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Research article

General toxicity studies of alpha mangostin from *Garcinia mangostana*: A systematic review

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ABSTRACT

Alpha mangostin (AM), the main xanthone derivative contained in mangosteen pericarp (*Garcinia mangostana*/GM), has many pharmacological activities such as antioxidant, antiproliferation, antiinflammatory, and anticancer. Several general toxicity studies of AM have been previously reported to assess the safety profile of AM. Toxicity studies were carried out by various methods such as on test animals, interventions, and various routes of administration, but the test results have not been well documented. Our study aimed to systematically summarizes research on the safety profile of GM containing AM through general toxicity tests to get the LD_{50} and NOAEL values, and so, can be used as a database related to AM toxicity profiles. This could facilitate other researchers in determining further development of GM-or-AM-based products. Pubmed, Google scholar, ScienceDirect, and EBSCO were chosen to collect the articles while ARRIVE 2.0 was used to evaluate the quality and risk-of-bias of the *in vivo* toxicity studies included in this systematic review. A total of 20 articles met the eligibility criteria and were reviewed to predict the LD_{50} and NOAEL of AM. The results showed that the LD_{50} of AM is between >15.480 mg/kgBW to ≤6000 mg/kgBW while the NOAEL value is between <100 and ≤2000 mg/kgBW.

1. Introduction

Mangosteen (*Garcinia mangostana* Linn./GM) is well known as the queen of fruits that grows in the Southeast Asian region. This fruit is widely consumed and the skin (pericarp) has also been widely studied containing active compounds with various pharmacological activities [1]. The main constituents found in the mangosteen pericarp are xanthones [2], with 50 types of xanthones isolated from the mangosteen pericarp [3]. However, the most abundant xanthone constituent is Alpha mangostin (AM) (Fig. 1) [2,4,5].

Many studies have proven the pharmacological activity of AM, through *in vitro, in vivo*, or *in silico* studies. AM is reported to have antioxidant [6–8], antiproliferation [9,10], wound healing, and anti-inflammatory activity [1,8,11–18]. AM also has activity as

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analgetic [15,19] and proven to be active against periodontal disease [20]. Besides that, AM has anticancer activity such as skin, colon, prostate, lung, breast, and colorectal cancers [2,4,10,21–27].

In central nervous system disease, AM is known to has activity as antidepressant [28]. AM also can act as antidermatitis, antibacteria, antifungal, and antiviral agent [29–34]. *In silico* study via molecular dynamics simulations showed that AM has activity as an estrogen-α receptor antagonist in breast cancer [35–37]. A computational study conducted by Megantara et al. (2022) also proved that AM can increase the effectiveness of breast cancer therapy using concomitant with trastuzumab [38]. Through molecular docking and dynamic simulations, mangosteen compounds are known to have activity as anti-diabetic agent [39]. This *in silico* finding is in line with the *in vitro* and *in vivo* studies conducted by Dyah (2011) and Djeujo (2022) [40,41].

Because of the bioactivities possessed by mangosteen, it has been widely developed into herbal products such as Mastin®, Meratrim®, Sari Skin Mangosteen®, Sunscreen Mangosteen, Mangosteen Body Lotion (products from Indonesia). Meratrim®, is a dietary supplement containing a mixture of mangosteen and *Sphaeranthus indicus* flower heads extracts. There are also attempts to develop AM as a radiopharmaceutical for breast cancer treatment [42–44]. In addition, a drug delivery system (DDS) for AM has been of interest with the aim to improve the physicochemical properties and increase the efficacy of AM, such as AM nanoformulations or AM microparticles coated with chitosan-alginate [22,45–47]. This indicates that mangosteen has been an interest as an alternative medicinal product.

