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Review

# The phytochemistry, pharmacokinetics, pharmacology and toxicity of *Euphorbia semen*

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Euphorbia Factor L2 (PubChem CID: 101071473)
Euphorbia Factor L3 (PubChem CID: 92043360)
Euphorbia Factor L4 (PubChem CID: 124511085)
Euphorbia Factor L5 (PubChem CID: 56841025)
Euphorbia Factor L6 (PubChem CID: 6442562)
Euphorbia Factor L7a (PubChem CID: 131676046)
Euphorbia Factor L8 (PubChem CID: 131876111)
β-sitosterol (PubChem CID: 222284)
Glycerol-monooleate (PubChem CID: 33022)
Glycerol-dioleates (PubChem CID: 33120)
Glycerol-trioleate (PubChem CID: 5497163)
Ingenol (PubChem CID: 442042)
16-hydroxy-ingenol (PubChem CID: 119057278)

*Keywords:*
*Euphorbia semen*
Botany
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Pharmacology
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Toxicity

ABSTRACT

*Ethnopharmacological relevance:* *Euphorbia semen*, the dried and ripe seed of *Euphorbia lathyris* Linnaeus, is widely cultivated for traditional medicine use. This semen is used to expel water, help with phlegm retention, promote blood circulation, remove blood stasis, cure tinea and scabies, and treat amenorrhea, snakebites, terminal schistosomiasis, anuria and constipation.

*Aim of the review:* This review provides updated, comprehensive and categorized information on the local and traditional uses, phytochemistry, pharmacokinetics, pharmacological activities and toxicity of *Euphorbia semen*. Future research to deepen the recognition and utilization of *Euphorbia semen* is proposed.

*Materials and methods:* This article conducted a literature review on information about *Euphorbia semen* in multiple Internet databases, including PubMed, Web of Science, Wiley, Science Direct, Elsevier, ACS publications, SciFinder, Google Scholar and China National Knowledge Internet, until March of 2018. In this manuscript, a number of books, PhD and MSc dissertations, and Chinese Pharmacopeia were also used as references.

*Results:* Approximately 240 chemical constituents have been isolated and identified from *Euphorbia semen*, namely, diterpenoids, coumarins, flavonoids, fatty acids, amino acids, and steroids. Pharmacokinetic study focused on investigating absorption, distribution, metabolism and excretion (ADME). The chemical constituents have extensive pharmacological effects, such as diuresis and anti-hyperuricaemia, anti-inflammation, antiviral, anticancer, antioxidant, antipigmentation, anti-platelet aggregation and anti-allergic activities, as well as hepatoprotection and neuroprotection. The toxicity of *Euphorbia semen*, including acute toxicity, target organ irritation and cocareinogenic effects, have been reported, and the detoxification methods are reviewed.

*Conclusion:* *Euphorbia semen* has extensive pharmacological activity and excellent clinical value, along with intense intestinal irritation. Although plenty of chemical constituents have been isolated and identified, the exact pharmacological and toxicological mechanisms still need to be explored.

## 1. Introduction

*Euphorbia semen*, the dried and ripe seed of *Euphorbia lathyris* Linnaeus, is widely used as an ethnic drug in East Asia. In Korea, *Euphorbia semen* is used for whitening (Masamoto et al., 2003). In China, these seeds are clinically used to expel water and phlegm retention, promote blood circulation, remove blood stasis, cure tinea and scabies, and treat amenorrhea, snakebites, terminal schistosomiasis, anuria and constipation (Appendino et al., 2003; Jiao et al., 2009; Zhang, 2009).

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As a herbal medicine, it is necessary to illuminate the material basis of *Euphorbia semen*. The major chemical constituents, including 15 types of the 240 compounds, have been extracted, separated and identified from *Euphorbia semen*. These compounds include lathyrane-type diterpenoids, ingenane-type diterpenoids, flavonoids, coumarins, steroids, amino acids, fatty oil and volatile oil, and so on (Luo et al., 2016). The lathyrane-/ingenane-type diterpenoids, flavonoids and coumarins exert multiple pharmacological effects, such as diuresis and antitumour, in both experimental models and clinical practice (Wong et al., 2018). In recent years, studies also reported that *Euphorbia semen* had antioxidant activity and antipigmentation (Teng et al., 2018).

However, *Euphorbia semen* is an irritant drug that causes severe toxicity in intestinal and nervous systems, including hyperemesis, stomach ache, diarrhoea, headache, dizziness, dysphoria, fervescence, perspiration, uneasiness, drops in blood pressure, and even respiratory and circulatory failure (Antcliff et al., 1994). It should be noted that Euphorbia factors and seed oil have a cocareinogenic effect (Adolf and Hecker, 1975).

It is imperative to develop more efficient methods to accelerate the evaluation of the toxicity-efficacy relationship and screening of corresponding constituents in *Euphorbia semen*. How to balance the benefits and risks poses a challenge to traditional medical researchers. This article provides a review of the botany, phytochemistry, pharmacokinetics, pharmacological activities and toxicity of *Euphorbia semen*, as well as the research perspectives.

## 2. Materials and methods

This review article collected the literatures published prior to March 2018 on traditional use, phytochemistry, pharmacokinetics, pharmacology, toxicity of *Euphorbia semen*. All available information on *Euphorbia semen* was retrieved by Internet databases (PubMed, Web of Science, Wiley, Science Direct, Elsevier, ACS publications, SciFinder, Google Scholar and China National Knowledge Internet) and library documents. The key words used to search were: "*Euphorbia lathyris* Linnaeus", "*Euphorbia semen*", "botany", "traditional use", "clinical study", "phytochemistry", "pharmacokinetics", "pharmacological effects", "toxicity" and "detoxification". Most of cited information in this review were from peer-reviewed journals with impact factor, and published in English or Chinese. Information of related books, PhD and MSc dissertations, and Chinese Pharmacopeia were also used as references. Scientific name of the plant was validated through www.theplantlist.org. All chemical compounds were identified via Substance Identifier of SciFinder and Pubmed Compound of NCBI.

## 3. Botanical description

*Euphorbia lathyris* Linnaeus (Fig. 1) was native to Middle and Southern Europe and Southern Russia, and was introduced into Northwest Africa, Korea, China, Australia, and North and South America (Ioannidis et al., 2009). *Euphorbia lathyris* Linnaeus is an erect biennial herb, up to 0.8–2 m tall (Adolf and Hecker, 1975). After maturing, flowers without petals produce in three-celled globular clusters containing three seeds compressed together (Sun, 2009). Average seed output per acre reaches 100 kg, and the seeds are rich in oil, up to 47–50% (Zheng, 2009).

## 4. Local and traditional uses

*Euphorbia semen* is a famous Chinese medicine with over a millennium of history (Zhu, 2008; Wei and Lin, 2004). A list of traditional medicine uses of *Euphorbia semen* in China is shown in Table 1. According to the 2015 edition of Chinese Pharmacopoeia, *Euphorbia semen* is orally used to treat oliguria, constipation, oedema, dyspepsia and amenorrhea, and externally used for tinea and wart. The Chinese Pharmacopoeia reminds that *Euphorbia semen* is a toxic herbal medicine, and the compulsive processing is decorticating and reducing the fatty oil, then makes into pills or powder for oral administration. The recommended oral dosage in adults is 1–2 g per day. For the identification of *Euphorbia semen*, thin-layer chromatography (TLC) has been used to analyze the esculetin in silica gel G plate. In spite of long traditional uses history of *Euphorbia semen*, the exact mechanisms for its pharmacological and toxicological effects remain to be explored.

## 5. Phytochemistry

There are 15 types of 240 compounds that have been extracted, isolated and identified from *Euphorbia semen*. Among which, lathyrane-type diterpenoids (No. 1–29), ingenane-type diterpenoids (No. 30–33), flavonoids (No. 34–41) and coumarins (No. 42–48) are the main active constituents for pharmacological and toxicological effects, their detailed information is shown in Tables 2–5. Among these 4 types of 48 chemical constituents, 32 compounds are unique to Euphorbia semen, presenting broad biomedical research prospects. The other 11 types of 192 chemical constituents, including steroids, amino acids, hydrocarbons, carboxylic acids, esters, aldehydes, alcohols, ketones, ethers, epoxides and phenols, are described in the Supplementary materials.

### 5.1. Lathyrane-type diterpenoids

Lathyrane-type diterpenoids have a 5/11/3-membered ring system. Thus far, 29 lathyranes have been isolated and identified from *Euphorbia semen*. Most of these have a double bond between C-12 and C-13 and a carbonyl group linked to C-14, some have an out-ring double-bond or an oxirane in C-6, and some have a double bond between C-6 and C-5 (or C-7) (Shi et al., 2008). Up to now, all lathyrane-type diterpenoids except Jolkinol B are unique to *Euphorbia semen*, not found in other plants. The chemical structures of these compounds are shown in Table 2.

### 5.2. Ingenane-type diterpenoids

Ingenane-type diterpenoids both have a 5/7/7/3-tetracyclic ring system, a ketone bridge between C-8 and C-10, a double bond between C-1 and C-2 in ring A, and another double bond between C-6 and C-7 in ring B (Wu et al., 2009). *Euphorbia semen* includes 4 ingenane-type diterpenoids, of which Euphorbia Factor L4, L5 and L6 have only been found in this seed. Their chemical structures are presented in Table 3.

### 5.3. Flavonoids

There are 8 flavonoids in *Euphorbia semen*, and all have a common flavone structure and an oxyhydryl group linked to C-5 (Zhang et al., 2017). All these flavonoids have been found in other plants, namely non-specific flavonoids in *Euphorbia semen*. The chemical structures of these flavonoids are shown in Table 4.

