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Jatropha curcas seed oil for possible human consumption: A toxicological assessment of its phorbol esters

Xinyuan Cao [a], Xiaoyang Wei [a], Yuanyuan Shao [a], Dongbing Li [a,*], Jesse Zhu [a,b]

[a] Nottingham Ningbo China Beacons of Excellence Research and Innovation Institute, University of Nottingham Ningbo China, Ningbo 315048, China
[b] Department of Chemical and Biochemical Engineering, Western University, London, Ontario N6A 3K7, Canada

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ABSTRACT

Jatropha curcas seeds are known for their high oil content, and the oil extracted from these seeds has been traditionally utilized in biodiesel production. The presence of toxic compounds, specifically phorbol esters (PEs), in *Jatropha curcas* seed oil (JCSO) has blocked its use for human consumption. This article presents a thorough literature review that summarizes the latest research on the toxicological effects, including acute toxicity, genotoxicity, carcinogenicity, and chronic toxicity associated with *Jatropha curcas* phorbol esters (JCPEs). It also provides an overview of current detoxification strategies. A quantitative risk assessment was performed using the Benchmark Dose (BMD) approach, revealing an Acute Reference Dose (ARfD) of 139.64 μg kg^{-1} body weight for JCPEs (expressed as 12-O-tetradecanoyl-phorbol-13-acetate equivalent). Moreover, a Health-Based Guidance Value (HBGV) for JCPEs in a sub-chronic exposure context was established at 0.0105 mg kg^{-1} body weight per day. These results have guided the formulation of detoxification goals for JCSO, targeting a detoxification rate of 99.5 %, along with recommendations for future research to investigate the feasibility of using JCSO in food products.

1. Introduction

Jatropha curcas, commonly known as physic nut or purging nut, is a pantropical species of the *Euphorbiaceae* family. It is believed to have originated in tropical America and the Caribbean. Today, it is extensively found across tropical and subtropical regions of Asia, Africa and the Americas [1–3]. *Jatropha curcas* seeds are notably high in oil content, approximately 60 % based on dry weight, as indicated by our laboratory findings. The fatty acid profile of *Jatropha curcas* seed oil (JCSO), detailed in Table 1, reveals a significant presence of oleic acid (18:1, 44.7 %) and linoleic acid (18:2, 32.8 %). This profile closely mirrors that of oils typically found in human diets [4]. Numerous studies have highlighted the oil's significant antimicrobial properties, enhancing its potential as a valuable edible oil [5–8]. Currently, JCSO is mainly used for biodiesel production [9]. Another application of JCSO under development is for use as insulating oil in distribution transformers within the electricity sector [10].

USDA statistics reveal that during the 2023/24 period, China's domestic oilseed production totaled 69.08 million tons, with imports amounting to 11.71 million tons [11]. The consumption of vegetable oil in China exceeded 40 million tons, with over 25 % of this supply coming from imports. The supply of edible oils in China remains in a state of crisis [12]. Consequently, it is essential to investigate new oilseed resources to ensure oil security in the country. The total cultivation area of *Jatropha curcas* in China was over 20×10^4 ha [13]. The natural seed yield is approximately 0.8 kg/m^2, resulting in an annual oil production of 45,000 tons [13]. Furthermore, significant investments in *Jatropha* plantations across China have led to a substantial increase in oil output [14]. The edible use of JCSO could effectively leverage these resources, providing a means to alleviate the deficit in vegetable oil production in China while also benefiting the local economy.

The by-product of oil extraction, known as *Jatropha curcas* seed cake (JCSC), contains about 60 % crude protein. Its amino acid profile is comparable to the Food and Agriculture Organization of the United Nations (FAO) reference, with the exception of lysine, making JCSC a promising protein source for both humans and livestock [15,16]. JCSC can also be used for biogas production or as an organic fertilizer [17]. *Jatropha curcas* has been utilized for medicinal purposes in various regions [18]. This plant has shown effectiveness in treating a range of infections [19]. Moreover, research indicates that phorbol esters (PEs) extracted from *Jatropha curcas* could be a viable alternative in cancer chemotherapy, as they may help downregulate proto-oncogenes and

* Corresponding author.
E-mail address: dongbing.li@nottingham.edu.cn (D. Li).

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Table 1
Fatty acid composition of JCSO.

Fatty acid	Composition (%)
Oleic 18:1	44.7
Linoleic 18:2	32.8
Palmitic 16:0	14.2
Stearic 18:0	7.0
Palmitoleic 16:1	0.7
Linolenic 18:3	0.2
Arachidic 20:0	0.2
Margaric 17:0	0.1
Myristic 14:0	0.1
Caproic 6:0	-
Caprylic 8:0	-
Lauric 12:0	-
Capric 10:0	-
Saturated	21.6
Monounsaturated	45.4
Polyunsaturated	33.0

trigger apoptosis in cancerous cells [20]. In addition, these PEs can serve as a precursor for the synthesis of prostratin, an anti-tumor agent that has also demonstrated potential in the fight against AIDS [21].

The JCSO exhibits significant potential for edible applications. However, despite their favorable composition and value-added properties, the presence of a group of toxic PE compounds in the *Jatropha curcas* products poses a challenge for their utilization for edible uses [22, 23]. An essential aspect in the effective utilization of *Jatropha curcas* resources involves finding efficient methods to detoxify or to separate the PEs present in JCSO. Currently, there have been some preliminary studies to detoxify the *Jatropha curcas* products. Solvent extraction and fermentation are the two prevailing methods for detoxification of PEs from JCSO and JCSC, typically in batch mode [24–26]. Following the detoxification process, it is crucial to conduct a thorough toxicity assessment for edible applications. There is lack of a consolidated summary regarding the existing toxicity data of *Jatropha curcas* phorbol esters (JCPEs). This review aims to fill that gap through a systematic analysis of the literature. It compiles current toxicological information on JCSO and JCPEs, includes some quantitative assessments to help establish detoxification targets, and outlines the future toxicological data needed to steer further research.

2. Methodology

A comprehensive search was conducted on the toxicology of JCPEs across three databases: Scopus, Web of Science, and Pubmed. The search utilized the keywords *Jatropha curcas* AND phorbol esters AND toxicity. Only articles published in English were selected for review. In total, 156 articles were gathered, and each was evaluated based on its title and abstract to ascertain its relevance to the subject matter.

2.1. Chemical structures of PEs

PEs are a group of esters derived from tigliane diterpenes (Fig. 1a), characterized by their four-ring structure. Hydroxylation can occur at various positions on these rings, leading to the formation of the foundational compound known as phorbol (Fig. 1b). Phorbol contains five hydroxyl groups, and the esterification of the hydroxyl groups located at C-12, C-13, and C-20 results in a diverse array of PEs. One notable derivative, prostratin (13-O-acetyl-12-deoxyphorbol), has demonstrated the ability to counteract HIV latency [27].

