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Acute and developmental toxicity of embelin isolated from *Embelia schimperi* Vatke fruit: *In vivo and in silico* studies

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ABSTRACT

Background: Embelin is a hydroxybenzoquinone constituent of the Embelia species that has anti-disease properties. However, its toxicity, particularly the *in silico*, acute, and developmental toxicity profiles, has yet to be thoroughly investigated. Hence, this study aims to assess these toxicity profiles.

Materials and Methods: In silico and *in vivo* experimental studies were conducted on embelin isolated from the fruits of *Embelia schimperi* Vatke. *In silico* toxicity predictions were computed using the ProTox model. The *in vivo* experiment was done by administering 5000 mg/kg of embelin to a single female albino Wistar rat, followed by three female rats in the absence of death, to determine the mean lethal dose (LD50). Afterwards, three groups of pregnant rats were treated with embelin at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg for the developmental toxicity test. Vehicle and *ad libitum* control groups were used to compare the acute and developmental toxicity variables.

Results: In silico toxicity predicted that embelin is free from hepatotoxic, carcinogenic, mutagenic, and cytotoxic effects. No inhibitory effect on hERG channels was observed. It has an immunotoxic property and an inhibitory effect on the CYP2D6 enzyme. Since mortality and signs of toxicities were not observed after treatment with 5000 mg/kg, the mean lethal dose (LD_{50}) is determined to be $>$ 5000 mg/kg. There was no significant difference in the morphological scores or number of somites among experimental animals. None of the embryonic systems possessed developmental delays. Nevertheless, the crown-rump length of the high-dose group became significantly shorter. Maternal food intake and weight gain exhibited significant dose-dependent differences between embelin-treated animals and controls. The number of implantations was significantly low in the treatment group, accompanied by a higher frequency of prior resorption.

Conclusion: Embelin is predicted to have a high probability of immunotoxicity potential and affect drug metabolism by inhibiting CYP2D6. In addition, it affects food intake, weight gain, and the number of implantations in pregnant rats. Therefore, it is highly recommended not to take embelin and embelin-rich plants during pregnancy. Further *in vitro* and *in vivo* studies need to be conducted to understand the mechanism behind the toxicity of embelin.

1. Background

Embelin is a well-known hydroxybenzoquinone constituent of *Embelia* species that has been identified to have therapeutic value against various infectious and non-infectious disease entities [1]. The common ethno-medicinal claim of embelin is related to its role against intestinal parasitic infestation [2]. There are reports declaring that embelin, isolated from *Embelia* species, is a much better anthelminthic agent than the standard drugs, even at lower doses without side effects [3]. It also became a promising compound as a potent analgesic and anti-inflammatory agent by inhibiting the pro-inflammatory cytokines IL-1β and TNF-α, which results in blockade of leukocyte accumulation [4]. Similarly, topical application of embelin-based cream exhibited a significant effect on the wound healing process by facilitating epithelialization and wound contraction [5]. Some studies regard embelin as an effective hepatoprotective substance with the potential to lower elevated enzymatic levels of transaminases and alkaline phosphatase. Moreover, histopathological manifestations of embelin treatment

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showed reductions in hepatic cord swelling and mononuclear cell infiltration [6]. Embelin has notable significance in neurological disorders as an anti-convulsant against grand mal and petit mal epilepsy. In addition, it is suggested as a treatment option to improve locomotion after ischemia or reperfusion-induced neuronal damage [7,8]. It is also a beneficial natural product acting as an anti-cancer agent through its complex cascades of molecular targeting activities [9]. Embelin is claimed to be an important therapeutic agent against metabolic disorders such as diabetes mellitus, obesity, hyperlipidemia, and oxidative stress [10,11].

Regarding the safety profile (toxicity) of embelin, prior animal studies have shown that administration of embelin up to 5000 mg/kg acutely and sub-acutely is free from any sign of overt toxicity [12]. However, administration of embelin for 6 weeks brought about an alteration of enzymatic activities in the kidneys and suprarenal glands, accompanied by some histological changes [13]. Several studies on the reproductive toxicity profile of embelin indicated that it significantly alters the morphology of sperm cells, lowers testosterone levels, and impairs semen quantity and quality [12,14]. Similarly, embelin interferes with estrogenic cycles, affects implantation, and lowers plasma levels of progesterone and oestradiol [15].

