Y, Kong T, Yang Z, Zhang Y, Lei J, Zhao P. Self-Assembled Folic Acid-Targeted Pectin-Multi-Arm Polyethylene Glycol Nanoparticles for Tumor Intracellular Chemotherapy. ACS Omega. 2021;6(2):1223–34.

70. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine. 2015;10:975–99.

71. Corvera E, Mouritsen OG, Singer MA, Zuckermann MJ. The permeability and the effect of acyl-chain length for phospholipid bilayers containing cholesterol: theory and experiment. BBA - Biomembr. 1992;1107(2):261–70.

72. Hansen AE, Petersen AL, Henriksen JR, Boerresen B, Rasmussen P, Elema DR, et al. Positron Emission Tomography Based Elucidation of the Enhanced Permeability and Retention Effect in Dogs with Cancer Using Copper-64 Liposomes. ACS Nano. 2015;9(7):6985–95.

73. Koda Y, Liang MT, Blanchfield JT, Toth I. In vitro stability and permeability studies of liposomal delivery systems for a novel lipophilic endomorphin 1 analogue. Int J Pharm. 2008;356(1–2):37–43.

74. Yoshimoto M, Tamura R, Natsume T. Liposome clusters with shear stress-induced membrane permeability. Chem Phys Lipids. 2013;174:8–16.

75. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and challenges of liposome assisted drug delivery. Front Pharmacol. 2015;6(DEC):1–13.

76. Swenson CE, Perkins WR, Roberts P, Janoff AS. Liposome technology and the development of Myocet™ (liposomal doxorubicin citrate). The Breast. 2001;10:1–7.

77. Chastagner P, Devictor B, Geoerger B, Aerts I, Leblond P, Frappaz D, et al. Phase I study of non-pegylated liposomal doxorubicin in children with recurrent/refractory high-grade glioma. Cancer Chemother Pharmacol. 2015;76(2):425–32.

78. Dawidczyk CM, Kim C, Park JH, Russell LM, Lee KH, Pomper MG, et al. State-of-the-art in design rules for drug delivery platforms: Lessons learned from FDA-approved nanomedicines. J Control Release. 2014;187:133–44.

79. Forssen EA. The design and development of DaunoXome® for solid tumor targeting in vivo. Adv Drug Deliv Rev. 1997;24(2):133–50.

80. Silverman JA, Deitcher SR. Marqibo® (vincristine sulfate liposome injection) improves the pharmacokinetics and pharmacodynamics of vincristine. Cancer Chemother Pharmacol. 2012/12/05. 2013 Mar;71(3):555–64.

81. Zhang H. Onivyde for the therapy of multiple solid tumors. Onco Targets Ther. 2016;9:3001–7.

82. Ventola CL. Progress in nanomedicine: Approved and investigational nanodrugs. P T. 2017;42(12):742–55.

83. Anselmo AC, Mitragotri S. Nanoparticles in the clinic: An update. Bioeng Transl Med. 2019;4(3):1–16.

84. Plummer R, Wilson RH, Calvert H, Boddy A V., Griffin M, Sludden J, et al. A Phase i clinical study of cisplatin-incorporated polymeric micelles (NC-6004) in patients with solid tumours. Br J Cancer. 2011;104(4):593–8.

10 Supplementary Material

None

REVIEWER 1

The manuscript submitted by Andang Miatmoko and colleagues is good work submitted for publication. However it can not be accepted in its present form, authors require extensive revisions for this manuscript.

1. The Manuscript content is not giving good idea about the actual work the authors did, at some instances it seems like research (what the need to discuss characterization of formulation, Histopathology discussed like the authors are not writing review instead they are discussing their own findings).

Answer:

Many thanks for the comments. In this study, we evaluated the use of nanoparticle for delivering ursolic acid by performing scoping review, which we analyzed the type of nanoparticles as well as their physical characteristics determining the efficacy and safety of these delivery systems. Therefore, we evaluated characterization of formulation depended on the components, and compared the efficacy and safety of these systems by doing analysis from the data presented by authors in their research publication. Thus, it has not only literature review describing potential use of each nanoparticle types for ursolic acid as anticancer agents, but we would like to show the relative efficacy and safety of each nanoparticle compared to negative control and native ursolic acid itself aiming for providing data-based review of the efficacy and safety of various types of Ursolic Acid-loading nanoparticles within the setting of preclinical and clinical anticancer studies, as we stated in the abstract line 17-19.