PEs are naturally found in several plant species, particularly in croton and *Jatropha*. The major phorbol ester presents in croton is 12-O-tetradecanoyl-phorbol-13-acetate, TPA (Fig. 1c). TPA is one of the most commonly used PEs in research as a PKC activator and serves as a reference compound for measuring phorbol ester content. Interestingly, TPA is not present in *Jatropha*; instead, PEs in this plant are derived from the isomer known as 12-Deoxy-16-hydroxy phorbol (Fig. 1d). There are 6 different PEs present in the *Jatropha curcas* seeds product [28]. They have been previously isolated and designated as *Jatropha* factor 1–6 [29], with their structures illustrated in Fig. 1(e).

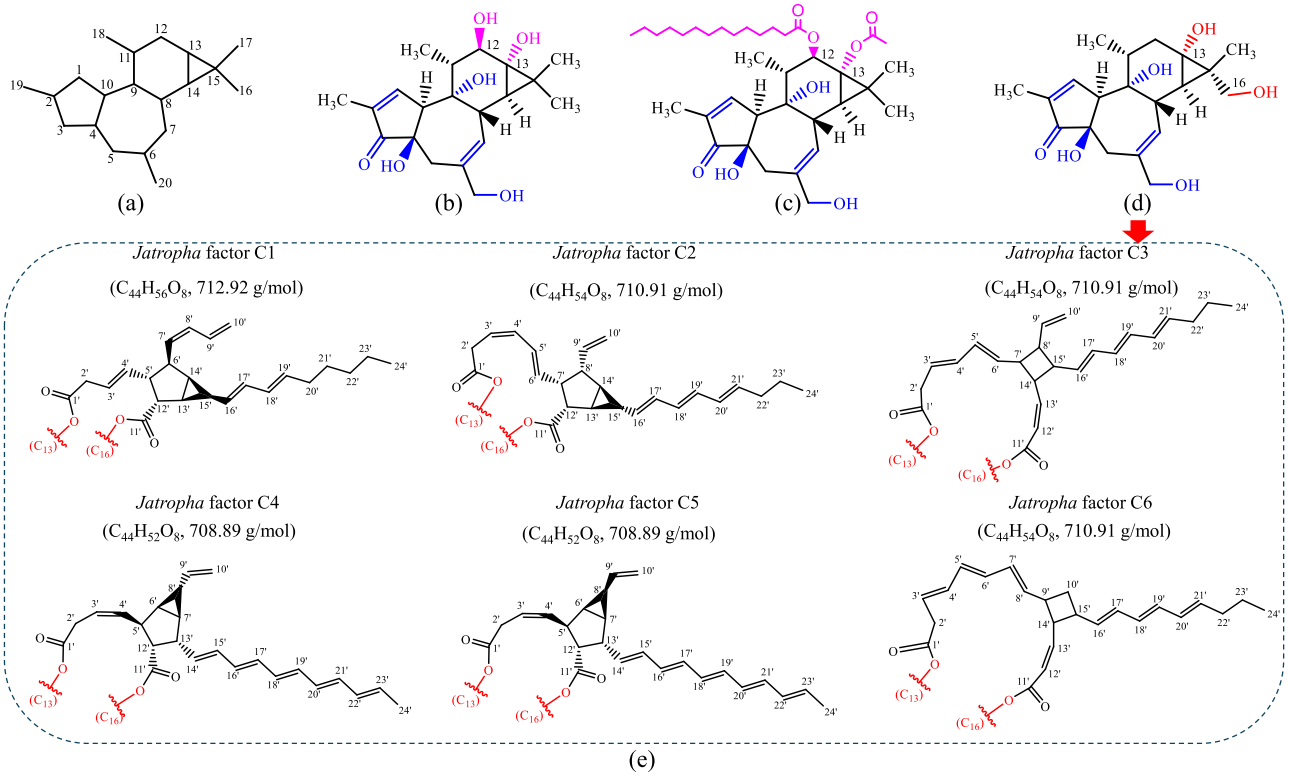

Fig. 1. Chemical structures of (a) tigliane, (b) phorbol, (c) TPA, (d) 12-deoxy-16-hydroxy phorbol, and (e) *Jatropha* factors C1–C6.

The contents of PEs in *Jatropha curcas* seeds vary from different origins and species. Previous studies have shown that the seed kernels of the toxic *Jatropha curcas* species contain 0.87–3.32 mg g^{-1} PEs in TPA equivalent [30]. Notably, the distribution of PEs within the seeds is not uniform. Approximately 95 % of these compounds are located in the tegments and endosperms, while the testas and embryos contribute the least [31]. Being lipophilic, PEs tend to be extracted into the seed oil during oil extraction, although some remain in the seed meal. Additionally, the germination process of *Jatropha curcas* seeds leads to the breakdown of PEs, primarily due to the action of lipase [32].

2.2. Measurement of JCPEs

The quantification of PEs is commonly performed using HPLC analysis, which is considered the most extensively employed technique. In a study by Makkar et al. [30], a HPLC-based method was developed to analyze PEs in *Jatropha curcas* seeds, which has since been adopted in various research efforts. Makkar et al. [33] offered a comprehensive review of various HPLC methods that are being utilized. In these methods, TPA is used as an external standard and the results are reported as TPA equivalents. While TPA is not naturally present in *Jatropha* products, its structure and properties are similar to those of other PEs. However, these studies did not confirm the identification of the separated compounds as PEs. The structure of one of the *Jatropha* factors was confirmed by Hirota et al. [34]. Another study by Haas et al. [29] successfully identified and characterized the six compounds (Fig. 1e) separated from *Jatropha* products using NMR analysis, confirming their structures and providing essential information on their elution order for HPLC applications. Baldini et al. [35] employed LC-MS for the precise quantification of each *Jatropha* factor, enhancing the accuracy of structural and content assessments. Typically, UV profiles of PEs were also obtained to assist in their identification during detection [35–37]. The HPLC chromatograph of our *Jatropha curcas* seed oil is depicted in Fig. 2.

3. Toxicity mechanism of PEs

The negative impacts associated with PEs are closely linked to their ability to activate protein kinase C. The toxic effects of PEs trigger inflammatory responses and promotes platelet aggregation. Additionally, the PEs are recognized as significant tumor promoters.

3.1. Activation of PKC

The role of PEs in activating protein kinase C (PKC) has been extensively researched. It is widely recognized that cells communicate through lipid-mediated signaling pathways. Phosphatidylinositol (PIs), a class of inositol-containing phospholipids, plays a crucial role in the membranes of eukaryotic cells. The pioneering research conducted by Hokin and Hokin [38] revealed that the stimulation of enzyme secretion by cholinergic drugs such as carbamylcholine, leading to a significant uptake of P^{32}, a radiolabeling on ATP, into PIs. This finding indicated a significant increase in the turnover of these lipids. Following this, Zhang and Majerus [39] characterized and summarized the pathway of PIs turnover. Essentially, when external agonists activate phosphatidylinositol-specific phospholipase C, it hydrolyzes PIs, generating the crucial second messengers diacylglycerol (DAG) and inositol-1, 4,5-trisphosphate (IP3), which are vital for activating protein kinase C.