### 5.4. Coumarins

Seven coumarins have been isolated and identified from Euphorbia semen, and most have a coumarin structure (Dutta et al., 1972, 1973; Jaretzky and Kohler, 1942; Li et al., 1994; Yang et al., 2016a, 2016b). Coumarins can be divided into simple coumarins and dicoumarins based on the complexity of the structures. Simple coumarins has a substituent group in C-5/6/7 on coumarin benzene rings, such as esculetin, daphnetin, scopoletin, fraxidin, esculin. Dicoumarins are dimers of two simple coumarins, euphorbetin and isoeuphorbetin are two dicoumarins. There are 7 coumarins in *Euphorbia semen*, of which isoeuphorbetin is unique to this seed. Table 5 shows the detailed structures of these coumarins.


**Fig. 1.** The plant (A) and dried seed (B, namely, *Euphorbia semen*) of *Euphorbia lathyris* Linnaeus. The plant (voucher specimen No. QYM0906071155, year of 2009) was identified and authorized by Yongming Qiao of Plant Photo Bank of China.

## 6. Pharmacokinetic study

Euphorbia Factor L (EFL), esculin, esculetin and daphnetin are regarded the main active constituents in *Euphorbia semen*. According to the 2015 edition of the Chinese pharmacopoeia, esculetin is used as a mark compound for quality control. The contents of EFL1 and fatty oil in *Euphorbia semen* should not be less than 0.35% and 35.0% (w/w), respectively.

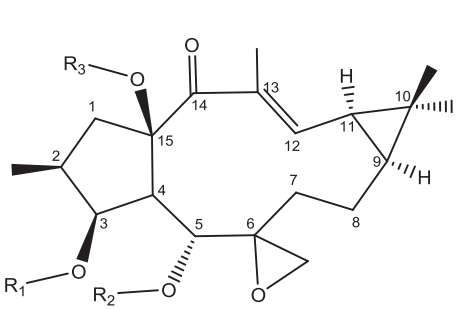
Simultaneous determination of EFL1, EFL2 and EFL3 from *Euphorbia semen* was performed by liquid chromatography-mass spectrometry (LC-MS). SD rats were orally administered with ethanol extract of *Euphorbia semen* containing different concentrations of EFLs. The main pharmacokinetic parameters are listed in Table 6. Additionally, EFLs began to be excreted in faeces at 6 h after gavage, the peak time appeared at 8 h, and the EFLs were totally excreted at 40 h (Meng et al., 2013).

Fu (2013) investigated the ADME process of EFL1 in rats and macaques, and the detailed data are shown in Table 6. At concentrations of 20, 100 and 1000 μg/L EFL1, the plasma protein binding rate in rats and macaques was in the range of 76.0–98.0%, without a dose-dependent manner. The metabolites of EFL1 (i.v. or i.g.) in blood and urine were analysed by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS). The metabolites in blood were mainly hydrolysed esterase, methylated hydroxyl, reduced C-6 epoxide and oxydic methyl, which were produced by the phase I reaction. The metabolites in urine were mainly produced by the phase II reaction

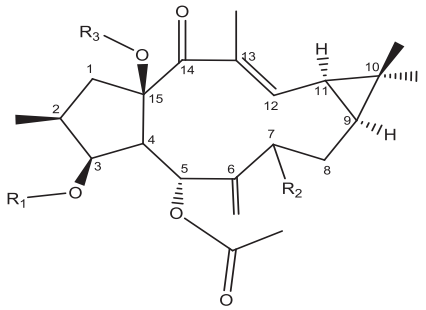
**Table 1**
Traditional medicine uses of *Euphorbia semen* in China.

| Preparation names | Composition | Traditional and clinical uses | Origins |
|---|---|---|---|
| Xusuizi Wan (续随子丸) | *Euphorbia semen*, sea clam, *Draba nemorosa* L., *Stephania tetrandra* S.Moore, *Euphorbia kansu* S.L.Liou ex S.B.Ho, *Pruni semen*, talc, calomel | Treatment of oedema, dyspnea, tachypnea, ventral tympanites and urinary obstruction | Taiping Shenghui Fang 《太平圣惠方》 |
| Xusuizi Wan (续随子丸) | *Euphorbia semen*, minium | Treatment of urinary obstruction, umbilical and abdominal distension pain | Shengji Zonglu 《圣济总录》 |
| Shengshou Duoming Dan (圣授夺命丹) | *Euphorbiae semen* pulveratum, Galla chinensis, *Cremastra appendiculata*, *Knoxia valerianoides Thorel et Pitard*, moschus | Detoxification | Yeshi Yanfang Lu 《叶氏验方录》 |
| Shenxian Jiedu Wanbing Wan (神仙解毒万病丸) | *Euphorbiae semen* pulveratum, Galla chinensis, *Cremastra appendiculata*, *Knoxia valerianoides Thorel et Pitard*, moschus | Detoxification, treatment of sore, promote barrier permeability | Shizhai Baiyi Xuanfang 《是斋百一选方》 |
| Wanbing Jiedu Wan (万病解毒丸) | *Euphorbiae semen* pulveratum, Galla chinensis, *Cremastra appendiculata*, *Knoxia valerianoides Thorel et Pitard*, moschus, scorpio, *Sophora tonkinensis* Gagnep, cinnabar, realgar | Detoxification, treatment of sore, snakebites and insect bites | Renzhai Zhizhi Fanglun 《仁斋直指方论》 |
| Xusuizi Wan (续随子丸) | *Euphorbia semen*, *Draba nemorosa* L., ginseng, *Aucklandia costus* Falc., *Stephania tetrandra* S.Moore, poria cocos, *Areca catechu* L., *Lygodium japonicum* (Thunb.) | Treatment of puffiness, chest congestion, cough and asthma | Yixue Faming 《医学发明》 |
| Taiyi Zijin Dan (太乙紫金丹) | *Euphorbiae semen* pulveratum, Galla chinensis, *Cremastra appendiculata*, *Santalum album* L., benzoinum, styrax, *Knoxia valerianoides Thorel et Pitard*, amber, borneolum, moschus, realgar | Treatment of cold-dampness syndrome | Suixiju Chongding Huoluan Lun 《随息居重订霍乱论》 |
| Zijin Ding (紫金锭) | *Euphorbiae semen* pulveratum, Galla chinensis, *Cremastra appendiculata*, *Knoxia valerianoides Thorel et Pitard*, scorpio, cinnabar, realgar, moschus | Detoxification, detumescence, analgesia, and treatment of heatstroke, epigastric abdominal distention and pain, nausea, vomit, diarrhoea, dysentery and pediatric phlegm syncope | Chinese Pharmacopoeia , 2015 edition |

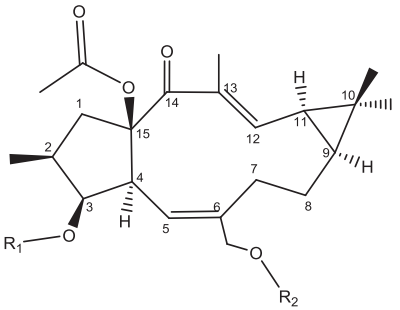
**Table 2**
Chemical structures of the lathyrane-type diterpenoids in *Euphorbia semen*.



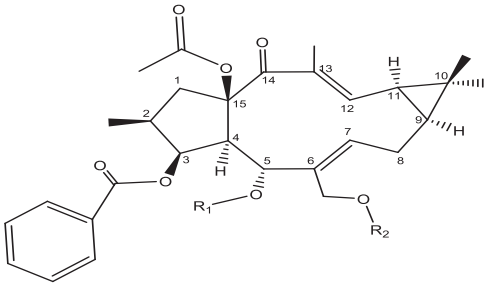
Nuclear 1



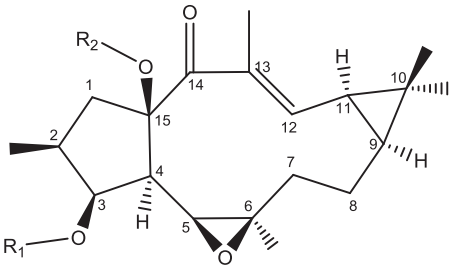
Nuclear 2



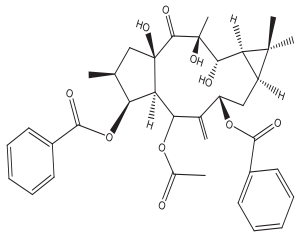
Nuclear 3



Nuclear 4



Nuclear 5



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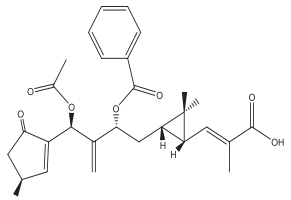

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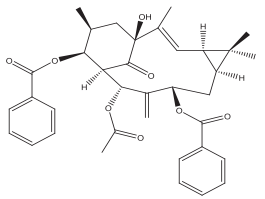

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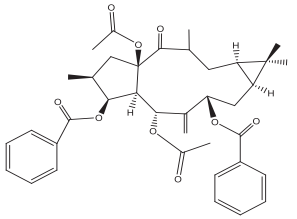