With the increasing number of commercial products containing mangosteen in the form of extracts, powders, and other forms, it is necessary to pay attention to their safety profile for human use. The safety profile of AM can be determined through the toxicity study to determine the safe dose for humans consumptions. The dose can be determined through general toxicity studies consisting of acute, subacute/subchronic, and chronic toxicity. From the acute toxicity test, the LD_{50} (the dose causing 50% death of the test animals) value can be obtained, while the NOAEL (the lowest dose that not harming the test subject) is determined from the subacute/subchronic and chronic toxicity test [48,49]. Several studies related to the toxicity of mangosteen have been performed but not well documented. Based on this, we conducted a systematic review that aims to summarize the safety profile from the general toxicity tests of AM so it can be used as a database and facilitate other researchers in determining the next stage of developing AM-based products. From the database obtained, we also suggest other general toxicity studies of AM, especially subchronic and chronic tests, as well as other *in vivo* toxicity studies in different routes of administration.

2. Results

2.1. Study selection

The total number of articles obtained from the related search results is 2015 articles with 295 articles from Pubmed, 593 articles from Google scholar, 133 articles from ScienceDirect, and 994 articles from EBSCO. The sorting of duplicate articles, titles, abstract and full text screening was done manually. A total of 20 articles were selected from the final screening based on the general toxicity of AM or GM. The article screening process is shown in Fig. 2.

The databases searches were conducted through Pubmed, Google Scholar, ScienceDirect, and EBSCO. 1241 duplicates were eliminated and 774 were further screened based on its titles and abstracts. 22 articles were chosen for full paper screening while 751 irrelevant articles and one article that was not written in English were excluded. Twenty articles were chosen for the final review.

2.2. Quality assessment

The quality of the selected articles was assessed using ARRIVE 2.0 guidelines, which is a reporting tools for *in vivo* tests in animal research. These evaluations include the study design, sample size, inclusion and exclusion criteria, randomisation, blinding, outcome measures, statistical method, experimental animals, experimental procedures, and results. Besides that, the bias or the quality was also assessed with an essential checklist of ARRIVE [50].

Based on the screening, most articles from the 20 selected articles met all 10 essential ARRIVE criteria (from study design to result) and were identified to be low-risk-of bias with an average indicator of 87.7% for each article (Fig. 4). The high-risk-of bias was due to lack of information regarding the randomisation and blinding process of the studies and lack of data availability. The sample

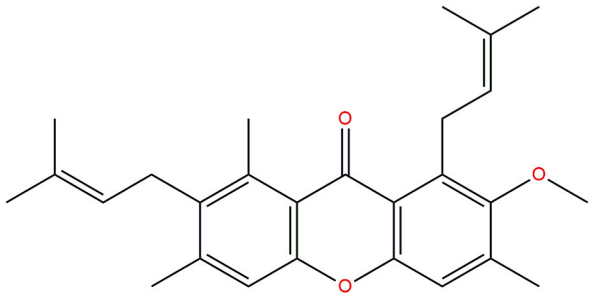

Fig. 1. Alpha mangostin structure.

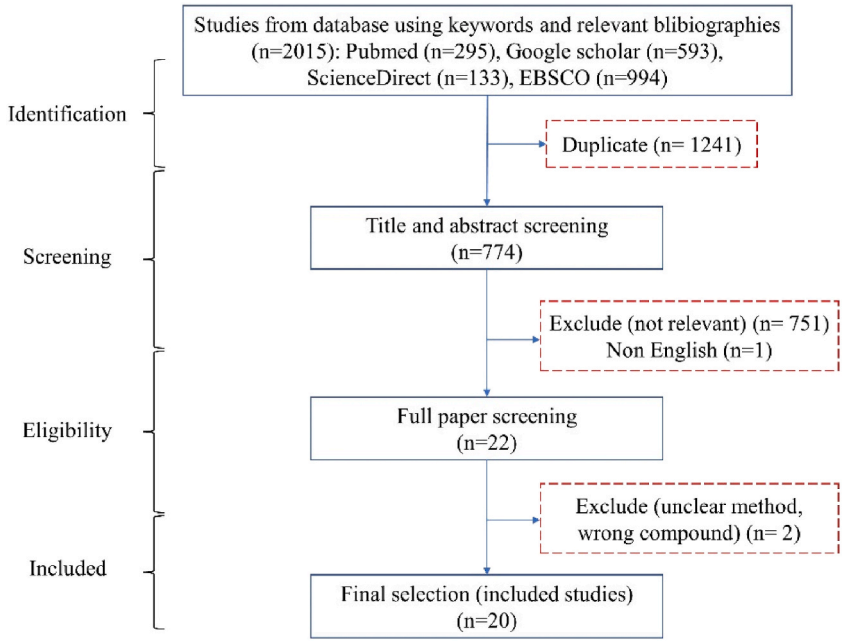

Fig. 2. Flow chart of screening and selection process for this review.