Protein kinase C, also referred to as Ca^{2+}-phospholipid-dependent protein kinase, consists of a family of serine-threonine kinases that are vital for various cellular functions. This family is divided into three categories: conventional (cPKCs), novel (nPKCs), and atypical PKCs

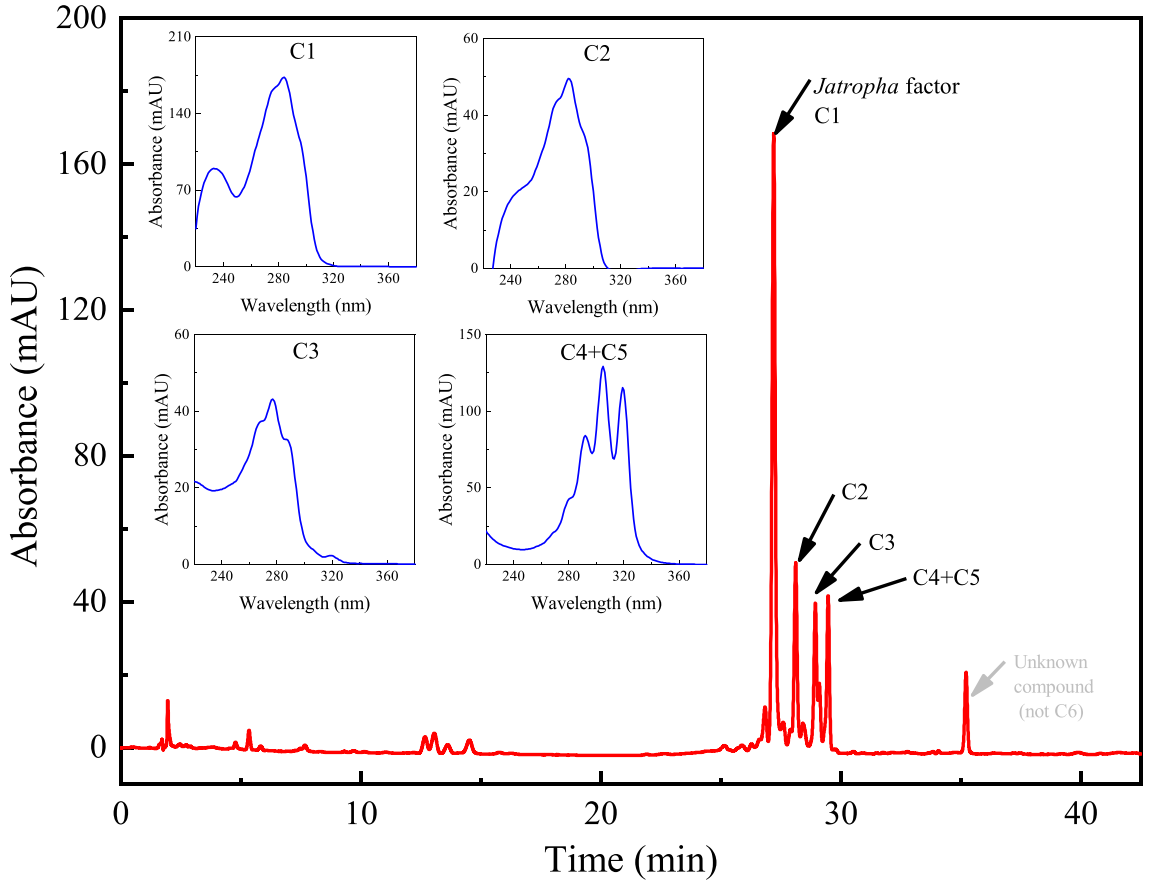

Fig. 2. HPLC chromatogram of *Jatropha* factors and their UV spectra.

(aPKCs). Conventional PKCs can be activated by Ca^{2+}, the second messenger DAG, or PEs. In contrast, novel PKCs are not activated by Ca^{2+} but can still respond to DAG and PEs, while atypical PKCs do not respond to either Ca^{2+}, DAG, or PEs [40,41].

PKC is widely distributed in the cytosol. All PKC isozymes contain two distinct domains: the regulatory domains at the amino-terminal and the catalytic domains at the carboxyl terminal [41]. The regulatory domains contain motifs that can bind to cofactors such as Ca^{2+} and DAG, while the catalytic domain interacts with ATP and substrates. Each PKC isozyme features four conserved regions: C1, C2, C3, and C4. The C2 domain is responsible for binding Ca^{2+} and facilitating the translocation of PKC to the cell membrane [41]. The C1 domain is where the binding of DAG and PEs occurs. Within this domain lies a pseudosubstrate that engages with the substrate binding site in the catalytic domain, leading to an inactive state. When PKC binds with DAG or PEs, it induces a conformational change that facilitates the release and cleavage of the pseudosubstrate from its binding site, ultimately resulting in PKC transitioning to an active form [42].

Due to their lipophilic nature, PEs readily integrate into cell membranes, where their hydroxyl and carbonyl groups interact with specific receptors [40,43,44]. PEs can mimic DAG and compete for binding with Ca^{2+}-phospholipid-dependent protein kinase, PKC [45]. DAG attaches to the C1 domain of PKC, which is also recognized as a binding site for PEs. While DAG is easily metabolized, PEs maintain a more stable structure, leading to an overactivation of PKC [40].

3.2. Inflammatory effects and cell apoptosis

Goel et al. [46] provided a comprehensive overview of the key inflammatory responses triggered by PEs (Fig. 3). In a study by Oskoueian et al. [47], the researchers explored the redox status and gene expression in bovine kidney cells subjected to JCPEs at a CC_{50} concentration. Their findings offered valuable insights into the mechanisms of inflammation and cell apoptosis. The study revealed that PEs not only induced oxidative stress within the cells but also stimulated the release of pro-inflammatory cytokines like IL-1β and Cox2. Additionally, PEs activated the PKC-βII signaling pathway, leading to the upregulation of proto-oncogenes, which further contributed to the expression of pro-inflammatory cytokines. Ultimately, this inflammatory response initiated the activation of caspase-3, resulting in apoptotic cell death through various signaling cascades. PEs are able to regulate the cell differentiation and proliferation, with effects that are diverse and largely dependent on the specific cell type and their interactions with different molecules [48]. For instance, research on BALB/3T3 T preadipocytes demonstrated that TPA at a concentration of 100 ng mL^{-1} inhibited cell proliferation after 6–18 h of incubation, but this effect shifted to stimulation over the following 3 days [49].