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| No. | Names | Nucleus | R1 | R2 | R3 | CAS numbers | References |
|---|---|---|---|---|---|---|---|
| 1* | Euphorbia Factor L1 | 1 | $C_6H_5CH_2CO$- | $CH_3CO$- | $CH_3CO$- | 76376-43-7 | Adolf et al. (1970) |
| 2* | Euphorbia Factor L2 | 2 | $C_6H_5CO$- | $C_6H_5COO$- | $CH_3CO$- | 218916-51-9 | Appendino et al. (1999) |
| 3* | Euphorbia Factor L3 | 2 | $C_6H_5CO$- | $H_2$ | $CH_3CO$- | 218916-52-0 | Adolf and Hecker (1971) |
| 4* | Euphorbia Factor L7a | 3 | $C_6H_5CH=CHCO$- | $CH_3CO$- | | 93550-94-8 | Adolf et al. (1984) |
| 5* | Euphorbia Factor L7b | 4 | $CH_3CO$- | $CH_3CO$- | | 93550-95-9 | Adolf et al. (1984) |
| 6* | Euphorbia Factor L8 | 2 | H | $H_2$ | $CH_3CO$- | 218916-53-1 | Adolf and Hecker (1971) |
| 7* | Euphorbia Factor L9 | 2 | $C_6H_5CO$- | $C_5H_4NCO$- | $CH_3CO$- | 129393-28-8 | Itokawa et al. (1990) |
| 8* | Euphorbia Factor L10 | 3 | $C_5H_{11}COCO$- | H | | 496776-71-7 | Appendino et al. (2003) |

(*continued on next page*)

**Table 2** (*continued*)

| No. | Names | Nucleus | R1 | R2 | R3 | CAS numbers | References |
|---|---|---|---|---|---|---|---|
| 9* | Euphorbia Factor L11 | 2 | $C_6H_5CO$- | $C_6H_5COO$- | H | 850560-45-1 | Liao et al. (2005) |
| 10* | Euphorbia Factor L12 | 5 | $C_6H_5CO$- | $CH_3CO$- | | 88202-63-5 | Lu et al. (2014) |
| 11* | Euphorbia Factor L13 | 5 | $C_6H_5COO$- | $CH_3CO$- | | 1613699-91-4 | Lu et al. (2014) |
| 12* | Euphorbia Factor L14 | 2 | $C_6H_5CO$- | $HOC_6H_4COO$- | $CH_3CO$- | 1613699-93-6 | Lu et al. (2014) |
| 13* | Euphorbia Factor L15 | 2 | $CH_3CO$- | $H_2$ | $CH_3CO$- | 1613699-95-8 | Lu et al. (2014) |
| 14* | Euphorbia Factor L16 | 2 | $HOC_6H_4CO$- | $H_2$ | $CH_3CO$- | 1613699-97-0 | Lu et al. (2014) |
| 15* | Euphorbia Factor L17 | 3 | $C_6H_5CO$- | $CH_3CO$- | | 1613699-99-2 | Lu et al. (2014) |
| 16* | Euphorbia Factor L18 | 3 | $C_6H_5CH_2CO$- | $CH_3CO$- | | 1613700-01-8 | Lu et al. (2014) |
| 17* | Euphorbia Factor L19 | 3 | $C_6H_5CH_2CO$- | H | | 1613700-03-0 | Lu et al. (2014) |
| 18* | Euphorbia Factor L20 | 4 | H | $CH_3CO$- | | 1613700-05-2 | Lu et al. (2014) |
| 19* | Euphorbia Factor L21 | | | | | 1613700-07-4 | Lu et al. (2014) |
| 20* | Euphorbia Factor L22 | 3 | $C_6H_5CH=CHCO$- | H | | 1613700-09-6 | Lu et al. (2014) |
| 21* | Euphorbia Factor L23 | 3 | $CH_3(CH_2)_4CO$- | $CH_3CO$- | | 1613700-11-0 | Lu et al. (2014) |
| 22* | Euphorbia Factor L24 | 4 | $CH_3CO$- | H | | 1613700-13-2 | Lu et al. (2014) |
| 23* | Euphorbia Factor L25 | 1 | C6H5CO- | CH3CO- | CH3CO- | 303174-98-3 | Lu et al. (2014) |
| 24* | 6,20-Epoxylathyrol | 1 | H | H | H | 28649-60-7 | Adolf et al. (1970) |
| 25* | Lathyranoic acid A | | | | | 850560-44-0 | Liao et al. (2005) |
| 26 | Jolkinol B | 5 | H | $C_6H_5CH=CHCO$- | | 62820-12-6 | Adolf et al. (1984) |
| 27* | Deoxy Euphorbia Factor L1 | 2 | $C_6H_5CH_2CO$- | $H_2$ | $CH_3CO$- | 247099-01-0 | Bicchi et al. (2001) |
| 28* | Lathyranone A | | | | | 947730-42-9 | Gao et al. (2007) |
| 29* | Euphorbia lathyris A | | | | | 1453194-30-3 | Li et al. (2013) |

* So far, this compound has only been found in *Euphorbia semen*.

binding to the glucuronic acid.

The above studies showed that EFLs were metabolized quickly after intragastric administration or intravenous injection and may be related to the plasma esterase. The bioavailabilities were at an extremely low level, due to low solubility or severe first-pass effect in the intestinal tract and liver.

A pharmacokinetic study of esculetin in SD rats was previously conducted (Kim et al., 2014), and data are shown in Table 6. Furthermore, only the kidney (20.29 ± 7.02 ng/g) and liver (30.87 ± 11.33 ng/g) had detectable esculetin, indicating that esculetin was also quickly distributed and eliminated from these tissues. Further studies are needed to investigate the entire ADME process of *Euphorbia semen* in more organs, including the stomach, intestine, kidney and liver, not just blood and excreta.

## 7. Pharmacological effects

In this article, all reviewed pharmacological studies were from peer-review journals, and set appropriate controls in experiments. The studies used very high dose were excluded, as well as in silico and in vitro studies with little meaning. As shown in Table 7, we summarized the main pharmacological effects of *Euphorbia semen* in human, animal, *Drosophila*, cell, bacteria and virus models. The tested constituents, effective dose, time intervals and assessment indicators were presented. In brief, Esculetin contributes to all mentioned effects. Esculin is related to diuresis and antibacterial activity. EFLs are involved in anticancer and antipigmentation. Daphnetin exhibits antibacterial and anti-inflammation properties.

The pharmacological effects and mechanisms of compounds from *Euphorbia semen* are revealed. However, only the antipigmentation activity of EFL3 and anti-platelet aggregation activity of esculetin have been studied in human. The antibacterial and antiviral activity have only been tested in vitro, lacking of evidences from animal and human studies. Future researches should be performed in vitro, in vivo and human systematically, for the supplements of existing pharmacological studies.

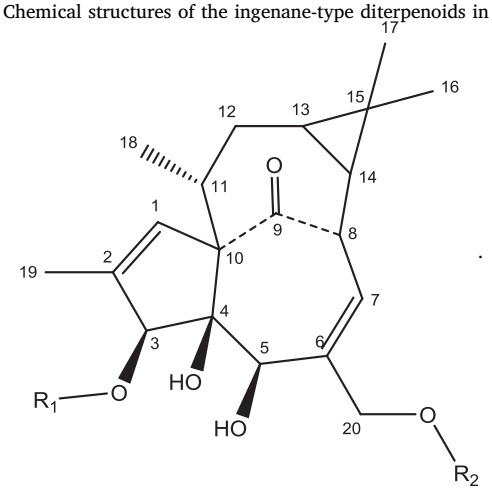
### 7.1. Diuresis and anti-hyperuricaemia

*Euphorbia semen* is a purgative herb, which might be related to the diuresis and anti-hyperuricaemia activity of esculin and esculetin. Esculin exerts diuretic activity via the elimination of uric acid and inhibition of xanthine oxidase activity to reduce uric acid generation, while esculetin only inhibits enzyme activity (Liu et al., 2015). In hyperuricaemia and renal dysfunctional mice, esculin and esculetin (20 and 40 mg/kg, i.g.) increased urine urate and creatinine (Cr) excretion and decreased serum uric acid, creatinine and blood urea nitrogen (BUN) levels (Li et al., 2011). Regarding the proposed mechanism, esculin and esculetin up-regulated the mRNA and protein expression of renal organic anion transporter (OAT1) and organic cation and carnitine transporters (OCT1, OCT2, OCTN1 and OCTN2) without gene or protein expression changes of glucose transporter 9 (GLUT9) and urate transporter 1 (URAT1).

### 7.2. Anti-inflammation activity

Daphnetin is the principal constituent in *Euphorbia semen* to treat inflammatory diseases. In rats with severe acute pancreatitis, daphnetin (4 mg/kg, i.p.) alleviated pathological oedema, inflammation, vacuolization, necrosis, neutrophil infiltration and cell apoptosis in the pancreas. Meanwhile, the serum Cr, alanine transaminase (ALT), amylase, lipase, pro-inflammatory cytokines as well as myeloperoxidase (MPO) activity and malondialdehyde (MDA) content were decreased, and anti-inflammatory cytokines were increased. The underlying mechanism was related to the suppressed Toll-like receptor-4/nuclear factor-κB (TLR-4/NF-κB) inflammatory pathway (Liu et al., 2016, 2014).

**Table 3**
Chemical structures of the ingenane-type diterpenoids in *Euphorbia semen*.




| No. | Names | $R_1$ | $R_2$ | CAS numbers | References |
|---|---|---|---|---|---|
| 30 | Ingenol | H | H | 30220-46-3 | Adolf and Hecker (1975) |
| 31* | Euphorbia Factor L4 | H | $CH_3(CH_2)_{14}CO$- | 39071-33-5 | Adolf and Hecker (1975) |
| 32* | Euphorbia Factor L5 | $CH_3(CH_2)_{14}CO$- | H | 52557-26-3 | Adolf and Hecker (1975) |
| 33* | Euphorbia Factor L6 | $CH_3(CH_2)_2(CH=CH)_5CO$- | H | 42483-56-7 | Adolf and Hecker (1971) |

* So far, this compound has only been found in *Euphorbia semen*.