Although embelin was said to have harmful effects on the reproductive system, nothing is known about its developmental toxicity profile, particularly how it will affect fetal and embryonic outcomes. Also, the *in silico* toxicity profile that aids in the prediction of other toxicological profiles of plants with high embelin content has not yet been computed. The main focus of this work is to ascertain the *in silico* toxicity prediction of embelin extracted from *Embelia schimperi* Vatke fruit and assess its impact on rat development. In this investigation, the acute toxicity of embelin was further examined because results from earlier trials were inconclusive.

2. Materials and methods

2.1. Isolation of embelin

Embelin was isolated by modifying a method used by Belete et al. [16]. Using an electric grinding mill, *E. schimperi* fruits were crushed into a coarse powder. The powder (1000 g) was macerated in ethyl acetate at a ratio of 1:10 (w/v) (plant material/solvent) for 48 h while being shaken at 160 rpm. After that, the sample was filtered *via* filter paper. Two times, the marc was re-macerated. The mixed filtrate was gathered in a glass container and kept cold for 72 h. Bright orange-colored crystals of embelin formed at the bottom of the glass container. The crystals were separated by decantation, and n-hexane was used to repeatedly wash the separated crystals until the solvent was colorless. NMR spectroscopic verification revealed that the isolated compound was a pure crystallized embelin (2,5-dihydroxy-3-undecyl-1, 4-benzoquinone) with a chemical formula of $C_{17}H_{26}O_4$ (Supplementary file), and it was then refrigerated for use in later investigations.

2.2. In silico toxicity prediction

The canonical Simplified Molecular Input Line Entry System (SMILES) **[CCCCCCCCCCCC1 =C(C(=O)C=C(C1 =O)O)O]** and PubChem CID (3218) of embelin were extracted from the PubChem database [17]. Both Canonical SMILES and the 2D structure of embelin, generated by ChemDraw, were used as inputs to generate *in silico* toxicity predictions at the ProTox II server [18]. ProTox-II is a freely available *in silico* toxicity predictive model built by a combination of machine learning algorithms to predict toxicity probabilities by incorporating *in vivo* and *in vitro* toxicological assays. The reason for preferring ProTox was that it incorporates both chemical and molecular target knowledge, making it a comprehensive tool for predicting toxicological endpoints. Moreover, it stands out from other models due to its classification scheme, which divides the prediction scheme into various levels of toxicity [19,20].

3. Acute toxicity

3.1. Experimental animal preparation

The acute toxicity study was conducted according to Organization for Economic Co-operation and Development (OECD) protocol number 425 [21]. Female albino Wistar rats of 12 weeks old weighing 220–250 g free from previous experimental procedures were used for the test. The animals were maintained in an environmentally optimal condition and left untouched for 5 days before the experiment to let them adapt to the experimental environment, during which they were given unlimited food and water.

3.2. Dose preparation and administration

A limit test was performed using rats that were successively dosed with 5000 mg/kg of embelin dissolved in 7% tween 80 in accordance with OECD 425 guidelines. After administering 5000 mg/kg of embelin to a single female rat at first, three additional animals were dosed after 48 h since there was no death. Five animals were used as the vehicle's control group. Prior to dosing, animals fasted for one night. Using a stomach tube, embelin and the vehicle were both given by gavage.

3.3. Toxicity signs and necropsy

Mortality, coma, convulsions or tremors, feces consistency, hair, itching, respiration, salivation, sleeping pattern, somato-motor activity, and behavior pattern were evaluated as signs of toxicity every 30 min for the first 4 h after administration [22]. Observation was continued on a daily basis for the rest of the experimental period, till the 14th day after administration. All animals were subjected to necropsy after anesthesia with sodium pentobarbital 150 mg/kg intraperitoneally in order to assess gross pathological changes of visceral organs such as the liver, kidneys, spleen, heart, and intestines.

4. Developmental toxicity

4.1. Animal preparation, mating

12-week-old albino Wistar rats weighing 220–250 g, which were not involved in any prior experiments, were used. They were held on to cages made from stainless steel, where their housing conditions were maintained with a regulated temperature of 22 ± 3 °C and 50–60% humidity. Mating was conducted by letting a male rat with confirmed fertility stay overnight inside a cage containing two female rats. Gestational age was calculated after the confirmation of sperm presence *via* microscopic evaluation of a vaginal swab the next morning. Details of the method employed were robustly described in the previous developmental toxicity studies [23–26].