3.3. Tumor promotion

PEs are widely recognized as a category of tumor promoters. A well-established two-stage chemical carcinogenesis model utilizes 7,12-dimethylbenz[*a*]anthracene (DMBA) in conjunction with TPA to effectively induce tumors in mouse skin as well as other tissues [50–53]. While a high dose of DMBA can independently trigger tumor formation, lower doses of DMBA do not lead to detectable tumors throughout the lifespan of the mice. Similarly, a single application of TPA does not result in tumor development. However, when TPA is combined with a subthreshold dose of DMBA, tumors become evident [54]. Notably, the effects of TPA as a tumor promoter are reversible; if DMBA and TPA are administered in reverse order, tumor formation is prevented, and tumors are also absent when there is an adequate time gap between the two applications [55]. The underlying mechanisms of these distinct effects are thought to be linked to the activation of PKC [56,57]. PKC plays an essential role for various cellular responses and influences cell proliferation and differentiation [58,59]. PEs have demonstrated diverse effects across different cell types. As previously noted, PEs can inhibit cell proliferation and induce apoptosis, while in certain cells, such as leukemic cells, they promote differentiation by modulating the cells' sensitivity to specific protein inducers [48,55]. This mechanism is considered a pathway through which PEs act as tumor promoters. Nevertheless, the intricate mechanisms and the interplay between the various effects of PEs require further investigation.

4. Current toxicology data

4.1. Acute toxicity

Research has primarily focused on *Jatropha* products, with limited attention given to the PEs themselves. Studies have assessed the acute toxicity of PEs across various organisms, including insects, aquatic species, and mice [60–66]. The quantitative results from these investigations are summarized in Table 2, while the acute toxicity profile of JCPEs is depicted and shown in Fig. 4.

The toxicity of a PEs-rich fraction was evaluated in snails (*Physa fontinalis*), brine shrimp (*Artemeia salina*), and daphnia (*Daphnia magna*) by Devappa et al. [62]. The bioassays utilized samples containing 43.8 mg g^{-1} PEs. The half maximal effective concentration (EC_{50}) values recorded were 0.33 mg L^{-1} for snails, 26.08 mg L^{-1} for brine shrimp, and 0.95 mg L^{-1} for daphnia, with all concentrations expressed as TPA equivalents. Roach et al. [63] isolated *Jatropha* factor C1, C2, C3, C4 + 5 and performed bioassays on snails and brine shrimp to compare the toxicity of these isolated compounds. For snails, the potency was ranked as follows (EC_{50}, μg L^{-1}, equivalent to *Jatropha* factor C1): factor C3 mixture (6.78, obtained as a mixture) < factor C2 (6.54) < factor C1 (4.12) < factor C4 + 5 (2.18). For brine shrimp, the potency was (EC_{50}, mg kg^{-1}, equivalent to *Jatropha* factor C1): factor C2 (11.8) < factor C3

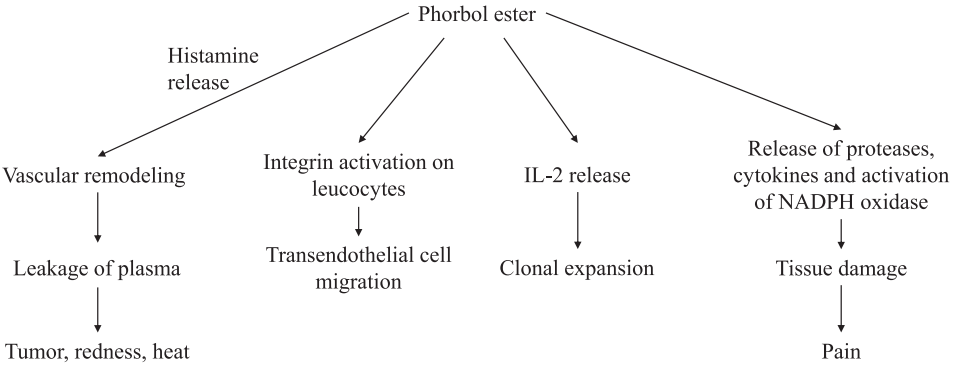

Fig. 3. Inflammatory responses induced by PEs.

Table 2
Overview of acute toxicity data for JCPEs and JCSO.

Matrix	Test animal	Concentration expressed	Results*	Ref.
PE-rich JCSO extract	Termites (*Odontotermes obesus*)	TPA equivalent	EC_{50} (24 h exposure): 71 mg mL^{-1}	[60]
	Third instar larvae (*Spodoptera frugiperda*)	TPA equivalent	EC_{50} (24 h exposure): 0.83 mg mL^{-1}	[61]
	Brine shrimp (*Artemia salina*)	TPA equivalent	EC_{50} (24 h exposure): 26.48 mg L^{-1}	[62]
	Brine shrimp (*Artemia salina*)	Equivalent of *Jatropha* factor C1	EC_{50} (24 h exposure), *Jatropha* factor C1: 0.43 mg L^{-1} EC_{50} (24 h exposure), *Jatropha* factor C2: 11.8 mg L^{-1} EC_{50} (24 h exposure), *Jatropha* factor C3: 1.08 mg L^{-1} EC_{50} (24 h exposure), *Jatropha* factor C4 + 5: 0.043 mg L^{-1}	[63]
	Daphnia (*Daphnia magna*)	TPA equivalent	EC_{50} (48 h exposure): 0.95 mg L^{-1}	[62]
	Snails (*Physa fontinalis*)	TPA equivalent	EC_{50} (24 h exposure): 0.33 mg L^{-1}	[62]
	Snails (*Physa fontinalis*)	Equivalent of *Jatropha* factor C1	PE-enriched fractions, EC_{50}: 11.33 μg L^{-1} EC_{50} (24 h exposure), *Jatropha* factor C1: 4.12 μg L^{-1} EC_{50} (24 h exposure), *Jatropha* factor C2: 6.54 μg L^{-1} EC_{50} (24 h exposure), *Jatropha* factor C3: 6.78 μg L^{-1} EC_{50} (24 h exposure), *Jatropha* factor C4 + 5: 2.18 μg L^{-1}	[63]
JCPEs diluted in corn oil	Swiss Hauschka male mice (18–20 g)	TPA equivalent	LD_{50}: 27.34 (24.90–29.89) mg kg^{-1} body weight (bw) LD_5: 18.87 mg kg^{-1} bw LD_{95}: 39.62 mg kg^{-1} bw	[64]
JCSO/ soil mixture	Brine shrimp (*Artemia salina*)	TPA equivalent	EC_{50} (24 h exposure): 2.7 % of oil or 67 mg L^{-1} PEs	[65]
JCSO	Haffkine Wistar strain (90–130 g)	-	LD_{50}: 6.0 (4.7–7.6) mL oil kg^{-1} bw; 100 % mortality at over 9.0 mL kg^{-1} bw dose	[66]

* EC_{50}, half maximal effective concentration; LD_{50}, median lethal dosage; LD_5 and LD_{95}, lethal dosage to 5 % or 95 % of the test animals.

mixture (1.08) < factor C1 (0.43) < factor C4 + 5 (0.043).