In arthritic rats, daphnetin (4 mg/kg/d, i.p., 21 d) decreased the swelling rate of the immunized foot, and the splenic retinoic acid-related orphan receptor γt (RORγt), as well as the mRNA expression of pro-inflammatory cytokines IL-6, transforming growth factor-β (TGF-β) and IL-17, (Tu et al., 2012). The protein contents of Th1 cells produced interferon-γ (IFN-γ); Th2 cells produced IL-4, and Th17 cells produced IL-22, all were decreased in the serum (Yao et al., 2011). However, the anti-inflammatory protein forkhead/winged helix transcription factor (Foxp3), mediated by regulatory T cells (Treg), was up-regulated in joints. Since IL-6 combined with TGF-β to direct the differentiation of Th17 cells from naïve $CD_4^+$ T cells, the protective effect was related to the inhibition of Th17 priming and activation. These studies supported that Th1/Th2/Th17/Treg balance played a vital role in daphnetin-mediated anti-inflammation.

Esculetin exhibited suppression to inflammation in neuroinflammatory ICR mice (40 mg/kg, i.g., 7 d), leading to the blocked NF-κB pathway, along with down-regulated inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression (Zhu et al., 2016).

Both daphnetin and esculetin exert anti-inflammatory effects via suppressed NF-κB inflammatory pathways, balance the inflammation/anti-inflammation system, and alleviate oxidative injuries.

### 7.3. Antibacterial activity

Coumarins are the chief constituents in *Euphorbia semen* to inhibit bacteria. The antibacterial activity of daphnetin was evaluated, tested strains (*Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus lentus*, *Escherichia coli* and *Morganella morganii*) had minimum inhibitory concentration (MIC) over 100 mg/L, and only *Pseudomonas aeruginosa* had a MIC of 50 mg/L. The inhibition was inferior to the positive control drug nalidixic acid (Cottiglia et al., 2001). Céspedes et al. (2006) appraised the antibacterial activities of esculetin for Gram-negative bacteria *Salmonella typhi*, *Shigella boydii*, and *Vibrio cholerae* (four strains: CDC-V12, NO-O1, INDRE-206 and clinic case), and esculetin (100 μg/ 6 mm disk, 24–72 h) significantly inhibited their growth.

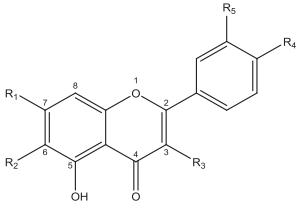
The antibacterial activities of daphnetin against *Ralstonia solanacearum* was evaluated with MIC of 64 mg/L. The C-6, C-7, or C-8 positions' hydroxylation enhanced the antibacterial activity of coumarins against *Ralstonia solanacearum*. Hydroxycoumarins mechanically destroyed the biomembrane and inhibited its formation, which was attributed to the down-regulated genes, *fli*A and *flh*C (Yang et al., 2016a, 2016b).

Duggirala et al. (2014) revealed that daphnetin and esculetin changed the secondary protein structure in bacteria; then, computational toxicological methods, molecular docking and 3D-quantitative structure-activity relationship (QSAR) were performed to predict potential mechanisms. Daphnetin and esculetin changed the morphology of bacterial cell division protein filamentous temperature sensitive protein Z (FtsZ), a crucial bacterial tubulin homologue, to exert antibacterial activity FtsZ comprised of 41.3% α-helix and 6.1% of β-strand, but daphnetin altered its secondary protein structure by reducing α-helix to 35% and increasing β-strand to 9%. In the presence of 100 μM daphnetin, agminated FtsZ monomers prevented the formation of protofilaments, thus inhibiting the polymerization activity and assembly of FtsZ. Losing the ability to undergo division, the bacterial length of *Bacillus subtilis* 168 was elongated from 4.40 μm to 9.06 and 16.16 μm. Molecular docking revealed that coumarins bound to the T7 loop of FtsZ triggered DNA repair in bacteria, resulting in overexpressed SulA, an endogenous inhibitor of FtsZ. 3D-QSAR study demonstrated the C-7 position's hydroxylation of the coumarins' benzene ring was required for antibacterial activity, consistent with the above experimental results.

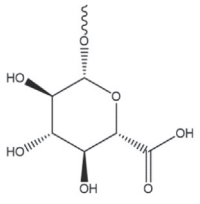
### 7.4. Antiviral activity

Galabov et al. (1996) investigated the antiviral activities of 10 kinds of hydroxycoumarin derivatives against Poliovirus 1 (PV1), influenza virus A/chicken/Germany/27/Weybridge (H7N7), Newcastle disease virus (NDV) and pseudorabies virus (PsRV); only esculetin showed antiviral activity against NDV at 2.8 mM, with a diameter of inhibition zone of 27.1 mm and toxicity zone of 7.5 mm. The chick embryo fibroblast cultured with 50% NDV infectious doses ($CCID_{50}$) of 10, 100 and 1000 replications/well had $MIC_{50}$ values of 30.3, 36.0 and 56.2 μM, respectively.

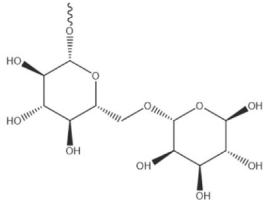
**Table 4**
Chemical structures of the flavonoids in *Euphorbia semen*.



Nuclear



β-D-Glucopyranosiduronic acid

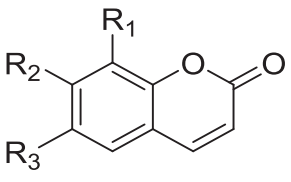


[6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy

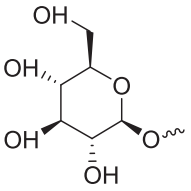
| No. | Names | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | CAS numbers | References |
|---|---|---|---|---|---|---|---|---|
| 34 | Baicalein | OH | OH | H | H | H | 491–67–8 | Zhang et al. (2017) |
| 35 | Kaempferol | OH | H | OH | OH | H | 520–18–3 | Zhang et al. (2017) |
| 36 | Quercetin | OH | H | OH | OH | OH | 117–39–5 | Zhang et al. (2017) |
| 37 | Vitexicarpin | $CH_3O$- | $CH_3O$- | $CH_3O$- | $CH_3O$- | OH | 479–91–4 | Zheng et al. (2009) |
| 38 | Artemetin | $CH_3O$- | $CH_3O$- | $CH_3O$- | $CH_3O$- | $CH_3O$- | 479–90–3 | Zheng et al. (2009) |
| 39 | Kaempferol-3-glucuronide | OH | H | β-D-Glucopyranosiduronic acid | OH | H | 22688–78–4 | Dumkow (1969) |
| 40 | Quercetin-3-glucuronide | OH | H | β-D-Glucopyranosiduronic acid | OH | OH | 22688–79–5 | Dumkow (1969) |
| 41 | Rutin | OH | H | [6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy | OH | OH | 153–18–4 | Zhang et al. (2017) |



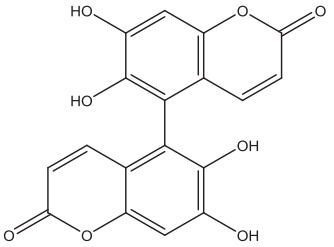
**Table 5**
Chemical structures of the coumarins in *Euphorbia semen*.



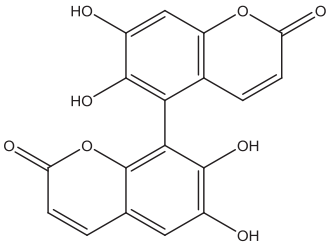
Nuclear



β-D-Glucopyranose



47



48

| No. | Names | $R_1$ | $R_2$ | $R_3$ | CAS numbers | References |
|---|---|---|---|---|---|---|
| 42 | Esculetin | H | OH | OH | 305-01-1 | Dutta et al. (1972) |
| 43 | Daphnetin | OH | OH | H | 486-35-1 | Jaretzky and Kohler (1942) |
| 44 | Scopoletin | H | OH | $CH_3O$- | 92-61-5 | Yang et al. (2016a, 2016b) |
| 45 | Fraxidin | OH | $CH_3O$- | $CH_3O$- | 525-21-3 | Yang et al. (2016a, 2016b) |
| 46 | Esculin | H | OH | β-D-Glucopyranose | 531-75-9 | Li et al. (1994) |
| 47 | Euphorbetin | | | | 35897-99-5 | Dutta et al. (1972) |
| 48* | Isoeuphorbetin | | | | 50886-61-8 | Dutta et al. (1973) |

* So far, this compound has only been found in *Euphorbia semen*.

### 7.5. Anti-allergic activity

In the Croton oil-induced ear irritation test, CD-1 mice showed oedema granulocyte infiltration and increased peroxidase activity (Tubaro et al., 1988). Esculetin (0.84, 1.17 and 1.68 μM/ear) attenuated the above anaphylactic reactions in a dose-dependent manner. Yamagami et al. (1968) observed that esculin and esculetin alleviated oedema caused by carrageenan, dextran and yeast in rats. Ovalbumin (OVA)-induced allergic asthma model was prepared in BALB/c mice. Esculetin (20 mg/kg, i.g., 25–29 d) relieved anaphylactic reactions by inhibiting eosinophils, IL-4, IL-5, IL-13 and IL-17A in bronchoalveolar lavage fluid (BALF), and down-regulated RORγt and GATA3 protein expressions in lung tissue (Long, 2016). In other ovalbumin-induced allergic asthma and mitochondrial dysfunctional BALB/c mice, esculetin (0.1, 1, and 10 mg/kg, i.g., 19–32 d) alleviated airway hyperresponsiveness and goblet cell metaplasia, and reduced lung eotaxin and OVA-specific IgG2a (Mabalirajan et al., 2009). The mitochondrial-related 15-lipoxygenase and its metabolites, lipid hydroperoxide, 8-isoprostane, cytochrome *c* and Caspase-9 activity were decreased, along with increased electron transport chain activity/total citrate synthase activity and ATP level in lung tissue or BALF. In conclusion, esculetin regulates allergic reaction via Th2/Th17 balance and mitochondrial functional restoration.