4.2. Dose preparation and administration

Based on the results of the acute toxicity study ($LD_{50} > 5000$ mg/kg), three groups were prepared that took 250 mg/kg, 500 mg/kg, and 1000 mg/kg of embelin. The fourth and fifth groups were labeled as vehicle and *ad libitum* control, respectively, and supplied with 7% tween 80 and unrestricted food, respectively. Each group contained 10 pregnant rats that were randomly assigned after microscopic evaluation of the vaginal plug. On a daily basis, both embelin and tween 80 were administered *via* oral gavage from day 6 up to day 12 of gestation, as it is a critical period of pregnancy in rats. Administration was carried out at a constant time. Further explanation of the methods used was covered in our previous work [26].

Table 1
In silico toxicicty out put of embelin after running ProTox toxicity model.

Model target		Prediction	Probability
Hepatotoxicity		Inactive	0.81
Carcinogenicity		Inactive	0.66
Immunotoxicity		Active	0.82
Mutagenicity		Inactive	0.79
cytotoxicity		Inactive	0.77
Cyp inhibitors	1A2	No	-
	3A4	No	-
	2D6	Yes	-
	2C19	No	-
hERG channel blocker		No	-

4.3. Clinical sign evaluation

Daily cage side clinical evaluation was performed to look for signs of visible toxicity all round the experimental period.

4.4. Embryonic and fetal toxicity experiments

12 and 20-day-old rat embryos and fetuses were evaluated for possible features of embryonic and fetal toxicity parameters after explanting them at 12 and 20 days of gestation, respectively, *via* the same procedures mentioned by previous studies that were adopted for *in vivo* developmental toxicity studies [26–30].

4.5. External gross morphology and soft tissue evaluation

External gross morphological alterations were evaluated in near term fetuses after thorough examination from head to tail. Similarly, soft tissue evaluation was carried out using a modified Willison's procedure after fixing rat fetuses with Bouin's solution [27].

4.6. Skeletal ossification evaluation

The effect of embelin on ossification was evaluated according to the Rigueur and Lyons method, characterized by a range of activities from skin removal up to bone staining by alizarin red [31]. After due process, bones from the axial and appendicular skeletons were evaluated as per Nash and Persaud's skeletal scoring mechanism [32].

4.7. Statistical analysis

The Statistical Package for Social Science (SPSS) version 24 software was utilized for data entry and analysis. A One-way Analysis of Variance (ANOVA) with a post Hoc (Turkey) test at $p < 0.05$ significance was used to evaluate mean differences among groups. The chi-square test was employed so as to evaluate the difference in proportion of retarded developmental indices.

4.8. Ethical consideration

An ethical approval letter was obtained from the College of Health Sciences, Addis Ababa University, in accordance with the OECD test guideline (TG-414/2018) [33]. Every procedure was carried out according to Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines. The experiment was also guided by the laboratory animal handling protocol of the Traditional and Modern Medicine Research Directorate (TMMRD) of the Ethiopian Public Health Institute (EPHI).

Table 3
Developmental characteristics of the circulatory system of embryos in the experimental group of pregnant rats following embelin treatment.

Group	Proportion of delayed development		
	Yolk sac circulation	Heart	Allantois
Group I (250 mg/kg)	0	0	0
Group II (500 mg/kg)	0	0	0
Group III (1000 mg/kg)	0	0	0
Group IV (vehicle)	0	0	0
Group V (*Ad libitum*)	0	0	0

Results: Proportion, Chi-square test

5. Results

5.1. In silico toxicicty

The ProTox toxicity model revealed that embelin is predicted to be free of hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity effects. However, the database indicated that embelin is predicted to have an immunotoxic effect and is suggested to be a CYP2D6 inhibitor. Furthermore, embelin is predicted to be devoid of an hERG-blocking effect (Table 1).

5.2. Acute toxicity and LD_{50}

The limit test of embelin at 5000 mg/kg in four animals did not exhibit mortality. Moreover, daily clinical evaluation revealed that the test animals did not show any observable signs of toxicity. As a result, the LD_{50} of embelin is declared to be $>$ 5000 mg/kg. There was no difference in weight gain or food intake between embelin-treated and vehicle-control animals. The gross necropsy also showed no gross pathological abnormalities in the experimental animals.

6. Developmental toxicity

6.1. Clinical observation

The daily cage-side clinical evaluation revealed that there was neither an abortion nor a maternal death report. Pregnant animals were also free from any sign of toxicity.

6.2. Embryonic outcomes

As embryonic outcome indicators, morphological scores and the number of somites did not exhibit significant differences across the experimental groups and their control counterparts (Table 2). But the embryonic crown-rump length (CRL) was found to be significantly shorter in the higher dose group, 1000 mg/kg, than in vehicle and *ad*

Table 2
Developmental characteristics of embryos in the experimental group of pregnant rats following embelin treatment.