Wang et al. [67] carried out a study where they administered JCSO to carp fingerlings (*Cirrhinus chinensis*). Their results indicated that even though the levels of PEs were beneath the detection threshold of HPLC, toxic effects were still evident in the carp fingerlings. In contrast, other studies suggested that the kernel extracts did not produce adverse effects after detoxification [68,69]. These discrepancies may arise from variations in detection limits associated with the analytical methods and techniques employed, as well as differences in the experimental subjects. It is essential to emphasize the significance of establishing the detection limit for any analytical approach used. Another study

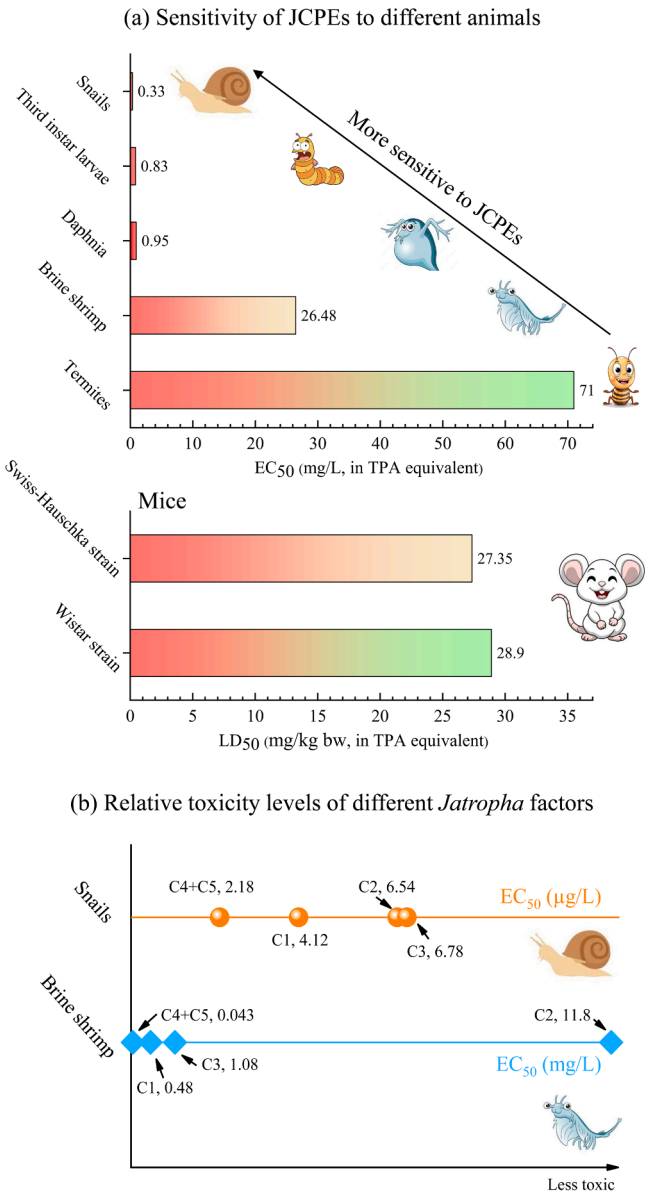

Fig. 4. Acute toxicity profile of JCPEs.

investigated the toxicity of a new food product made from *Jatropha curcas* [70]. It was noted that the detection limit for PEs was as low as 0.75 μg PEs per g of kernel, in TPA equivalent, using HPLC-UV. Samples that fell below the limit of detection (LOD) did not exhibit any acute or genetic toxicity, providing a reference point for the detoxification of *Jatropha curcas* products intended for human consumption.

Comparable studies have also been carried out on mammals. In a study by Gandhi et al. [66], mice were administered JCSO. The median lethal dosage (LD_{50}) of this oil was found to be 6.0 mL kg^{-1} body weight (bw), with a dosage of 9 mL kg^{-1} bw resulting in 100 % mortality. The report used *Jatropha curcas* seeds from India, but did not give the PE content in the seeds oil. Similarly, Roach et al. [63] used *Jatropha curcas* seeds from India and reported a PE content of 5.45 mg g^{-1} in terms of TPA equivalent. Previous research by Belewu et al. [71] measured the density of Indian JCSO, which was recorded at 0.884 g mL^{-1}. Using this data, the LD_{50} for the JCPEs in the oil was estimated to be 28.9 mg kg^{-1} bw, in TPA equivalent. This finding is consistent with the results from Li et al. [64], who investigated the acute toxicity of JCPEs in Swiss Hauschka mice. They reported an LD_{50} of 27.34 mg kg^{-1} bw, in TPA equivalent, for male mice. The LD_5 and LD_{95} values were found to be

18.87 and 39.62 mg kg^{-1} bw, respectively, in TPA equivalent. The quantitative acute toxicity data derived from mice is valuable for assessing potential toxicity in humans.

4.2. Genotoxicity

A study assessed the genotoxic effects of *Euphorbia tirucalli* extract, which contains TPA [72]. The findings revealed that TPA led to a five-fold increase in micronuclei, a 40-fold rise in DNA damage, and approximately a 68-fold increase in chromosomal aberrations. While TPA is categorized as a phorbol ester, it is absent in *Jatropha* plants. The PEs found in *Jatropha* have a different structure than TPA, suggesting a variation in their toxic potential [28]. Another investigation explored the tumor-promoting effects of JCPEs using an *in vitro* Bhas 42 cell transformation assay, which demonstrated a dose-dependent transformation activity [73]. To assess the genotoxicity, cytotoxicity, and mutagenicity of PEs in JCSO, researchers turned to *Lactuca sativa* as a substitute for traditional animal models [74–76]. Metrics such as mitotic index (MI), chromosomal aberrations, and nuclear aberrations were recorded. The results showed that JCSO reduced the MI, implying a suppression of cell proliferation. Observations of sticky chromosomes, c-metaphase cells, and nuclear alterations associated with abnormal cell proliferation were noted even at a concentration of 5 % JCSO. These results confirmed that JCPEs have the potential to disrupt the normal cell cycle and induce mutations. Additionally, *in vitro* cell proliferation assays were performed on human hepatocyte (Chang) and African green monkey kidney (Vero) cells, providing quantitative insights into the cytotoxicity of JCPEs [77]. In Chang cells, proliferation inhibition was observed at doses of 100 μg mL^{-1} and higher, while in Vero cells, it was noted at 50 μg mL^{-1} and above (both in TPA equivalent). The CC_{50} values for Chang and Vero cells were determined to be 125.9 and 110.3 μg mL^{-1} (in TPA equivalent), respectively. In summary, PEs in *Jatropha curcas* disrupt normal cell proliferation, potentially leading to alterations in genetic material.