The anti-inflammation, antibacterial, antiviral and anti-allergic activity of the chemical constituents in *Euphorbia semen* might be a reflection of its clinical efficacy in curing tinea, scabies and snakebites.

### 7.6. Anticancer activity

Growing attention has been paid to the excellent antitumour activity of Euphorbia factors. In mice, EFL5 (0.1 mg/kg/day, i.p., 5 d) was effective against Sarcoma 180 ascites carcinoma (Itokawa et al., 1989). EFL1 inhibited human oral epidermoid carcinoma cell line KB and human breast carcinoma cell line MCF-7 with $IC_{50}$ of 50.05 and 65.31 μM, respectively (Zhang et al., 2011b). EFL2 and EFL3 induced human lung carcinoma A549 cell cytotoxicity with $IC_{50}$ of 36.82 and 34.04 μM, respectively, and mediated apoptosis via mitochondrial pathway (Lin et al., 2017; Zhang et al., 2011a). While Euphorbia factors differed in the inhibitory effect of gynaecologic cancer cells, HeLa, HEC-1, SHIN3, HOC-21 and HAC-2 (Wang et al., 2011). This variation was due to the exo-double bond and the substitution adjacent to the double bond in lathyrane diterpenes-L factors (Shi et al., 2008). Multidrug resistance (MDR) enables pathological cells to gain resistance to an extensive spectrum of structurally and functionally different therapeutic drugs, thus, becomes the main obstacle of successful cancer chemotherapy. P-glycoprotein (P-gp), the major transporter to efflux drugs from cells, mediates MDR through a process driven by ATP hydrolysis-produced energy (Lopes-Rodrigues et al., 2016). Zhang et al. (2011b) treated P-gp overexpressed vincristine-selected KBv200 cells and doxorubicin-selected MCF-7/ADR cells with EFL1 (2.5, 5 and 10 μM, 3 h), and intracellular drug accumulations were elevated, along with increased P-gp ATPase activity, whereas the mRNA and protein expressions of P-gp were unchanged. Besides, EFL2, EFL3, EFL7a, EFL7b and EFL8 showed the ability of anti-MDR. (Jiao et al., 2009; Zhang et al., 2013). The potential mechanism could be that EFLs increased the accumulation of anticancer drugs in cells, thus induced stronger cytotoxicity in cancer cells.

In human HepG2 cells, esculetin inhibited mitogen-activated protein kinase (MAPK) pathway and enhanced apoptosis by Fas/FasL-mediated Caspase pathway (Kuo et al., 2006). After treating human leukaemia cells HL-60 with esculetin (100 μM, 24 h), DNA breakage and condensed, and fragmented nuclei were observed. The apoptosis was related to the activated mitochondrial pathway (Chu et al., 2001). Furthermore, esculetin arrested HL-60 cells in G0/G1 phase via reduction of cyclin-dependent kinases (CDKs), downregulation of Cyclin D1 and inhibition of hypophosphorylated retinoblastoma protein (pRb) phosphorylation (Wang et al., 2002). The methanol extract of *Euphorbia semen* inhibited human cervical cancer cells (HeLa), human erythroleukaemia cells (K652), human monocyte leukaemia cells (U937), human acute lymphocytic leukaemia cells (HL60) and human hepatocellular carcinoma cells (HepG2), with $IC_{50}$ of 15.5, 13.1, 10.5, 17.5 and 29.6 μg/ml, respectively (Huang et al., 2004).

In mice or cells models, the compounds or extraction from *Euphorbia semen* were effective to various types of cancer, including ascites carcinoma, oral epidermoid carcinoma, breast carcinoma, lung carcinoma, leukaemia, gynaecologic cancer and hepatocellular carcinoma. However, no clinical trials have been reported, further evidences from human are needed to assess the anticancer activity of *Euphorbia semen*.

### 7.7. Antioxidant activity

Esculetin exerts antioxidant activity both in vitro and in vivo. In albino rats with oxidative injuries, esculetin (10, 20 and 40 mg/kg, i.g., 45 d) recovered enzymic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), GPx, glutathione-S-transferase (GST), and GSH, as well as non-enzymic antioxidants such as Vitamins C and E, in the liver and kidney, in a dose-dependent manner (Prabakaran and Ashokkumar, 2013). Kim and Lee (2017) treated 3T3-L1 preadipocytes

**Table 6**
Non-compartmental plasma pharmacokinetic parameters after different administration of EFL1–3 or esculetin in animal models.

| Species | Drugs | Routes | Dose (mg/kg) | Analytical methods | $AUC_{(0\text{-}t)}$ (μg h/L) | $AUC_{(0\text{-}\infty)}$ (μg.h/L) | $T_{1/2}$ (h) | $T_{max}$ (h) | $C_{max}$ (μg/L) | F (%) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD rats | EFL1* | i.g. | 9.5 | LC–MS | 100.9 ± 46.0 | 158.0 ± 82.4 | 15.9 ± 3.3 | 1.1 ± 0.5 | 30.4 ± 12.0 | – | (Meng et al., 2013) |
| SD rats | EFL2* | i.g. | 6 | LC–MS | 830.0 ± 113.0 | 2668.0 ± 336.0 | 4.2 ± 1.8 | 6.2 ± 2.0 | 81.3 ± 35.5 | – | (Meng et al., 2013) |
| SD rats | EFL3* | i.g. | 10.5 | LC–MS | 680.6 ± 230.1 | 765.9 ± 172.8 | 10.7 ± 3.3 | 3.2 ± 0.8 | 73.6 ± 22.7 | – | (Meng et al., 2013) |
| SD rats | EFL1 | i.g. | 100 | HPLC-MS/MS | 201.4 ± 46.1 | 224.2 ± 48.6 | 12.6 ± 1.9 | 0.3 ± 0.1 | 87.3 ± 32.4 | 2.0 | (Fu, 2013) |
| SD rats | EFL1 | i.v. | 10 | HPLC-MS/MS | 1051.7 ± 518.8 | 1128.9 ± 531.6 | 6.9 ± 4.5 | – | 3418.6 ± 3486.2 | | (Fu, 2013) |
| Rhesus macaques | EFL1 | i.g. | 30 | HPLC-MS/MS | 644.1 ± 194.9 | 659.0 ± 195.9 | 9.9 ± 2.9 | 0.3 ± 0.1 | 139.1 ± 21.1 | 4.6 | (Fu, 2013) |
| Rhesus macaques | EFL1 | i.v. | 5 | HPLC-MS/MS | 2243.6 ± 277.2 | 2380.2 ± 291.2 | 3.9 ± 0.4 | – | 1359.5 ± 236.0 | | (Fu, 2013) |
| SAIDS rhesus macaques | EFL1 | i.g. | 30 | HPLC-MS/MS | 251.7 ± 97.3 | 255.3 ± 97.0 | 8.2 ± 1.7 | 0.6 ± 0.3 | 56.9 ± 58.2 | 1.8# | (Fu, 2013) |
| SD rats | Esculetin | i.g. | 25 | HPLC | 5167.5 | 86.1 | 0.8 | 0.1 | 173.3 ± 25.8 | – | (Kim et al., 2014) |

**LC–MS**: liquid chromatography–mass spectrometry; **HPLC-MS/MS**: high-performance liquid chromatography-tandem mass spectrometry; **F**: bioavailability; **SAIDS**: simian immunodeficiency virus-induced acquired immune deficiency syndrome; **i.g.**: intragastric administration; **i.v.**: intravenous injection. *The EFL contents in ethanol extract of *Euphorbia semen*. #The bioavailability of SAIDS rhesus macaques was calculated from the data of Rhesus macaques (i.v.) simultaneously. -Unattained data.

with esculetin (12.5, 25 and 50 μM, 6 d), and the intracellular ROS generation was decreased. The antioxidant enzymatic defence system was activated with increased glutathione (GSH) and glutathione peroxidase (GPx), as well as up-regulated heme oxygenase-1 (HO-1) and glutamate-cysteine ligase catalytic subunit (GCLC).

### 7.8. Antipigmentation activity

The antipigmentation activity of *Euphorbia semen* contributes to its whitening effect of traditional use, and this activity has been verified in volunteer, cells and artificial tyrosinase system. Melanin is synthesized by the tyrosinase enzyme through the tyrosine precursor in melanocytes. To suppress hyperpigmentation lesions, such as melasma and ephelides, the role of tyrosinase in melanin biosynthesis has been studied, and phytochemical ingredients with tyrosinase inhibitory activity have been isolated from *Euphorbia semen* (Masamoto et al., 2003). EFL3 has been widely used as a raw material to treat pigmentation in Korea. 30 volunteers were recruited and received ultraviolet b (290–320 nm, 20 W for 24 h) to induce hyperpigmented lesions. Then, EFL3 was used (0.2%, w/w, twice daily, 7 weeks) for treatment; the objective chromameter and subjective evaluation of the double-blinded researcher and subjects were consistent with positive drug arbutin (Suh et al., 2009). Moreover, After treating B-16 melanoma cells with EFL3 (5 μg/ml, 3–5 d), cell numbers, melanin content and tyrosinase activity were decreased. EFL3 exerts a whitening effect via the inhibition of tyrosinase activity. Esculetin exerted inhibitory effect on 3-(3,4-dihydroxyphenyl)-L-alanine oxidation by mushroom tyrosinase, in a competitive inhibition mode, with $IC_{50}$ 0.043 μM. The hydroxyl groups in the C6 and C7 positions of the coumarin skeleton may contribute to the inhibition activity. Combined with the anti-inflammation and ultraviolet absorption effect, esculetin has broad application prospects to treat dermal hyperpigmentation.