Group	Embryonic developmental variables		
	Morphological score/ litter	Number of somites/litters	CRL (mm)/litter
Group I (250 mg/kg)	44.84 ± 1.7	28.44 ± 1.23	4.86 ± 0.13
Group II (500 mg/kg)	44.52 ± 1.5	28.52 ± 1.24	4.79 ± 0.14
Group III (1000 mg/kg)	44.5 ± 1.45	28 ± 0.78	4.51 ± 0.35*
Group IV (vehicle)	45.54 ± 1.95	29.08 ± 1.1	4.86 ± 0.49
Group V (*Ad libitum)*	45.35 ± 1.17	29.09 ± 1.11	4.96 ± 0.42

Data were shown as mean ± standard deviation of the means (μ ± SD), CRL: Crown-rump length.*statistically significant (p-value<0.05)

libitum control animals.

7. Embryonic developmental indices

7.1. Circulatory system

The current study revealed that the yolk sac circulation and heart development showed no sign of developmental delay across all experimental groups (Table 3; Fig. 1).

7.2. Nervous system and sense organs

As indicated in Table 4, the caudal neural tube, hindbrain, forebrain, otic system, and optic system were not subjected to developmental delay after treating pregnant rats with a high dose of embelin.

7.3. Musculoskeletal system

As depicted in Table 5, the current experiment showed that musculoskeletal development parameters were not significantly delayed in the treatment groups compared to the controls.

7.4. Food intake and weight gain

The mean food intake of animals in Group III (1000 mg/kg treatment group) showed a statistically significant increment, (p-value<0.001), compared with both pair-fed and *ad libitum* control animals. Similarly, the mean food intake of Group I (250 mg/kg) and Group II (500 mg/kg)

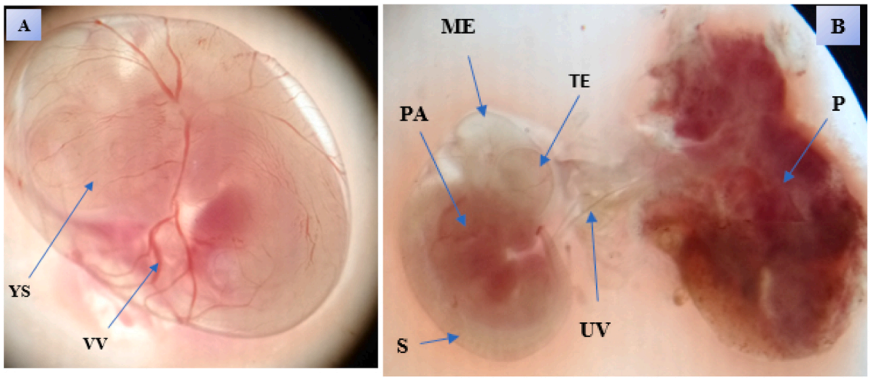

Fig. 1. : 12-Day-old embryos from high dose group (1000 mg/kg). [A]: Embryo inside its yolk sac (YS) with vitelline vessels (VV); [B]: ME (Mesencephalon); P (placental tissue); PA (Pharyngeal apparatus); S (Somite);TE (Telencephalon); and UV (umbilical vessels).

Table 4
Nervous system and sense organs characteristics of the embryos in the experimental group of pregnant rats following embelin treatment.

Group	Proportion of delayed development				
	Caudal neural tube	Hind brain	Fore brain	Otic system	Optic system
Group I (250 mg/kg)	0	0	0	0	0
Group II (500 mg/kg)	0	0	0	0	0
Group III (1000 mg/kg)	0	0	0	0	0
Group IV (vehicle)	0	0	0	0	0
Group V (*Ad libitum*)	0	0	0	0	0

Results: Proportion (%). Chi-square test.

Table 5
Musculoskeletal system characteristics of the embryos in the experimental group of pregnant rats following embelin treatment.

Group	Proportion of retarded development					
	Pharyngeal apparatus	Maxillary process	Mandibular process	Fore limb	Hind limb	Flexion
Group I (250 mg/kg)	0	0	0	0	0	0
Group II (500 mg/kg)	0	0	0	0	0	0
Group III (1000 mg/kg)	0	0	0	0	0	0
Group IV (vehicle)	0	0	0	0	0	0
Group V (*Ad libitum*)	0	0	0	0	0	0

Results: Proportion (%), Chi-square test

Table 6
Food intake and weight gain of pregnant rats treated with embelin.