2. The authors included figure, do they have copyright?

Answer:

Many thanks for the comments. We have cited the reference of the figures used in this manuscript; however, we still have no permission for their use. Therefore, we deleted figures used in this manuscript, but we ensure that all statements are still clear and informative for the readers.

We have revised and reordered the figure numbers in the manuscript.

3. The discussion is not drafted properly, flow of the content is not good too. In its present form readers will not have an idea about the concept of the review, authors must have straight forward approach for the readers (In results and discussion authors described their methodology of literature survey)

Answer:

Many thanks for the comments. Regarding the concept of the review, we proposed to give information about the type of nanoparticle used in delivering ursolic acid, as well as physical characteristics determined by the components of formulation, since these parameters greatly determine the successful delivery into cancer target in the presence of EPR effects. Moreover, the safety is also being important consideration as the requirement of nanoparticle for uses in clinical therapy. Therefore, in the manuscript, we have comparative data studies about these nanoparticles.

Ee have stated in the Metehods Section in Line 114-116 as the following: “This study uses the scoping review method involving literature accessible through the PubMed, Sciencedirect, Scopus, and Google Scholar databases consisting of online research publications dating from 2011 to 2021.” In this section, we have also informed about how the data were calculated and

presented in the manuscript, started from the physical characterization, efficacy and safety of the nanoparticle use for ursolic acid delivery.
We have also stated the review method in the early part of Result and Discussion in the following statement:
Line 247-250: "This study provides a literature review focusing on the anticancer effectiveness and safety of UA delivered with various types of nanoparticles to increase its anticancer effects as confirmed by both pre-clinical and clinical trials. Literature searches of all four databases using pre-determined keywords identified 237 articles in the prescreening stage as can be seen in Figure 1."
We have added some information regarding data achieved in this study into the following:
Line 274: "From the data analysis of the 18 articles"
Line 354: Pharmacokinetic data review on *in vivo* studies
Line 387: Table 4. Pharmacokinetic data summary of preclinical studies of nanoparticles containing UA

4. References should be revised to match with given text. For example, Author reported the Ursolic Acid belong to BCS-IV but reference number 10 which not match with these text.
Answer:
Many thanks for the comments. We have revised location of the reference citation as the following:
Line 61-62: we have ervised the references into the following:" BCS class IV compound demonstrating low permeability and solubility (10) which, consequently, requires a nanotechnology-based drug delivery system to reach the desired target (11)."
Line 329: we have revised the following statement: "UA has poor permeability and low water (10), thus causing possibly encapsulated within the membrane bilayer of lipid vesicles"

5. The authors should also refer some review/research articles published recently on novel nanotechnology based drug delivery systems, which will be more beneficial for their work.
✓ Novel nanotechnology approaches for diagnosis and therapy of breast, ovarian and cervical cancer in female: A review
✓ Nanomedicine in treatment of breast cancer – A challenge to conventional therapy
✓ Bioactive Apigenin loaded oral nano bilosomes: Formulation optimization to preclinical assessment
✓ Implications of Solid Lipid Nanoparticles of Ganoderic Acid for the Treatment and Management of Hepatocellular Carcinoma
✓ Nanocrystals: Characterization Overview, Applications in Drug Delivery, and Their Toxicity Concerns
✓ Anticancer effect of ursolic acid stearoyl glucoside in chemically induced hepatocellular carcinoma

Answer:
Many thanks for the suggestions. However, we focused on the use of nanoparticles for delivery of ursolic acid. The review article and derivates of ursolic acid have been excluded from the study, as seen in the methods section (Table 1, line 123) and the results (Figure 1, line 256-257)