### 7.9. Anti-platelet aggregation activity

Antiplatelet agents inhibit platelet hyperaggregability to prevent vascular thromboembolic events. GPIIb/IIIa receptor antagonists are most commonly prescribed. Zaragoza et al. (2016) tested the anti-platelet aggregation activity of multiple polyphenols against the blood of healthy volunteers, of which esculetin (0.5 mM) had an antiplatelet activity of 53.83% and significantly greater percentage occupation of GPIIb/IIIa receptors than that of fraxetin and coumarin. In *Helicobacter pylori* urease-induced platelet aggregation, the phospholipase A2 was activated and made arachidonic acid available for the 12-lipoxygenase enzyme, ultimately leading to the secretion of platelet-dense granules (Wassermann et al., 2010). Esculetin acts as a 12-lipoxygenase inhibitor to block the synthesis of 12-hydroxyperoxy-eicosatetranoic acid, thus avoiding the release reaction (Olivera-Severo et al., 2006).

### 7.10. Hepatoprotection

The hepatoprotection activity of esculetin was mainly based on the up-regulated of antioxidant system. After N-nitrosodiethylamine induced hepatotoxicity, including hepatocyte necrosis, loss of architecture, acute inflammation, lipid peroxidation (LPO) and ALT increase, and GSH depletion in rats, esculetin (0.5% w/w in the diet, 7 d) ameliorated the above injuries and up-regulated the protein expression of protective enzymes HO-1, quinone oxidoreductase 1 (NQO1) and glutathione S-transferase P1 (GSTP1) (Subramaniam and Ellis, 2016).

Esculetin (50 mg/kg/d, i.g.) reduced triglycerides (TG), total cholesterol (TC) alkaline phosphatase (ALP), aspartate aminotransferase (AST), ALT and LDH in the plasma of nonalcoholic fatty liver rats. The underlying mechanism was related to up-regulated phospho-FoxO1 and down-regulated TGF-β1 and Fibronectin, as well as reduced collagen deposition (Pandey et al., 2017).

Briefly, t-butyl hydroperoxide (t-BHP), a short-chain analogue of lipid hydroperoxide, is metabolized into free radical by cytochrome P450 in hepatocytes, thus affecting cell integrity and leading to cell injury. In t-BHP-treated primary rat hepatocytes, esculetin (5, 10 and 20 μg/ml, 1 h) decreased the leakage of lactate dehydrogenase (LDH), serum alanine transaminase (ALT) and malondialdehyde (MDA). In hepatic-injured rats, esculetin (0.5 and 5 mg/kg, i.p., 5 d) decreased serum levels of hepatic enzyme and oxidative stress (Lin et al., 2000).

### 7.11. Neuroprotection

In a middle cerebral artery occlusion model, ICR mice intracerebroventricularly received esculetin after cerebral ischaemia/reperfusion (I/R) injury, and the infarct volume and neurological deficit were relieved in a dose-dependent manner (Wang et al., 2012). The mechanism is related to the down-regulated Caspase-3 and Bax, as well as the up-regulated Bcl-2. Missense mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the leading cause of autosomal dominant and sporadic Parkinson's disease. In LRRK2 mutational *Drosophila*, esculetin exerted kinase inhibitor properties and alleviated oxidative dysfunction (Angeles et al., 2016). Esculetin and daphnetin remarkably inhibited butyrylcholinesterase (BChE), acetylcholinesterase (AChE) and β-site amyloid precursor protein cleaving enzyme 1 (BACE1), which are responsible for the progression of Alzheimer's disease (Ali et al., 2016).

## 8. Toxicological study

### 8.1. Acute toxicity

The acute toxicity of *Euphorbia semen* varies in different extraction solvents. As shown in Table 8, the methanol, ethyl acetate, petroleum ether and aqueous extracts had totally different $LD_{50}$ in mice (Huang et al., 2004; Liang et al., 2011). Since their toxic symptoms are similar, such as having reduced spontaneous activities and tic, the authors inferred that liposoluble EFL1 may be the toxic constituent. However, high dose EFL1 (2 g/kg) did not induce any acute toxicity in mice, suggesting that EFL1 was not the chief toxic constituent in *Euphorbia semen*. Volatile oil and esculetin had high MTD or $LC_{50}$ and excluded the possibility for toxicity (Tubaro et al., 1988). Compared to the dose of esculetin (0.1–100 mg/kg for i.g.) to exert pharmacological effects, its toxic dose ($LD_{50}$ > 2000 mg/kg) is much higher to avoid potential toxicity. The acute toxicity should be focused on the fatty oil for its high content in *Euphorbia semen* and relatively low $LD_{50}$ in rats.

### 8.2. Intestinal tract and ear irritation

As early as the 1940s, a colourless resin from methanol-solution fraction of seed oil was separated from biologically inactive crystals and was considered the main intestinal toxic ingredient of the seed oil (Dublyanskaya, 1941). As shown in Table 8, the toxicity of the seed oil was reported at a dose of 2.5 ml/100 g in rats. The seed oil had three times the diarrhoeal toxicity as castor oil but had less toxicity than croton oil. By means of further fractionation and identification, EFL1, EFL2 and EFL4 were deemed the intestinal tract stimulant constituents to induce diarrhoea, by accelerating the push movement of the small intestine (Jaretzky and Köhler, 1943). Since these three constituents belong to lathyrane or similar structure ingenol, whether the dozens of other diterpenes in *Euphorbia semen* can promote small intestine movement is still unknown. It should be noted that the diarrhoea induced by *Euphorbia semen* is also the pharmacological effect for constipation, thus the dose for clinical use needs to be strictly controlled.

The ear irritation of *Euphorbia semen* was assayed by Adolf and Hecker (1975). EFL1–4, EFL7–8, β-sitosterol, glycerol-monooleate, glycerol-dioleates, glycerol-trioleate, ingenol and 16-hydroxy-ingenol were non-irritants in mice. Compared to the classic irritant, croton oil factor A1 ($ID_{50}$ = 0.016 nM/ear), seed oil, EFL5 and EFL6 had lower