Maternal variables	Experimental groups				
	Group I	Group II	Group III	Group IV	Group V
	250 mg/kg	500 mg/kg	1000 mg/kg	Vehicle control	*Ad libitum*
Food intake (g)	183.87 ± 23.49*	185.24 ± 10.16*	198.09 ± 13.62**	171.07 ± 14.45	167.54 ± 21.01
Weight gain (g)	71.72 ± 3.27**	68.4 ± 7.64**	67.7 ± 7.31**	87.81 ± 5.48	89.73 ± 4.31

Data were shown as mean ± standard deviation of the means (μ ± SD).*statistically significant (p-value<0.05) and **statistically significant (p-value<0.001).

animals was also higher than that of vehicle and *ad libitum* control animals (p-value<0.001). As illustrated in Table 6, experimental pregnant animals in all three treatment groups showed a significant dose-dependent decrease in the mean of their weight when compared to animals in both control groups.

7.5. Pregnancy outcomes

As shown in Table 7 and Fig. 2 the number of implantations is significantly low in all of embelin-treated groups when compared to vehicle control and *ad libitum* groups. Similarly, the number of resorp-

Table 7
Pregnancy outcomes in the experimental group of pregnant rats following embelin treatment.

Pregnancy outcomes	Experimental groups				
	Group I (250 mg/kg)	Group II (500 mg/kg)	Group III (1000 mg/kg)	Group IV (vehicle control)	Group V (*Ad libitum*)
Number of implantation/dams	5.4 ± 0.55*	5.4 ± 1.14*	4.2 ± 0.84*	10 ± 1.58	10.4 ± 1.14
Number of prior resorptions/dams	0.4 ± 0.55*	0.8 ± 0.45*	1.4 ± 0.55*	0	0
Alive pups	5 ± 1*	4.6 ± 1.52*	2.8 ± 1.09*	10 ± 1.58	10.4 ± 1.14
Dead pups	0	0	0	0	0

Data were shown as mean ± standard deviation of the means (μ ± SD). *Statistically significant (p-value<0.001).

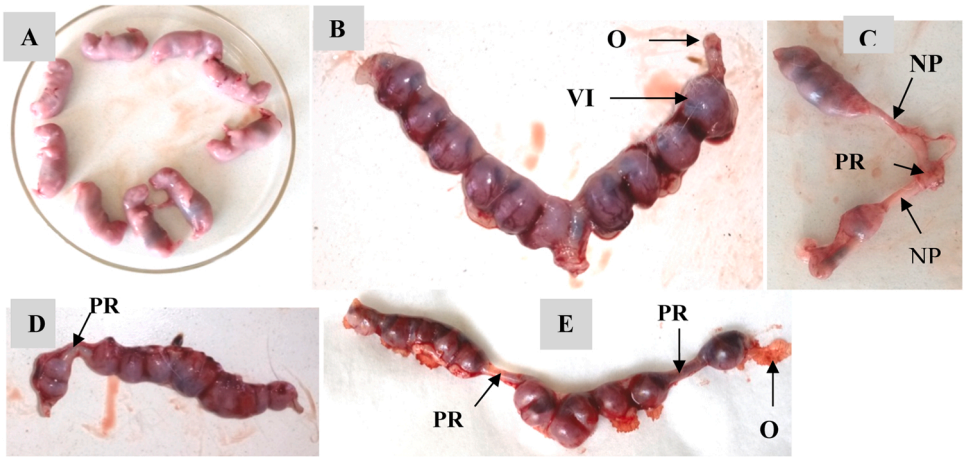

Fig. 2. : Implantation on a gravid uterus of rats treated with embelin. A: Alive near term fetuses; B: a gravid uterus from rats treated with tween 80 showing the ovary (O) and a viable implantation site (VI); C: A gravid uterus from high dose treatment group (1000 mg/kg) with visibly impaired implantation sites designated as NP and prior resorption (PR); D: Gravid uterus from rats treated with 250 mg/kg of embelin; E: uterus from rats treated with 500 mg/kg of embelin.

Table 8
Fetal outcomes in the experimental group of pregnant rats following embelin treatment.

Fetal outcomes	Experimental groups				
	Group I (250 mg/kg)	Group II (500 mg/kg)	Group III (1000 mg/kg)	Group IV (vehicle control)	Group V (*Ad libitum*)
Fetal weight (g) per dam	3.78 ± 1.02	3.98 ± 0.87	4.02 ± 0.86	3.47 ± 1.00	3.66 ± 0.76
Crown-ramp length (cm)	4.86 ± 0.11	4.7 ± 0.41	4.67 ± 0.21	4.7 ± 0.41	4.87 ± 0.21

Data were shown as mean ± standard deviation of the means (μ ± SD), CRL: Crown-rump length.