preparation and administration of the test compounds in *in vivo* toxicity tests are usually carried out directly by the main researcher, so that there is no blinding process in the study. In most cases, the raw data is not provided but can be obtained by contacting the author directly. Fig. 3 showed the risk-of-bias assessment and Fig. 4 shows the risk of bias included articles based on ARRIVE essential indicator.

2.3. Toxicity studies

The studies from the selected articles included in this review are summarized in Table 1. Generally, toxicity studies are performed to determine the safety level of the compound tested. Results of this review showed that AM is classified as non-toxic to slightly toxic (LD_{50} 500–5000 mg/kgBW) compound according to toxicity class [51,52].

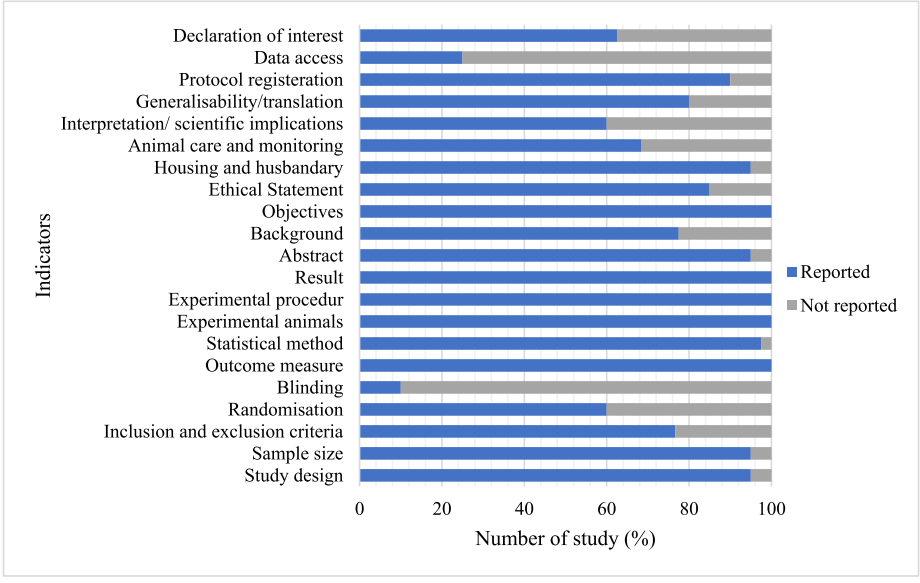

Fig. 3. The risk-of-bias assessment.

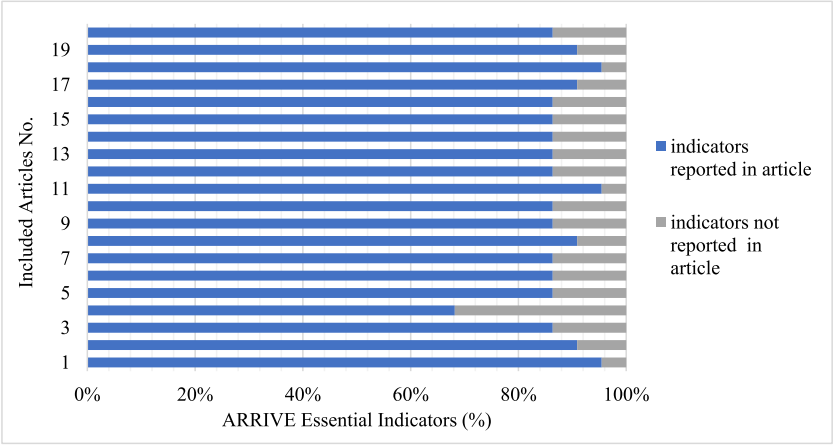

Fig. 4. Risk of bias assessment of included articles based on ARRIVE essential indicator.