4.3. Carcinogenicity

Two-stage carcinogenesis experiments were performed on mouse skin to determine if the PEs found in the JCSO contributed to tumor development [78,79]. The findings indicated that JCPEs indeed acted as tumor promoters. When applied topically, these JCPE-containing samples increased ornithine decarboxylase activity and activated PKC. Van Duuren et al. [80] established a dose-response relationship for TPA in mice, ranging from 0.02 to 25 μg per application, administered 3 times a week. Their research revealed that a dosage of 2.5 μg, given 3 times weekly, resulted in a 100 % of occurrence of papilloma, while no tumors were observed at the 0.1 μg level. Further investigations indicated that PEs derived from JCSO exhibited a weaker tumor-promoting effect compared to TPA [34]. To gain a clearer understanding of the carcinogenic potential of JCPEs and to determine safe dosage levels, more extensive quantitative studies, especially those involving oral administration, are necessary.

4.4. Chronic toxicity

Data on chronic toxicity remain relatively scarce. Poon et al. [81] explored the negative effects of a 28-day oral administration of JCSO in mice, with dosages ranging from 0.5 to 500 mg kg^{-1} bw per day. The study revealed significant adverse effects, such as stunted growth, were only noted in the 500 mg kg^{-1} bw group, which corresponds to a daily exposure of 1–2 mg kg^{-1} bw in TPA equivalent [24]. Additionally, See et al. [82] conducted a 45-day chronic toxicity assessment on *Jatropha curcas* leave extract at doses of 200, 500, and 1000 mg kg^{-1} bw. Alarmingly, 21 out of 30 mice succumbed during the 45-day treatment period. However, the specific concentrations of PEs were not quantified in this investigation.

5. Exposure assessment

Statistical data indicates that the average daily intake of edible vegetable oil among rural residents in China is approximately 35–39 g per person, while urban residents consume slightly less [83,84]. The Chinese Dietary Guidelines (2016) suggest limiting edible oil consumption to between 25 and 30 g per person daily [84]. Previous research has found that the concentration of PEs in JCSO was 5.45 mg g^{-1} in TPA equivalent [63]. On the other hand, our measurements of PEs in JCSO from Sichuan showed a concentration of 3.45 mg mL^{-1}, translating to about 3.91 mg g^{-1} in TPA equivalent (unpublished data). To ensure a conservative estimate of toxic compounds, the higher value of 5.45 mg g^{-1} will be used for subsequent calculations. In a hypothetical scenario where all vegetable oil consumption is substituted with JCSO, the total intake would amount to 39 g per person per day, leading to a daily PEs intake of 212.55 mg (in TPA equivalent). For an adult weighing 60 kg, the estimated daily intake (EDI) of JCPEs would be calculated at 3.54 mg kg^{-1} bw.

6. Quantitative risk assessment of JCPEs with benchmark dose approach

Li et al. [64] provided dose-response data related to mouse mortality from a single exposure acute toxicity test, as shown in Table 3. Following the guidelines set by the European Food Safety Authority (EFSA), a Benchmark Dose (BMD) approach was employed in this study to determine the $BMDL_{10}$, i.e., 95 % lower bound on the benchmark dose corresponding to a 10 % extra risk, for PEs in JCSO [85]. The analysis was conducted using the BMSA-Online Web App [86]. The benchmark response level (BMR) was set at a 10 % extra risk, with a confidence level of 0.95. The dataset was analyzed using a probit model (Fig. 5), resulting in an acute toxicity $BMDL_{10}$ for JCPEs of 13.96 mg kg^{-1} bw.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a safety factor of 100 when comparing mice to humans, which includes a factor of 10 for interspecies differences and another 10 for intraspecies variations [87]. By utilizing the $BMDL_{10}$ value from acute toxicity tests, an Acute Reference Dose (ARfD) can be calculated using the following equation [88]:

$$ARfD = \frac{BMDL_{10}}{Safety\,factor} \quad (1)$$

As a result, the ARfD for JCPEs has been determined as 139.64 μg kg^{-1} bw, in TPA equivalent. For a 60 kg adult, this means that the maximum amount of JCPEs that is unlikely to cause severe acute effects, including death, is estimated to be 8.38 mg (in TPA equivalent). Given the previously stated PE concentration of 5.45 mg g^{-1}, the ARfD for JCSO is calculated to be 1.54 g. If a 60 kg adult ingests 25 g of oil in one sitting, the JCPEs must be detoxified to 93.8 % to avoid severe toxic effects from a single exposure.

Poon et al. [81] investigated the changes in body weight of mice over a 28-day, sub-chronic administration of JCSO. The doses administered were 0, 0.5, 5, 50, and 500 mg kg^{-1} bw per day. The study did not specify the exact concentration of JCPEs. However, using the

Table 3
Relationship between dosage and mortality rates in mice following a single exposure to JCPEs, as reported by Li et al. [64].

Dose (mg kg^{-1} bw)	Mortality (%)
0	0
21.26	10
23.62	30
26.24	40
29.16	70
32.4	70
36	90

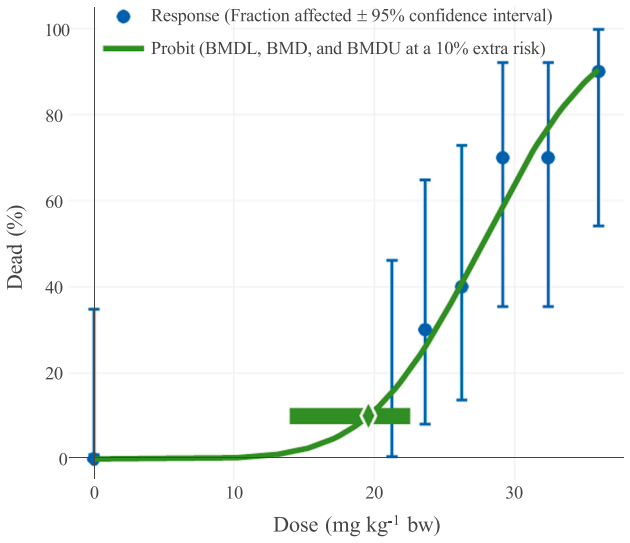

Fig. 5. Benchmark dose (BMD) analysis on the dataset presented in Table 3, using a Probit model. ($BMDL_{10}$, BMD_{10}, and $BMDU_{10}$ are 13.96, 19.58, 22.55 mg kg^{-1} bw, respectively.).