Table 9
External gross malformation characteristics in the experimental group of pregnant rats following embelin treatment.

Group	Proportion of external malformations (%)							
	Nervous system defects			Musculoskeletal defects			Others	
	ExE	AnE	SB	KY	SC	LD	MT	EGA
Group I (250 mg/kg)	0	0	0	0	0	0	0	0
Group II (500 mg/kg)	0	0	0	0	0	0	0	0
Group III (1000 mg/kg)	0	0	0	0	0	0	0	0
Group IV (Pair-fed)	0	0	0	0	0	0	0	0
Group V (*Ad libitum*)	0	0	0	0	0	0	0	0

Results: Proportion, Chi-square test. ExE: Exencephaly, AnE: Anencephaly, SB: Spina bifida, KY: Kyphosis, SC: Scoliosis, LD: Limb defect, MT: Missed tail, EGA: External genitalia agenesis.

tion sites and live fetuses were also possessed significant difference between experimental groups and the controls.

7.6. Fetal outcomes

As a measure of fetal outcome indicators, fetal weight and crown-rump length did not show differences within experimental groups (Table 8).

7.7. External and visceral morphology

Fetuses from both treatment and control groups did not exhibit significant external morphological defects or malformations (Table 9). Furthermore, there were no visible structural defects in visceral structures among all groups of animals (Fig. 3).

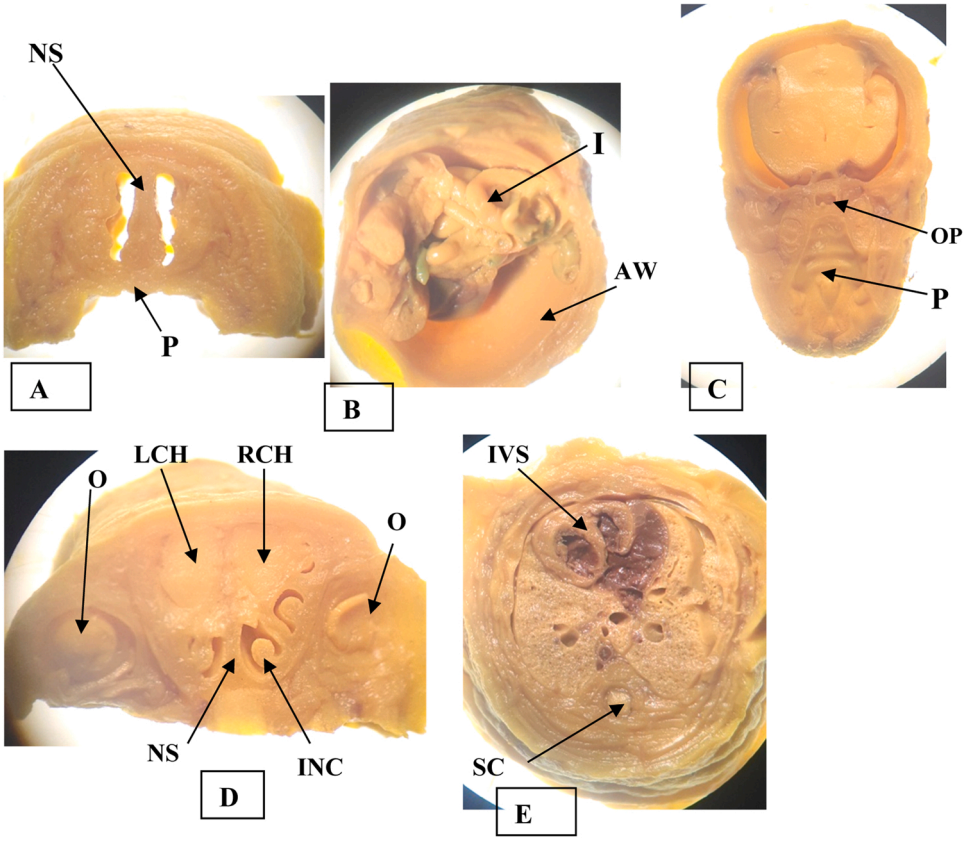

Fig. 3. : (A) (NS: nasal septum, P: palate); (B) (AW: abdominal wall, I: intestinal content); (C) (OP: oropharynx, P: palate); (D) (INC: inferior nasal conchae, LCH: left cerebral hemisphere, O: optical tissue and RCH: right cerebral hemisphere); (E) (IVS: interventricular septum, SC: spinal cord).