3. Discussion

Toxicity studies are one of the important pre-clinical tests. Hence, it is a requirement for drug development and approval by regulatory bodies. General toxicity studies consist of acute, subacute or subchronic, and chronic studies. Each study has different duration and purpose. The acute study was performed to obtain the LD_{50} value while the subacute or chronic study were conducted to generate the NOAEL value.

In this review, a total of 20 articles reporting the toxicity of AM or GM were selected. Among the studies, GM extract was used as an intervention (15 studies) while the remaining five articles used AM as an intervention. Two out of 15 articles that used GM extract reported the usage of GM extract in combination with other plant extracts as intervention, while two studies used GM in the form of DDS. For the studies that used AM as an intervention, four studies used isolated and purified AM while a study used pure AM that is commercially available.

From 20 studies, seven studies performed both acute and subacute/subchronic toxicity evaluations, 10 studies performed acute toxicity tests, two studies performed subacute toxicity tests, and one study performed chronic toxicity tests. Most studies were conducted by the oral administration route (po), while two studies used the intra peritoneal (ip) injection. The oral administration is widely chosen because it is relatively easy and safe to administer [70].

Regarding test subject, all studies were performed on rodent (mice and rat). A total of five studies used BALB/c mice while four other studies used ICR mice. Seven studies used *Wistar* rat while the remaining four studies used *Sprague dawley* rat. As recommended by *The Organisation for Economic Co-operation and Development* (OECD), rodents is a suitable test subject for the toxicity study because it is considered to have similar physiology as human, hence resemble the toxicity response in human [71–74]. Besides, rodent is also cheap, easy to breed, and have a short life cycle [75].

The *in vivo* acute studies listed in Table 1 were conducted using GM extracts or AM in various concentration of AM as marker compound. The highest dose used is 6000 mg/kgBW. Nine studies showed that GM extract contains AM with oral administration is classified as class 5 toxicity (LD_{50} 2000–5000 mg/kgBW) in GHS classification and OECD which considered as non toxic compound [51]. Compared to two other studies of GM extract with different administration route (ip administration), the results showed lower LD_{50} (231.5 and 1000 mg/kgBW) which classified as class 3 (toxic) and class 4 (moderate toxic) [55,66]. Studies using isolated AM with oral administration showed an LD_{50} value from 1250 up to 2000 mg/kgBW indicating class 4 and 5 toxicities since the highest dose (2000 mg/kgBW) used by Nelli et al. (2013) did not exhibit any sign of toxicity [8,67,68]. In comparison, an acute toxicity study of AM from another plant, *Cratoxylum arborescens* also showed similar LD_{50} value (1000 mg/kgBW) with LD_{50} of AM from GM [76].

From these information, it is evident that both GM extract or AM when given orally will give a lower toxicity value than the compounds given intraperitoneally. However, the LD_{50} value of GM extract were higher than pure AM. This might be due to the differences of the AM concentration present in the extracts which is considered as the active compound responsible for the pharmacological activity [77,78]. The GM extract not only contains AM as its marker compound, but also other compounds in low concentration. When the extract is purified, the pure isolate with high concentration will be obtained. While high doses are given, it can increase the toxic response in the biological system [79,80]. This is in accordance with previous studies. Jujun et al. (2008) reported the LD_{50} value of 5000 mg/kg when using GM extract containing AM 11.45% while Chayaburakul et al. (2015) obtained LD_{50} 2000 mg/kg from GM extract containing AM 21.23% [61,64].