concentration of 5.45 mg g^{-1} PEs in seed oil reported by Roach et al. [63], the JCPE doses were calculated as 0, 0.003, 0.027, 0.0272, and 2.725 mg kg^{-1} bw. Various models, including the exponential, hill, inverse exponential, and log-normal models, were employed to analyze the dataset based on the EFSA BMD webtool [89]. The analysis revealed that the lowest $BMDL_{10}$ was 1.58 mg kg^{-1} bw per day for male mice and 1.05 mg kg^{-1} bw per day for female mice (see Table 4). To ensure safety, the lower value of 1.05 mg kg^{-1} bw will be used for further calculations to avoid underestimating toxicity. The Health-based Guidance Value (HBGV) for humans can then be derived by dividing the $BMDL_{10}$ by a safety factor [90]. With a safety factor of 100, the HBGV for JCPEs in humans was determined to be 0.0105 mg kg^{-1} bw per day in a sub-chronic context. For a 60 kg adult, this equates to a daily limit of 0.63 mg. In cases where there is a daily intake of 25 g of oil, the concentration of PEs in JCSO must be reduced to at least 0.0252 mg g^{-1} (in TPA equivalent). Given the original PE concentration of 5.45 mg g^{-1}, this requires a detoxification rate of 99.5 %.

It is important to note that the calculations mentioned earlier were based on body weight responses and represent a worst-case scenario. Given their role as potential tumor promoters, it is essential to further investigate the dose-response relationship of the tumor-promoting effects of JCPEs.

7. Existing detoxification approaches

A variety of research efforts have explored the detoxification processes of *Jatropha curcas* products, primarily focusing on the seed cake, while only a handful have looked into the seed oil [65,68,91–102]. The conventional detoxification methods can be divided into three main categories: physical, chemical, and biological treatments. An overview of these detoxification techniques and their effects is provided in Table 5. Although some studies claim to achieve complete detoxification rates of 100 %, the validity of these results is uncertain because of the

Table 4
Model fitting results with the EFSA BMD webtool.

Model used	$BMDL_{10}$ (male mice)	$BMDL_{10}$ (female mice)
Exponential	1.61	1.05
Hill	1.60	1.05
Inverse exponential	1.58	1.10
Log-normal	1.60	1.08

Table 5
Overview of detoxication techniques and their effects on JCSC or JCSO.

Substrate	Method	Condition	PE reduction (%)	Ref.
JCSC	Methanol extraction	50 % methanol, 1:10 (w/v), extraction for 8 h	97.3	[91]
	Heat treatment + methanol wash	Heat treatment at 121 °C, 30 min (with 66 % moisture) followed by 4 times washing with 92 % methanol	94.9	[68]
	Chemical treatment	2.5 % NaOH (w/w) + 10 % NaOCl (v/w)	87.6	
		3.0 % NaOH (w/w) + 10 % NaOCl (v/w)	87.6	
		3.5 % NaOH (w/w) + 10 % NaOCl (v/w)	92.1	
		4.0 % NaOH (w/w) + 10 % NaOCl (v/w)	92.7	
		2.0 % NaOH (w/w)	50.0	
		2.5 % NaOH (w/w)	80.9	
		3.0 % NaOH (w/w)	83.7	
		3.5 % NaOH (w/w)	89.9	
		2.0 % NaOH (w/w) + 15 % NaOCl (v/w)	74.2	
		2.0 % NaOH (w/w) + 20 % NaOCl (v/w)	73.6	
		2.0 % NaOH (w/w) + 25 % NaOCl (v/w)	86.5	
		4.0 % NaOH followed by 2 times washing with 92 % methanol	100.0	
		4.0 % NaOH followed by 4 times washing with distilled H_2O	100.0	
	H_2O_2 treatment	15 % H_2O_2, 3:5 (w/w), pH 8.0, 60 °C, 8 h	100.0	[92]
	Enzyme treatment + methanol/ethanol wash	Cellulase (5 mg g^{-1}) and pectinase (10 mg g^{-1}) treatment at 50 ℃ for 1 h	14.2	[93]
		Cellulase + pectinase treatment + 60 % methanol wash	100	
		Cellulase + pectinase treatment + 65 % ethanol wash	100	
		90 % Methanol (10:1 v/v) treatment	66.0	
		90 % Ethanol (10:1 v/v) treatment	59.7	
	Chemical treatment	90 % Methanol (with 0.1 mol L^{-1} NaOH), 10:1 (v/w) treated, heat in a refluxing unit at 50 °C for 30 min, repeat 2 times	86.4	[94]
	UV irradiation	53.4 mW cm^{-2} UV irradiation for 30 min	16.0	
	Multistage ethanol extraction	Aqueous ethanol, 1:3 (w/v) extraction at 50 ℃ for 30 min, repeat 3 times	95.2	[95]
	Ozone treatment + chemical treatment + irradiation treatment	$NaHCO_3$ moistening combined with 3 min ozone (50 mg L^{-1}) treatment	75.3	[96]
		Irradiation 50 kGy for 30 min	71.4	
		$NaHCO_3$ moistening combined with heat treatment 90 °C for 30 min	56.0	

(*continued on next page*)

Table 5 (*continued*)

Substrate	Method	Condition	PE reduction (%)	Ref.
		$NaHCO_3$ moist treatment	44.5	
	Chemical treatment	0.1 NaOH & 90 % methanol treatment at 65 ℃ for 30 min	98.0	[97]
		85 % ethanol treatment at 40 ℃ for 30 min	98.2	
		90 % methanol and 85 % ethanol (50:50) treatment at 25 ℃ for 8 h	95.4	
	Physical treatment (ultrasonic and microwave)	Ultrasonic in 80 % methanol (200 W, 59 kHz) for 1 h	87.6	
		Microwave treatment for 6 min	86.3	
		Microwave for 6 min follow by ultrasonic in 80 % methanol for 6 h	88.4	
	Enzyme treatment	Lipase (extracted from germinated *Jatropha* seeds) treatment at 30 ℃ for 12 h	98.4	
	Sunlight irradiation	Sunlight exposure at open air for 24 h, 48 h, 72 h	31.1–77.9	[98]
	Ozone treatment	Ozonated treatment for 5–100 min at 0.43–8.14 mg ozone per g seed cake	57.9–82.5	
JCSO	Sunlight irradiation	Mixed soil with JCSO (no matter autoclaved or non-autoclaved), and sunlight treatment	100	[65]
	Refining (degumming and deodorization)	200 °C and steam distilled for 2 h at normal pressure	0.0	[99]
	Refining (deacidification and bleaching)	NaOH and KOH to neutralize the free fatty acids and treated with bleaching reagent	55	
	UV treatment + ethanol wash	Treated by 220–400 nm ultraviolet irradiation combined with 65 % ethanol washing	100	[100]
	Refining (deodorization)	260 °C and 3 mbar treatment for 1 h and with 1 % steam injection	100	[101]
	Methanol extraction	Methanol extraction (1:1) for 15 min at room temperature	99	[102]

constraints associated with the analytical methods used.