Table 10
Number of ossification centers in the axial skeleton of rat fetuses from experimental groups treated with embelin.

Group	Sternum	Thoracic vertebrae	Lumbar vertebrae	Caudal	Ribs
Group I (250 mg/kg)	5.68 ± 0.12	12 ± 0	5 ± 0	4.22 ± 0.24	24 ± 0
Group II (500 mg/kg)	5.48 ± 0.21	12 ± 0	5 ± 0	4.11 ± 0.12	24 ± 0
Group III (1000 mg/kg)	5.64 ± 0.2	12 ± 0	5 ± 0	4 ± 1.38	24 ± 0
Group IV (vehicle)	5.77 ± 0.14	12 ± 0	5 ± 0	4.21 ± 0.23	24 ± 0
Group V (*Ad libitum*)	5.69 ± 0.18	12 ± 0	5 ± 0	4.31 ± 0.22	24 ± 0

Data were shown as mean ± standard deviation of the means (μ ± SD)

Table 11
Number of ossification centers in the appendicular skeleton of rat fetuses from experimental groups treated with embelin.

Group	Forelimb phalanges	Hind limb phalanges	Metacarpus	Metatarsus
Group I (250 mg/kg)	3.81 ± 0.42	3.49 ± 0.39	3.87 ± 0.31	4.07 ± 0.27
Group II (500 mg/kg)	3.84 ± 0.26	3.48 ± 0.41	3.81 ± 0.38	4 ± 0.35
Group III (1000 mg/kg)	3.82 ± 0.31	3.42 ± 0.51	3.88 ± 0.31	4.01 ± 0.43
Group IV (vehicle)	3.8 ± 0.28	3.51 ± 0.32	3.89 ± 0.29	4.14 ± 0.27
Group V (*Ad libitum*)	3.85 ± 0.32	3.44 ± 0.31	3.87 ± 0.31	4.08 ± 0.14

Data were shown as mean ± standard deviation of the means (μ ± SD)

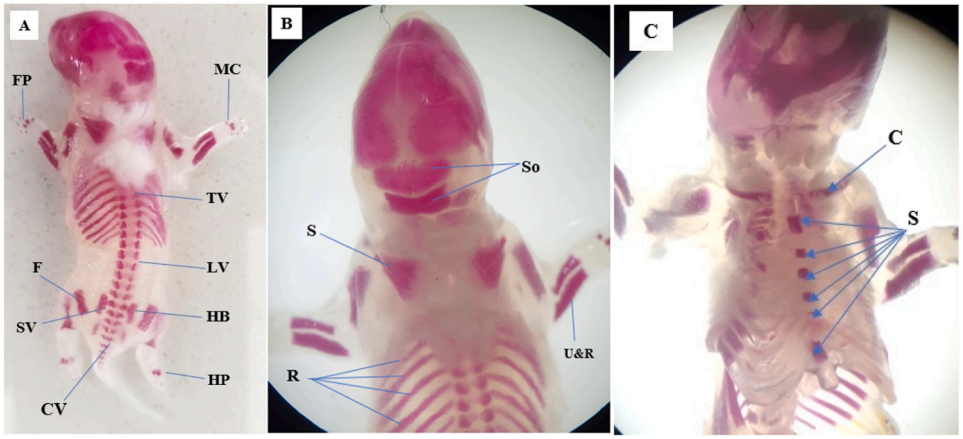

Fig. 4. : Skeletal ossification with alizarin red. C: clavicle, CV: Caudal vertebrae, F: femur, FP: Forelimb phalanges, HB: Hip bone, HP: Hindlimb phalanges, LV: Lumbar vertebrae, MC: Metacarpus, R: Ribs, So: Supraoccipital, S: Sternum, SV: Sacral vertebrae, TV: Thoracic vertebrae, U & R: ulna and radius.

7.8. Skeletal evaluation

As shown in Table 10, Table 11, and Fig. 4, there is no significant difference in the number of ossification centers in both the axial and appendicular skeletons.

8. Discussion

There have been shreds of evidence generated pertaining to the therapeutic relevance of embelin isolated from *Embelia* species to manage various ailments such as dermatological problems, gastrointestinal-related diseases, pain, metabolic disorders, fertility problems, neurological disorders, and cancer [12]. However, its safety profile has not yet been explicitly explored, other than a few suggested safety margins for the compound. Moreover, the *in silico* toxicity profile and its effect on the developmental process remain less known despite the fact that they are crucial parts of the toxicological endpoint, especially when dealing with such a compound of strong pharmaceutical importance. Hence, this study investigated the aforementioned toxicity profile of embelin.