Choi et al. (2014) reported that AM with ip administration has LD_{50} 150 mg/kgBW. This study corroborated with Kosem et al. (2013), indicating that the ip administration of compounds tested can give a lower LD_{50} value. The ip administration has a fast absorption rate, great bioavailability, and almost similar to iv administration due to its wide peritoneal cavity area and the presence of microvilli on mesothelial cells that help the absorption process [81,82]. Although the ip administration must first perform the metabolism in liver, but due to its high absorption, it will also provide high bioavailability of the compound. Compared to oral administration, besides undergoing the first metabolism in the liver, the compounds have the potential to be degraded by gastric fluid

Table 1
Data extraction of toxicity studies.

No	Author (year)	Type of Study	Rute of administration	Subject and sample size	Intervention (I) and Comparator (C)	Dose	Treatment duration	Outcome	Reference
1	Dodda et al. (2020)	acute and subacute	po	•Acute oral: female Wistar rats (n = 5) •Subacute: male and female Wistar rats (n = 10, 5 female and 5 male/group)	•I: herbal formulation CinDura (aqueous-ethanol extracts of *Garcinia mangostana* fruit rind and the Cinnamomum tamala leaf; contain at least 3.5% α-mangostin and 0.1% rutin) •C: negative control (0.5% CMC-Na) and reversal groups	•Acute oral: 2000 mg/kgBW •Subacute: four primary groups (0, 250, 500, 1000 mg/kgBW/d) and two reversal groups (0 and 1000 mg/kgBW/d)	•Acute: 1 d, monitored for 14 d •Subacute: 28 d, additional 14 d monitoring without treatment for reversal groups	$LD_{50} \geq 2000$ mg/kgBW NOAEL 1000 mg/kg	[53]
2	Saiyed et al. (2015)	acute and subchronic	po	•Acute oral: Sprague Dawley female rats (n = 10) •Subacute: male and female Sprague Dawley rats (n = 40, 20 male and 20 female/group)	•I: Meratrim (*Sphaeranthus indicus* flower heads and *Garcinia mangostana* fruit rind extract; 3:1 extract ratio; the final blend contains at least 3% 7-hydroxyfrullanolide and 2% α-mangostin, and other minor constituents at levels below 0.5%) •C: negative control and recovery group	•Acute oral: 5000 mg/kgBW •Subacute: 4 test group (0, 250, 500 and 1000 mg/kg/day) and 2 recovery group (1 and 1000 mg/kg/day	•Acute: 1 d, monitored for 14 d •Subacute: 91 d (with four weeks recovery period)	$LD_{50} \geq 5000$ mg/kgBW NOAEL 1000 mg/kgBW	[54]
3	Choi et al. (2014)	acute	ip	•Male Institute of Cancer Research (ICR) mice (n = 6/group)	•I: α-mangostin (>98% purity) and mangosteen extrtact (ME) (containing α-mangostin as 25% of total ME) •C: negative control (PEG 400 and distilled water 6:4 v/v)	•0, 5, 10, 20, 50, 100, 200, or 400 mg (5 ml)/kg of α-mangostin or ME	•1 d, 72 h monitoring	LD_{50} α-mangostin 150.0 mg/kgBW LD_{50} ME 231.5 mg/kgBW	[55]
4	Navya et al. (2012)	acute	po	•Wistar rats (n = 6/group)	•I: α-mangostin (40% purity) C: -	•10,100, 250, 500, 1000, and 1500 mg/kgBW	•1 d, monitored for 14 d	$LD_{50} >$ 1500mg/kgBW	[8]
5	Sunarjo et al. (2017)	acute	po	•BALB/c mice (n = 5/group)	•I: mangosteen peel extract (contained 0.16% xanthones and 0.74% mangostin per 100 gr) •C: negative control (CMC)	•0, 5, 50, 300, 2000 and 5000 mg/kg	•1 d, monitored for 14 d	$LD_{50} \leq 5000$ mg/kgBW	[56]
6	Satriadinatha et al. (2019)	subacute	po	•BALB/c mice (n = 10/group)	•I: *Garcinia mangostana* ethyl acetate fraction and coated within chitosan-alginate capsules •C: negative control group, and normal group (Aquadest)	•aquades, 0, 0.5, 1, and 2 g/kgBW/d	•14 d	NOAEL ≤2000 mg/kgBW	[22]
7	Rahmayanti et al. (2016)	acute	po	•Female Sprague Dawley rats (n = 5/group)	•I: ethyl acetate fraction of *Garcinia mangostana* pericarp (concentration 3094 ppm) •C: normal control (water)	•8, 18 mg/kgBW, water	•1 d, monitored for 14 d	$LD_{50} >$ 15.480 mg/kgBW	[57]
8	Diba et al. (2019)	acute	po	•Female BALB/c mice (n = 5/group)	•I: chitosan-alginate microparticles encapsulated mangosteen •C: negative control (distilled water and gummi arabicum)	•0, 2, 3 and 5 g/kgBW	•1 d, monitored for 14 d	$LD_{50} \leq 3$ g/kg BW	[46]
9	Chivapat et al. (2011)	chronic	po	•Wistar rats (n = 15 for each sex/group)	•I: mangosteen pericarp extract (MPE) (contained 24.42% α-mangostin) •C: normal control (distilled water) and satellite group	•10, 100, 500, 1000 mg/kg/day and satellite group (1000 mg/kg/day) of MPE; distilled water 10 ml/kgBW/d	•6 mo, additional 14 d monitoring without treatment for satellite groups	NOAEL <500 mg/kgBW	[58]