7.1. Physical detoxification

The most commonly used method for detoxifying *Jatropha curcas* products is solvent extraction. Given that PEs feature multiple hydroxyl groups, they exhibit high solubility in alcohol solvents such as methanol and ethanol. Studies have shown that methanol is more effective than ethanol in extracting PEs from *Jatropha curcas* seed kernels or seed oil, achieving reduction rates exceeding 95 % [91,93,102]. Dichloromethane has also been used for this extraction process [17,103]. Although methanol is an effective solvent for PEs, it poses food safety concerns due to its toxicity, making it unsuitable for food production. Ethanol, while less efficient, could serve as an alternative. A significant challenge with both methanol and ethanol in industrial detoxification is the large volume required, typically around a 1:10 ratio of material to solvent [91,93,94]. Furthermore, conventional extraction methods often involve multiple stages, usually requiring at least five extraction cycles [102], which complicates their industrial application. Strategies to minimize solvent usage and extraction stages are essential for industrial settings. One promising approach could be adsorption. To enhance industrial feasibility, strategies to reduce solvent usage and extraction stages are crucial. One potential solution is adsorption. Studies have shown that adsorbents like activated carbon and bentonite can effectively adsorb PEs [99,104]. Nevertheless, their effectiveness is much higher in liquid phases than in oil phases due to the physical properties of oil, which necessitates external forces like pressure for separating oil from solid adsorbents. To achieve cost-effective, continuous, and sustainable detoxification of JCSO, integrating solvent extraction with adsorption may be a viable solution.

Heating has been explored as a means to break down various compounds, including PEs. Makkar et al. [101] studied the changes in PE levels throughout the oil transesterification process. They discovered that when oil was deodorized at a high temperature of 260 °C and low pressure of 3 mbar for 1 h with 1 % steam injection, PEs were completely degraded. In contrast, Haas and Mittelbach [99] reported that PE levels remained stable during oil refining, even at 200 °C and normal pressure for 2 h. This discrepancy may be attributed to the more extreme conditions of high temperature and low pressure during deodorization, indicating that PEs have a relatively stable structure that resists degradation even under harsh conditions. Additionally, researchers have explored the use of irradiation processes to eliminate PEs. Evidence indicates that a gamma irradiation dose of 70 kGy is inadequate for effective detoxification of PEs, while a higher dose of 125 kGy can reduce PEs by 95.8 % [96,105]. Various UV exposure doses have also been tested, but these only resulted in minimal degradation of PEs. The decrease in PEs during irradiation treatment has been attributed to the oxidation of these compounds [94,98,102]. Unfortunately, previous studies did not provide enough information about the final products resulting from heating or irradiation treatments. Gaining a clearer understanding of the specific end products produced by these methods would significantly aid in clarifying the underlying mechanisms.

7.2. Chemical detoxification

Numerous investigations have examined different chemical methods for detoxifying *Jatropha curcas* products. For instance, earlier research has implemented ozonation and H_2O_2 treatment [92,98], as well as enzymatic approaches using cellulase, pectinase, and lipase [93,97]. Additionally, alkali treatment with NaOH has been investigated [68,97], along with experiments using $NaHCO_3$ [96]. However, most of these studies have focused on the seed meal rather than the seed oil. The chemical treatments primarily affect PEs by disrupting their structure through processes such as ester hydrolysis and oxidation, which may also modify the composition of the resulting products. For instance, lipase can break down triacylglycerol, while oxidative agents can lead to the degradation of oil quality. NaOH can denature the protein structures in the JCSC. Similar to physical treatments, the end products after chemical treatments require further characterization and analysis. Notably, hydrolysis mainly targets ester bonds, suggesting that the resulting hydrolyzed product is likely phorbol, which resembles PEs and is also toxic to humans. Therefore, the effectiveness of chemical hydrolysis in detoxifying *Jatropha* products remains questionable.

7.3. Biological detoxification

The literature on the biological degradation of PEs is more extensive, mainly focusing on solid-state and submerged fermentation processes involving fungi and bacteria for JCSC. Various fungal species, such as

Bjerkandera adusta, *Ganoderma resinaceum*, *Phlebia rufa*, *Trichoderma spp*., and *Pleurotus pulmonarius*, have been utilized in these degradation processes [106–108]. It has been suggested that the lipase produced by these fungi acts as a detoxifying agent. Additionally, bacteria such as *Pseudomonas spp.* and *Bacillus spp.* Have been investigated for their ability to detoxify PEs [25,109,110]. While many studies have explored the degradation of PEs by various microbial species, it is important to note that most of this research has concentrated on *Jatropha* seed meal. This focus may stem from the fact that the specific growth conditions for these microbes, i.e., oxygen and moisture, are readily available in both submerged and solid-state fermentation of seed meal. However, the bio-detoxification process carried out by microorganisms generally requires more time compared to chemical and physical methods. Furthermore, many studies have assumed that the action of microorganisms on PEs is primarily through esterase activity. Some research has delved into the production and activity of enzymes during incubation [25,108,109,111]. There is a notable lack of literature addressing the specific end products of this degradation and their potential toxicity. Moreover, the quality and characteristics of *Jatropha*-derived products could be affected by the metabolites generated by the fermentation microbes, highlighting the need for further investigation into these ambiguous aspects.

8. Additional data needed

At present, there is a substantial body of acute toxicity data available, demonstrating that JCPEs are genotoxic and can act as tumor promoters. However, the quantitative data and the dose-response relationship related to these genotoxic and tumor-promoting effects remain inadequate and require further investigation. Moreover, due to the lengthy nature of long-term studies, most toxicity research has focused on short-term or sub-chronic effects. For substances meant for human consumption, it is vital to confirm that they do not present health risks from prolonged use. Thus, it is crucial to enhance the data regarding the dose-response relationship and chronic toxicity of JCPEs. Furthermore, to accurately apply these findings to humans, investigating toxicokinetic data is necessary, as it will provide a thorough understanding of how PEs are absorbed, distributed, metabolized, and excreted.

9. Summary

This review has collected the up-to-date research on the toxicity mechanisms and data related to PEs derived from *Jatropha curcas*. Quantitative analyses were performed based on the information currently available. While the PEs from JCSO show toxicity, the results suggest a potential for detoxification, making it feasible for use as an edible oil. This could broaden consumer options and reduce China's dependence on imported vegetable oils. Nonetheless, a considerable amount of detailed toxicity data still needs to be generated by research to ensure the safe consumption of JCSO in the future.

CRediT authorship contribution statement

Jesse Zhu: Supervision. **Dongbing Li:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis. **Xinyuan Cao:** Writing – original draft, Visualization, Formal analysis. **Yuanyuan Shao:** Supervision. **Xiaoyang Wei:** Supervision.

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.

Data availability

Data will be made available on request.

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