**Table 7**
Pharmacological effects of chemical constituents or extracts of *Euphorbia semen*.

| Pharmacological effects | Constituent or extracts | Models | Effective Dose and time intervals | Assessment indicators | References |
|---|---|---|---|---|---|
| Diuresis and anti-hyperuricaemia | Esculin Esculetin | Hyperuricaemia and renal dysfunctional KM mice | 20, 40 mg/kg, i.g., once | Increased urine urate and creatinine excretion, decreased serum uric acid, Cr and BUN. Up-regulated mRNA and protein expression of OAT1, OCT1, OCT2, OCTN1 and OCTN2. | (Li et al., 2011) |
| Anti-inflammation | Daphnetin | Severe acute pancreatitis Wistar rats | 4 mg/kg, i.p., once | Alleviated oedema, inflammation, vacuolization and necrosis in pancreas slice. Decreased serum amylase, lipase, TNF-α, IL-1β and MDA content as well as MPO activity | (Liu et al., 2014) |
| | Daphnetin | Severe acute pancreatitis Wistar rats | 4 mg/kg, i.p., once | Decreased neutrophil and apoptotic cells in pancreas slice. Reduced serum ALT and Cr, increased anti-inflammatory cytokines IL-10. Suppressed TLR-4/NF-κB inflammatory pathway. | (Liu et al., 2016) |
| | Daphnetin | Arthritis Wistar rats | 4 mg/kg/day, i.p., 21 d | Attenuated swelling rate of the immunized foot in X-ray films. Decreased RORγt. Down-regulated mRNA expression of IL-6, IL-17 and TGF-β. | (Tu et al., 2012) |
| | Daphnetin | Arthritis Wistar rats | 1, 4 mg/kg/day, i.p., 21 d | Reduced inflammatory cell infiltration of joint cavity, attenuated pannus formation, and decreased synovial hyperplasia in slice. Decreased CD77, IL-4, IL-22, IL-23, and IFN-γ in serum. Up-regulated anti-inflammatory protein Foxp3. Th1/Th2/Th17/Treg balance. | (Yao et al., 2011) |
| | Esculetin | Neuroinflammatory ICR mice | 20, 40 mg/kg, i.g., 7 d | Decreased NF-κB p65 expression in hippocampus slice. Decreased IL-6, IL-1β and TNF-α in serum and hippocampus. Down-regulated iNOS and COX-2 protein expression, and suppressed NF-κB inflammatory pathway. | (Zhu et al., 2016) |
| Antibacterial | Esculetin | 7 kinds of bacteria | 100 μg/ 6 mm disk, 24–72 h | Positive to *Salmonella typhi*, *Shigella boydii*, and *Vibrio cholerae* (four strains: CDC-V12, NO-O1, INDRE-206 and clinic case). | (Céspedes et al., 2006) |
| | Daphnetin | 6 kinds of bacteria | 1.5, 3, 6, 12, 50 and 100 mg/L, 48 h | Positive to *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus lentus*, *Escherichia coli* and *Morganella morganii*, with MIC over 100 mg/L. Positive to *Pseudomonas aeruginosa* with MIC of 50 mg/L. | (Cottiglia et al., 2001) |
| | Daphnetin | *Ralstonia solanacearum* | 16,32 and 64 mg/L, 12 h | Daphnetin was positive to *Ralstonia solanacearum* with MIC of 64 mg/L. At 100 mg/L, the antibacterial rate was 97.4%. | (Yang et al., 2016a, 2016b) |
| Antiviral | Esculetin | virus NDV | 30.3, 36.0 and 56.2 μM, 72 h | In cells with 50% NDV infectious dose of 10, 100 and 1000 replication/well, the esculetin $MIC_{50}$ were 30.3, 36.0 and 56.2 μM, respectively. | (Galabov et al., 1996) |
| Anti-allergic | Esculetin | Ear irritant CD-1 mice | 0.84, 1.17 and 1.68 μM/ear, external use, once | Attenuated oedema granulocyte infiltration and decreased peroxidase activity in a dose-dependent manner. | (Tubaro et al., 1988) |
| | Esculetin | Allergic asthma BALB/c mice | 20 mg/kg, i.g., day 25–29 | Decreased eosinophils, IL-4, IL-5, IL-13, IL-17A and IgE in BALF. Down-regulated RORγt and GATA3 protein expressions in lung tissue. | (Long, 2016) |
| | Esculetin | Allergic asthma and mitochondrial dysfunctional BALB/c mice | 0.1, 1, and 10 mg/kg, i.g., day 19–32 | Alleviated airway hyper-responsiveness and goblet cell metaplasia, and reduced lung eotaxin and IgG2a. Mitochondrial functional restoration. | (Mabalirajan et al., 2009) |
| Anticancer | EFL5 | Sarcoma 180 ascites carcinoma-transplanted ICR mice | 0.1 mg/kg/d, i.p., 5 d | Ameliorative body weight and growth ratio. | (Itokawa et al., 1989) |
| | EFL1 | KB and MCF-7 cells | 50.05 and 65.31 μM, 72 h | $IC_{50}$ of 50.05 and 65.31 μM, respectively. | (Zhang et al., 2011b) |
| | EFL2 | A549 cells | 40 and 80 μM, 48 h | $IC_{50}$ = 36.82 μM. Increased intracellular ROS, decreased ΔΨm, and activated apoptosis mitochondrial pathway. | (Lin et al., 2017) |
| | EFL3 | A549 cells | 45 and 90 μM, 48 h | $IC_{50}$ = 34.04 μM. Increased intracellular ROS, activated apoptosis mitochondrial pathway. | (Zhang et al., 2011a) |
| | EFL1, EFL3 | HeLa, HEC-1, SHIN3, HOC-21 and HAC-2 cells | 1, 10 and 100 μM, 48 h | EFL1, $IC_{50}$ = 16.74 μM for HeLa, > 100 μM for others.<br>EFL3, $IC_{50}$ = 1.01 μM for HeLa, 18.8 μM for SHIN3, 51.02 μM for HOC-21, 76.49 μM for HAC-2, > 100 μM for HEC-1. | (Wang et al., 2011) |
| | Esculetin | HepG2 cells | 25, 50 and 100 μM, 24 h | Inhibited MAPK pathway, enhanced apoptosis by Fas/FasL mediated Caspase pathway. | (Kuo et al., 2006) |
| | Esculetin | HL-60 cells | 100 μM, 24 h | DNA breakage, activated apoptosis mitochondrial pathway. | (Chu et al., 2001) |
| | Esculetin | HL-60 cells | 100 μM, 0,3,6,12,24,36 h | Inhibited cell cycle progression. | (Wang et al., 2002) |
| | Methanol extract | HeLa, K562, U937, HL60 and HepG2 cells | 1–250 μg/ml, 24 h | $IC_{50}$ of 15.5, 13.1, 10.5, 17.5 and 29.6 μg/ml, respectively. | (Huang et al., 2004) |

(*continued on next page*)

**Table 7** (*continued*)

| Pharmacological effects | Constituent or extracts | Models | Effective Dose and time intervals | Assessment indicators | References |
|---|---|---|---|---|---|
| Antioxidant | Esculetin | Albino Wistar rats | 10, 20 and 40 mg/kg, i.g., 45 d | Recovered Antioxidant enzymes SOD, CAT, GPx and GST as well as antioxidant Vitamin C and E, in dose-dependent manner. | (Prabakaran and Ashokkumar, 2013) |
| | Esculetin | 3T3-L1 preadipocytes | 12.5, 25 and 50 μM, 6 d | Activated antioxidant enzyme system GSH, GPx HO-1 and GCLC against ROS generation. | (Kim and Lee, 2017) |
| Antipigmentation | EFL3 | Human received UV light | twice daily, 7 weeks | Effective antipigmentation by objective chromameter and subjective evaluation of the double-blind researcher and subjects. | (Suh et al., 2009) |
| | EFL3 | B-16 melanoma cells | 5 μg/ml, 3–5 d | Inhibited cells numbers. Decreased melanin content and tyrosinase activity. | (Suh et al., 2009) |
| | Esculetin | 1000 U/ml mushroom tyrosinase system | 1 min | $IC_{50}$ = 0.043 mM, A competitive inhibition mode. | (Masamoto et al., 2003) |
| Anti-platelet aggregation | Esculetin | Blood of healthy volunteers | 0.5 mM | Occupation of GPIIb/IIIa receptor. Antiplatelet activity of 53.83%. | (Zaragoza et al., 2016) |
| Hepatoprotection | Esculetin | Oxidative damaged SD rats | 0.5 and 5 mg/kg, i.p., 5 d | Decreased serum levels of ALT, AST and reduced oxidative stress. | (Lin et al., 2000) |
| | Esculetin | Hepatic injured Wistar rats | 0.5% w/w in the diet, 7 d | Attenuated injuries, including hepatocytes necrosis, loss of architecture, LPO and ALT increase, and GSH depletion. Up-regulated antioxidant enzymes HO-1, NQO1 and GSTP1. | (Subramaniam and Ellis, 2016) |
| | Esculetin | Nonalcoholic fatty liver Wistar rats | 50 mg/kg/d, once, i.g. | Decreased TG, TC, ALP, AST, ALT and LDH in plasma. Up-regulated protein of phospho-FoxO1, down-regulated TGF-β1 and Fibronectin. | (Pandey et al., 2017) |
| | Esculetin | Oxidative damaged primary rat hepatocytes | 5, 10 and 20 μg/ml, 1 h | Decreased leakage of LDH, ALT and MDA. | (Lin et al., 2000) |
| Neuroprotection | Esculetin | I/R injured ICR mice | 10, 50 and 100 mg/kg, intracerebroventricularly injection | Relieved infarct volume and neurological deficit in a dose-dependent manner. | (Wang et al., 2012) |
| | Esculetin | LRRK2 mutational *Drosophila* | 0.5 μM, 24 h | Strong antioxidant and kinase inhibitor properties, alleviated oxidative dysfunction, loss in dopaminergic neurons, and locomotor defects. | (Angeles et al., 2016) |

**i.g.**: intragastric administration; **i.p.**: intraperitoneal injection; **MEC**: minimal active concentration; **Cr**: creatine; **BUN**: blood urea nitrogen; **OAT**: organic anion transporter; **OCT/OCTN**: organic cation and carnitine transporters; **TNF-α**: tumour necrosis factor-α; **IL-1β**: interleukin-1β; **MPO**: myeloperoxidase; **MDA**: malondialdehyde; **TLR-4**: Toll-like receptor-4; **RORγt**: retinoic acid-related orphan receptor γt; **TGF-β**: transforming growth factor-β; **Foxp3**: forkhead/winged helix transcription factor; **iNOS**: inducible nitric oxide synthase; **COX-2**: cyclooxygenase-2; **LPS**: lipopolysaccharide; **ROS**: reactive oxygen species; **NDV**: Russeff strain Newcastle disease virus; **MIC**: minimum inhibitory concentration; **ΔΨm**: mitochondrial membrane potential; **MAPK**: mitogen-activated protein kinase; **GSH**: glutathione; **GPx**: glutathione peroxidase; **HO-1**: heme oxygenase-1; **GCLC**: glutamate-cysteine ligase catalytic; **BALF**: bronchoalveolar lavage fluid; **P-gp**: P-glycoprotein; **LDH**: lactate dehydrogenase; **ALT**: alanine transaminase; **MDA**: malondialdehyde; **AST**: aminotransferase; **LPO**: lipid peroxidation; **NQO1**: quinone oxidoreductase 1; **TG**: triglycerides; **TC**: total cholesterol; **ALP**: alkaline phosphatase; **I/R**: ischaemia/reperfusion.