(*continued on next page*)

Table 1 (*continued*)

No	Author (year)	Type of Study	Rute of administration	Subject and sample size	Intervention (I) and Comparator (C)	Dose	Treatment duration	Outcome	Reference
10	Avinash et al. (2016)	acute	po	•Male Swiss albino mice (n = 3)	•I: ethonolic extract of *Garcinia mangostana* (EEGM) •C: -	•2000 mg/kg	•1 d	LD_{50} > 2000 mg/kgBW	[59]
11	Bae et al. (2021)	acute	po	•Male Institute of Cancer Research (ICR) mice (n = 5/group)	•I: water extract of mangosteen pericarp (contained 0.95% and 0.10% (w/w) of α-mangostin and γ-mangostin) •C: -	•20, 50, 100, 500 mg/kgBW/d	•7 d	LD_{50} > 500 mg/kgBW	[60]
12	Jujun et al. (2008)	acute and subacute	po	•Sprague Dawley rats Acute: n = 6 for each sex/group •Subacute: n = 13 for each sex/group	•I: Ethanolic extracts of G. mangostana rind (contained 11.45% w/w of α-mangostin) •C: negative control (25% ethanol in water for acute study, 10% ethanol in water for subacute study) and satellite group	•Acute: 0, 2, 3 and 5 g/kgBW •Subacute: 0, 50, 500 and 1000 mg/kgBW •satellite group (1000 mg/kg BW)	•Acute: 1 d, monitored for 14 d •Subacute: 28 d, additional 14 d monitoring without treatment for satellite groups	LD_{50} < 5 g/kgBW NOAEL ≤1000 mg/kgBW	[61]
13	Bunyong et al. (2014)	acute and subacute	po	•Imprinting control region (ICR) mice (n = 3 for each sex/group)	•I: crude ethanolic extract of *Garcinia mangostana* •C: control (25% Tween-80)	•Acute: 5000 mg/kgBW, 25% Tween-80 •Subacute: 2000 mg/kgBW, 25% Tween-80	•Acute: 1 d, monitored for 14 d •Subacute: 14 d, additional 14 d monitoring without treatment for satellite groups	LD_{50} ≤ 5000 mg/kgBW NOAEL ≤2000 mg/kgBW	[62]
14	Pongsawat et al. (2017)	subacute	ip	•Wistar rats (n = 10 for each sex/group)	•I: purified alpha-mangostin pericarp extract (purity 96%) •C: negative control (0.1% CMC)	•100 mg/5 ml/kgBW/d	•5 d/week for a month	NOAEL <100 mg/kgBW	[63]
15	Chayaburakul et al. (2015)	acute and subchronic	po	•Sprague Dawley rats •Acute: n = 5 for each sex •Subchronic: n = 10 for each sex/group •Sentinel group: n = 5	•I: crude mangosteen pericarp hydro extract (contained 21.23% of α-xanthone) •C: control (distilled water), satellite group (100 mg/kg/day), sentinel group	•Acute: 2000 mg/kg BW •Subchronic: 0, 10, 50, 100, mg/kgBW/d	•Acute: 1 d, monitored for 14 d •Subchronic: 90 d, additional 14 d and 28 d monitoring without treatment for satellite groups and sentinel group	LD_{50} ≤ 2000 mg/kgBW NOAEL <100 mg/kgBW	[64]
16	Priya et al. (2010)	acute	po	•Specific-pathogen-free bred Wistar rats (n = 6/group)	•I: G. mangostana pericarp extract C: normal control (distilled water)	•0, 1.0, 2.0 and 3.0 g/kgBW	•1 d, monitored for 14 d	LD_{50} ≤ 3000 mg/kgBW	[65]
17	Kosem et al. (2013)	acute and subacute	ip	•Female BALB/c mice (n = 5/group)	•I: Crude methanolic extract (CME) mangosteen pericarp (contained 25.19 ± 0.22% of α-mangostin) •C: control (PBS)	•Acute: 50–2000 mg/kg CME in PBS •Subacute: 0–500 mg/kg/day	•Acute: 1 d •Subchronic: 14 d	LD_{50} ≤ 2000 mg/kgBW NOAEL <200 mg/kgBW	[66]
18	Nelli et al. (2013)	acute	po	•Male albino Wistar rats (n = 6/group)	•I: isolated α-mangostin •C: -	•100, 500, 1,000, and 2000 mg/kgBW α-mangostin in 40% ethanol	•1 d, 48 h observation	LD_{50} ≥ 2000 mg/kgBW	[67]
19	Kumar et al. (2016)	acute and subacute	po	•Male Swiss albino (Wistar strain) rats •Acute: n = 6/group Subacute: n = 3 for each sex/group	•I: α-mangostin •C: normal control	•Acute: 10, 50, 250, 1250 mg/kgBW •Subacute: 10, 50, 250, 1250 mg/kgBW/d	•Acute: 1 d, 48 h observation •Subacute: 28 d	LD_{50} ≥ 1250 mg/kgBW NOAEL ≤1250 mg/kgBW	[68]
20	Trya et al. (2019)	acute	po	•BALB/c mice (n = 5/group)	•I: ethyl acetate mangosteen fraction •C: distilled water	•0, 2, 4 and 6 g/kgBW	•1 d, 14 d observation	LD_{50} ≤ 6000 mg/kgBW	[69]