REVIEWER 2

The authors are compiling the literature on the various nanoparticles that were formulated to encapsulate a potent anti-cancer agent, ursolic acid. The review comprises detail mechanism of data collection and secondary data from in vivo to clinical trials.
Strengths of the study:
- Comprehensive review supported with the flow of study selection and data collection
- Authors extracted comprehensive data and discussed extensively from efficacy, pharmacokinetics and toxicity in animal to human studies
Limitations:
1. Half of the references are not updated (in recent 5 years)
Suggestions:
- It is suggested to include the period/date of data collection Answer:
Answer:
Many thanks for the comments. In this scoping review, we collected the articles published within the last ten years, therefore it was within 2010-2021. We have stated in the manuscript in the following parts:
Line 114-117: "This study uses the scoping review method involving literature accessible through the PubMed, Sciencedirect, Scopus, and Google Scholar databases consisting of online research publications dating from 2011 to 2021"

2. Lack of significant outcome: for example: comparison between different cancer or types of nanoparticles, the dose of UA in different studies that might contribute to discrepancy in data analysis.
Answer:
Many thanks for the comments. We have added information regarding the cancer cell types and administered dose in Table 2 (line 260).

3. Explain why the clinical trial data is limited to "liposome nanoparticles"?
Answer:
In the method section, we have stated that we collected the articles published within the last ten years, which is 2010-2021. Within this scope, the articles published about clinical trial of ursolic acid nanoparticle are limited only for liposomes. Therefore, in this manuscript, the data for clinical trial is limited to liposome.

4. For table 2, it us suggested to include the dose/concentration of UA being formulated, with the type of cancer tumours
Answer:
Many thanks for the comments. We have added information regarding the cancer cell types and administered dose in Table 2 (line 260).

5. It is unclear that the In vivo anti-cancer efficacy was presented in both tumour tissue (only in liver cancer?) and tumour growth inhibition (without mentioning the type of cancer?).
Answer:

Many thanks for the comments. For tumor tissue analysis, from 18 articles, we have summarized the results into Table 5. And, the tumor tissue histopathology is not limited only to liver cancer, it is accordingly to cancer cells induction used in the study. We have revised the column title of Table 5 into Tissue Histopathology (line 411).

6. The authors are comparing the efficacy of different type of nanoparticles without mentioning the dose comparison

Answer:

Many thanks for the comments. We have added information regarding the administered dose in Table 2. However, the different route of administration would be an important parameter to do the comparative analysis; however, our analysis calculated the relative comparison with negative control or native ursolic acid treatment groups that would be fair justification of drug efficacy.

7. It would be great if authors could derive some outcomes/impact of the research such as "which nanoparticles could enhance the efficacy, pharmacokinetic or reduce toxicity of UA"??

Answer:

Many thanks for the comments. we have stated the findings of the review in line 622 as the following: "The findings of this scoping review suggest that liposomes provide more comprehensive data than other forms of nanoparticles. This is demonstrated by the existence of in vivo studies of anticancer effectiveness assessed using several parameters such as increasing relative survival rate; more robust tumor growth inhibition (increasing relative inhibition rate, decreasing relative tumor weight, and reducing tumor volume); and improvements in tumor tissue histopathology. In addition, in vivo studies related to safety were also evaluated employing several parameters, i.e., weight loss, and other toxicity (lowering AST, ALT, and WBC), and well-tolerated toxicity by healthy volunteers and patients with advanced tumors."

8. It is suggested that authors may include the limitations of this comprehensive review.

Answer:

Many thanks for the comments. We have added some sentences regarding the limitation of this study in line 630 as the following:

"There needs multi-faceted views of the use of nanoparticles for reviewing drug delivery. The components of the nanoparticle formulation would greatly affect the characteristics of the nanoparticles including particle size, potential charges, stealth and biomimetic properties, and others, which are closely related to drug delivery to cancer tissue, due to the Enhanced Permeation and Retention (EPR) effects. In addition, *in vivo* analysis of different types of cancer, where each type of cancer cell has different biological properties, also requires an in-depth study to provide data on supporting the effectiveness of drug delivery to target cancerous tissues. Moreover, the route of administration, dose, and frequency of drug administration related to the physicochemical properties and pharmacokinetic profile of the drug also greatly affect the systemic bioavailability and effective drug amount capable of reaching cancer tissue as the target of drug delivery. All these aspects provide important views for comprehensive study of the drug delivery system in cancer therapy."