**Table 8**
*Euphorbia semen* induced toxicological effects in experimental models.

| Toxicity | Constituents | Animals | Exposure | Toxic parameters | Toxic symptoms | References |
|---|---|---|---|---|---|---|
| Acute toxicity | Methanol extract | NIH mice | i.g., once | $LD_{50}$ = 40.06 g crude drug /kg | Reduced spontaneous activities, watery stool, wet perioral hair, urinary incontinence and tic | (Huang et al., 2004) |
| | Ethyl acetate extract | KM mice | i.g., once | $LD_{50}$ = 160.23 g crude drug /kg | | (Liang et al., 2011) |
| | Petroleum ether extract | KM mice | i.g., once | $LD_{50}$ > 912 g crude drug /kg | | (Liang et al., 2011) |
| | Aqueous extract | KM mice | i.g., once | $LD_{50}$ > 912 g crude drug /kg | Reduced spontaneous activities and tic | (Liang et al., 2011) |
| | Volatile oil | KM mice | i.g., once | MTD = 266.8 g crude drug /kg | No abnormal symptoms | (Liang et al., 2011) |
| | EFL1 | KM mice | i.g., once | – | No abnormal symptoms at dose of 2.0 g/kg | (Liang et al., 2011) |
| | Esculetin | CD-1 mice | i.p., once | $LD_{50}$ = 1450 mg/kg | – | (Tubaro et al., 1988) |
| | Esculetin | CD-1 mice | i.g., once | $LD_{50}$ > 2000 mg/kg | – | (Tubaro et al., 1988) |
| | Fatty oil | Wistar rats | i.g., once | $LD_{50}$ = 20.78 g/kg | – | (Cao, 2000) |
| Intestinal irritation | Seed oil | Rats | i.g., once | 2.5 ml/100 g | Diarrhoea | (Jaretzky and Köhler, 1943) |
| Ear irritation | Seed oil | NMRI mice | Ear skin, once | $ID_{50}$ = 0.19 nM/ear | – | (Adolf and Hecker, 1975) |
| | EFL1 | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | EFL2 | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | EFL3 | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | EFL4 | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | EFL5 | NMRI mice | Ear skin, once | $ID_{50}$ = 0.13 nM/ear | – | (Adolf and Hecker, 1975) |
| | EFL6 | NMRI mice | Ear skin, once | $ID_{50}$ = 0.09 nM/ear | – | (Adolf and Hecker, 1975) |
| | EFL7 | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | EFL8 | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | β-sitosterol | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | Glycerol-monooleate | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | Glycerol-dioleates | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | Glycerol-trioleate | NMRI mice | Ear skin, once | $ID_{50}$ > 100 μg/ear | Non-irritant | (Adolf and Hecker, 1975) |
| | Ingenol | NMRI mice | Ear skin, once | $ID_{50}$ > 100 nM/ear | – | (Adolf and Hecker, 1975) |
| | 16-hydroxy-ingenol | NMRI mice | Ear skin, once | $ID_{50}$ > 100 nM/ear | – | (Adolf and Hecker, 1975) |
| Cocareinogenic effect* | Seed oil | NMRI mice | Back skin, 5000 μg, twice weekly, 48 w | Tumour rate: 13/23<br>Tumour yield: 44/23 | Multiple tumours; 2 malignant squamous cell carcinomas | (Adolf and Hecker, 1975) |
| | EFL1 | NMRI mice | Back skin, 0.1 μM, twice weekly, 48 w | Tumour rate: 0/27<br>Tumour yield: 0/27 | – | (Adolf and Hecker, 1975) |
| | EFL2 | NMRI mice | Back skin, 0.1 μM, twice weekly, 48 w | Tumour rate: 0/27<br>Tumour yield: 0/27 | – | (Adolf and Hecker, 1975) |
| | EFL3 | NMRI mice | Back skin, 0.1 μM, twice weekly, 48 w | Tumour rate: 0/28<br>Tumour yield: 0/28 | – | (Adolf and Hecker, 1975) |
| | EFL4 | NMRI mice | Back skin, 0.1 μM, twice weekly, 60 w | Tumour rate: 0/27<br>Tumour yield: 0/27 | – | (Adolf and Hecker, 1975) |
| | EFL5 | NMRI mice | Back skin, 0.1 μM, twice weekly, 36 w | Tumour rate: 21/26<br>Tumour yield: 102/26 | Multiple tumours; 1 malignant squamous cell carcinoma | (Adolf and Hecker, 1975) |

**$LD_{50}$**: median lethal dose; **MTD**: maximum tolerated dose; **$ID_{50}$**: irritation dose 50; **i.g.**: intragastric administration; **i.p.**: intraperitoneal injection. **Tumour rate**: total number of tumour bearing mice/number of survivors. **Tumour yield**: tumour number of skin tumours/number of survivors. ¯Not mentioned. *The tumour rate and tumour yield were the results at the 20th week, and the initial sample size in each group was 28.

toxicity. Both intestinal tract and ear irritation of *Euphorbia semen* were weaker than croton.

### 8.3. Cocareinogenic effect

The cocareinogenic activities were evaluated using 7,12-dimethyl [*a*] benzanthracene as the initial carcinogen in mice, and then, a single dose of croton oil factor A1, seed oil, and EFL1–5 were administered for two weeks (Adolf and Hecker, 1975). At the 20th week, the tumour rate and tumour yield in the back skin were calculated, of which seed oil and EFL5 were positive, and even malignant tumours were observed.

### 8.4. Detoxification

For safe medication, determining how to attenuate the toxicity of *Euphorbia semen* merits more attention. The above toxicity studies indicated that the main toxic constituents in *Euphorbia semen* were the oil and liposoluble EFLs. In the Chinese pharmacopoeia, the compulsive processing is decorticating via reducing the fatty oil to 18–20% (Pharmacopoeia of China, 2015). In an experimental model, rats received daily petroleum ether extract of *Euphorbia semen* or *Euphorbiae semen* pulveratum (5.84 g/kg, i.g., 4 d), and then, the LDH, malate dehydrogenase and lactase in the intestinal mucosa of the *Euphorbiae semen* pulveratum group were much closer to the normal level (Nie et al., 2018). Furthermore, *Euphorbia semen* caused intestinal flora disorders, including *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, and *Enterococcus* in rats, while *Euphorbiae semen* pulveratum did not (Sun et al., 2017). The different toxicity may be ascribed to the fact that *Euphorbia semen* induced a higher absorption rate constant ($K_a$) and apparent permeability coefficient ($P_{app}$) of EFL1–3 in the intestine than *Euphorbiae semen* pulveratum, resulting in more absorption of intestinal irritants (Li et al., 2017). Therefore, decreasing the oil in *Euphorbia semen* is an effective way to reduce toxicity.

Once poisoned by *Euphorbia semen*, the suggested treatments are repeated for lavage, catharsis, furosemidum injection and vitamin C supplementation. Attention should be paid to possible dysphoria and serious respiratory and circulatory failure (Du and Fang, 2003).

## 9. Discussion and future perspectives

*Euphorbia semen* has been used as ethnic drug in China since year 992, and its efficacy of catharsis, detumescence, detoxification, and so on, has been witnessed. But it was not until the 1970s, the main chemical constituents for pharmacological and toxicological effects were isolated and identified, thus led to growing attention on *Euphorbia semen*. With the development of new analysis technics, 201 compounds with relative low content and bioactivity were reported after year 2000. In this review, we firstly collected all the known compounds hitherto existed in *Euphorbia semen*. Highlighted achievements have been performed on the phytochemistry, pharmacokinetics and pharmacological studies of *Euphorbia semen*. But there are still scientific gaps in the researches of *Euphorbia semen*, therefore we summary several challenges which should have priority for further investigation.

A single constituent usually has relatively clear metabolism processes and efficacy (Shen et al., 2017). What needs reminding is that *Euphorbia semen* is clinically applied as an herb. It induces complicated ADME process and pharmacology effects (Zhang et al., 2015). It would be better to use *Euphorbia semen* rather than a single constituent to account for pharmacokinetic and pharmacological properties of *Euphorbia semen*. In addition, some crucial issues of ADME still need to be explored. For instance, the in vivo metabolic pathway of *Euphorbia semen* is still unknown. The exact tissue distribution and action of chemical constituents and secondary metabolites are unclear.

In *Euphorbia semen*, the seed oil has a high level of over 47% (w/w). As shown in Table 8, the oil has obvious acute toxicity and is an intestinal tract and ear irritant, and it has even induced malignant tumours. To avoid misleading the recognition of the oil, more attention should be paid to its liposoluble toxic constituents. Advanced separation and identification are in urgent need to discern the poisonous constituents rather than simply reducing the total oil in *Euphorbia semen*.

The target organ toxicity induced by *Euphorbia semen* is focused on the gastrointestinal tract. However, the normal cell lines, such as human gastric non-tumoural epithelial cells (GES-1) and human intestinal epithelial cells (HIEC-6), have not been used in related studies up to now. In vivo studies reported toxic effects without further mechanism studies (Song et al., 2010). In brief, the comprehensive gastrointestinal toxic effects and exact mechanisms have not been conclusively or totally clarified.

The Euphorbia species, such as *Euphorbia kansu S.L.Liou ex S.B.Ho*, *Euphorbia cornigera* Boiss, *Euphorbia genistoides* and *Euphorbia pekinensis* Rupr., induced obvious liver and kidney toxicity (Man et al., 2012; Shen et al., 2016). Ternary ring diterpenoids and tetracyclic diterpenoids are regarded as the main toxic constituents in Euphorbia species that induce liver injuries (Baloch et al., 2006). In *Euphorbia semen*, 29 compounds of lathyrane-type diterpenoids and 4 compounds of ingenane-type diterpenoids belong to ternary and tetracyclic ring systems, respectively. It is worth exploring the potential liver and kidney toxicity induced by *Euphorbia semen* for safe medical use in clinic.

In summary, the phytochemistry, pharmacokinetics of main compounds, pharmacological effects as well as underlying mechanisms of *Euphorbia semen* have been extensively investigated. The pharmacokinetics of *Euphorbia semen* rather than its single constituent remain open for investigation. The potential toxic effects and exact mechanisms are still need to be further explored. There are a few challenges to be overcame for the extensive and safe use of *Euphorbia semen*.

## Authors' contributions

Qi Wang and An Zhu conceived and designed the review. An Zhu and Tao Zhang searched the literature, collected the data, and drafted the paper. Qi Wang, An Zhu and Tao Zhang revised the manuscript. All authors read and approved the final manuscript.

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## Conflict of interest

All authors do not have conflicts of interest.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jep.2018.08.024.

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