and undergo biopharmaceutical changes which can affect or reduce the concentration or bioavailability of the compounds [70,81]. It can be concluded that the routes of administration will affect the pharmacokinetic response [70].

The NOAEL values obtained from subacute and subchronic toxicity studies of GM extract when given orally showed a wide range value of 1000–2000 mg/kgBW [61,62]. However, another study using GM extract by ip administration has lower NOAEL values (<200 mg/kgBW) [66]. Studies using AM also give similar NOAEL value compared to studies using GM extracts (NOAEL ≤1250 mg/kgBW) [68]. This is because the exposure routes affects the pharmacokinetic profile of the compounds [70]. Although there have been many studies on acute and subchronic toxicity, only one study performed the chronic toxicity study of GM. Chivapat et al. (2011) reported that GM extract contained 24.42% AM gave NOAEL values < 500 mg/kgBW [58].

In this review, we acknowledged that there is inadequate data on the toxicity studies of pure AM isolate. Further research is needed to investigate the toxicity of AM, particularly for subchronic and chronic tests, to generate an alpha mangostin safety database. It is hoped that this database will facilitates the development of a safe alpha mangostin-based product. Different types of natural compounds (extracts or isolates), their concentration, and the routes of administration might give different toxicity values. Therefore, it is very important for researchers to perform another toxicity studies through various routes of administration, to determine the safe dose of AM, ensuring that the dose used in products development is safe for human.

4. Materials and methods

4.1. Study design

No specific registered protocol was used to compile this systematic review. This review was prepared based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [83].

4.2. Search strategies

Article searches were conducted using EBSCO, Pubmed, ScienceDirect, and Google scholar platforms. The databases were screened from January 2000 until February 2023. The search strategy was carried out using keywords related to the research question, such as "*toxicity of alpha mangostin*; *toxicity of Garcinia mangostana*; *toxicity of mangosteen*; *acute, subacute, subchronic, chronic toxicity study of alpha mangostin Garcinia mangostana mangosteen*; *toxicity evaluation of mangosteen, alpha mangostin, Garcinia mangostana*; *in vivo toxicity study of mangosteen*; *LD_{50} alpha mangostin, Garcinia mangostana, mangosteen*; *NOAEL alpha mangostin, Garcinia mangostana, mangosteen*; *Acute and subchronic toxicity evaluation of mangosteen pericarp alpha mangostin, Garcinia mangostana*". The search strategies were also carried out using the words "*or*" and/or "*and*" to expand or narrow the search results.

4.3. Inclusion and exclusion criteria

Articles reporting *in vivo* general toxicity tests (acute, subacute/subchronic, and chronic) of GM and AM were included in the inclusion criteria. Articles reporting the toxicity test of GM in combination with other plants or those that have been formulated in various drug formulation or drug delivery systems were also included as inclusion criteria. In addition, the availability of the full article was also being considered. The exclusion criteria were articles reporting on the general toxicity of GM without AM (xanthones), special toxicity of GM and AM, toxicity studies of AM from other plants, and articles published in languages other than English.

4.4. Study selection

The selection of relevant articles was carried out by screening the title and abstract to determine the eligibility criteria by two authors (LUS and WN). The full text of the relevant published article was then reviewed. The reference manager EndNote 20 was used to compile the selected articles to be used in this review.

4.5. Data extraction

The data taken for this review include the author's name, year of publication, type of study, route of administration, subject and sample size, the dose of intervention and comparator (control) given, treatment duration, and result/outcome summarized in the table.

4.6. Quality assessment

Quality and risk of bias in the *in vivo* general toxicity studies were estimated using a checklist recommended by the ARRIVE 2.0 guideline [50]. Quality assessment was carried out independently by two authors (LUS and WN).

5. Conclusions and perspective

This systematic review collected and summarized the information on the toxicities profile of alpha mangostin (AM) from *Garcinia mangostana* (GM). Information from the animal studies provides the predicted safety doses of AM. It is hoped that this review may facilitate researchers in developing a safe alpha mangostin-based product. However, the data related to the toxicity of pure AM is still

very limited because most of the studies conducted use mangosteen extract which contains only small amount of AM. Meanwhile, to develop new drugs from herbal, the pure isolate with high concentrations is recommended.

From the data, the concentration of the tested compound influences the toxicity values (LD_{50} and NOAEL). The higher the purity of the test compound, the smaller the LD_{50} and NOAEL values, indicating that the compound is more toxic. In addition, differences in administration routes also can affect the toxicity values. This can be influenced by the pharmacokinetic profile given by the compound.

Despite the limited *in vivo* toxicity test data of AM isolates, we acknowledged that this can be a challenge as well as an opportunity for other researchers in doing research on AM toxicity to generate the complete AM safety profile data through general toxicity tests. The researchers can consider performing the subchronic and chronic toxicity studies of AM, to determine the safest dose and the long-term side effects of AM after repeated administration.

The information provided in this review provide insights to perform the toxicity test in other routes of administration, because most of the studies listed were given orally. The AM toxicity test through intravenous, intraperitoneal, intramuscular, and other routes also need to be considered for testing. This effort is beneficial to obtained the complete safety data of pure AM in various route of administration.

Author contribution statement

Luthfi Utami Setyawati: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nur Kusaira Khairul Ikram: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Wan Ezumi Mohd Fuad: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Muchtaridi Muchtaridi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

No data was used for the research described in the article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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