In silico studies of chemicals and compounds are becoming prefered predictive computer-based computational methods before diving into *in vivo* and *in vitro* studies. Such studies also showed the strong clinical success of drug targets [34]. The current study revealed that *in silico* toxicity output predicted embelin to be a non-hepatotoxic, non-carcinogenic, non-mutagenic, and non-cytotoxic agent. In line with these findings, animal studies confirmed that embelin is safe on the liver and does not have a carcinogenic effect; rather, it has hepatoprotective and anti-cancer activity [1,14,35].

The hepatoprotective property of embelin is explained by its role in lowering elevated liver enzymes and boosting protein and albumin concentrations during the carbon tetrachloride-induced hepatotoxicity test [6]. The same study signified the hepatoprotective role of embelin through histopathological findings in such a way that it reduced hepatic cord dilation and minimized the inflammatory response, accompanied by lowering mononuclear cellular infiltration. The anti-cancer activity of embelin is suggested based on its potency in inhibiting cell growth, inducing apoptosis, and activating caspase-9 in BIR3 domains of XIAP, inhibiting programmed cell death, especially during prostate cancer [36].

The *in silico* output also indicated that embelin has an immunotoxic property. Similar reports stated that plant-based natural products have the potential to possess an immunotoxicity effect [37–40]. This might be due to the possible effect of embelin on immune regulatory molecules such as PD-1 (programmed cell death 1), CTLA-4 (cytotoxic T-lymphocyte antigen 4), and galectins since there are evidences showing that medicinal plants have a potential of immune checkpoints inhibition [41,42]. On the contrary, there are medicinal plants with immunostimulant activity that make them a potential treatment option against immunotoxic agents [43]. Hence, further *in vivo* and *in vitro* studies need to be conducted to validate and further understand the effect of embelin on the immune system. Another *in silico* finding from the current study is the predicted inhibitory effect of embelin against the CYP2D6 enzyme, an important polymorphic enzyme that serves as a catalyst for the metabolism of various drugs [44]. In this regard, various reports emerged showing that both medicinal plant extracts and conventional drugs exhibit similar inhibitory effects against the CYP2D6 enzyme [45–47]. Regarding the cardiac toxicity potential of embelin, hERG channel (K^+ channel facilitating the cardiac conducting system) inhibition is considered a key indicator [48,49]. In this respect, the current *in silico* prediction reveals that embelin has no inhibitory effect on hERG channels. This is a good indication of a higher safety margin for *Embelia* extract to be free of cardiac toxicity effects as compared to *in silico* and *in vitro* evaluations of some medicinal plant extracts and chemicals that were found to have an inhibitory potential against hERG channels and hence be cardiotoxic [50,51].

The acute oral toxicity test of embelin isolated from the fruit part of *E. schimperi* showed no recorded mortality and no observed signs of toxicity for two weeks on female rats treated with a single dose of 5000 mg/kg. Thus, the mean lethal dose (LD_{50}) is determined to be above 5000 mg/kg. This result indicated that the compound had higher margin of safety in rats during the acute oral toxicity test. This finding is in agreement with reports from animal studies that demonstrated that embelin is safe when given acutely at higher doses [12].

In the present study, developmental toxicity of embelin was evaluated based on the effect of the plant isolate during the early pregnancy period (Day 12 of gestational age) and the late pregnancy period (Day 20 of gestational age). During both early and late developmental toxicity experiments, embelin did not exhibit any observable signs of toxicity, including abortion and maternal death, for the whole duration of the treatment period. This might indicate that embelin is a non-abortifacient yet tolerable compound for pregnant rats, even at a higher dose of 1000 mg/kg. In line with this, a previous study revealed that administration of an 80% ethanol extract of *E. schimperi* vatke fruits elicited neither maternal mortality nor abortion [26]. However, there should be no misconception that medicinal plants are non-abortifacient because there have been reports that medicinal plants may have abortifacient capacity *via* various mechanisms such as inducing utrine contraction, damaging placental tissue, causing multiple organ damage, and death [52–54].

Early pregnancy developmental toxicity was assessed based on morphological score, number of somites, and embryonic crown-rump

length, in addition to the extent of developmental delay in the embryonic circulatory, musculoskeletal, and nervous systems. In this regard, the current study found no statistically significant difference in the mean of morphological scores or the number of somites. Furthermore, none of the embryonic systems exhibited delayed development. However, the crown-rump length of embryos from the high dose group is significantly shorter than their counterparts from vehicle and *ad libitum* controls. These findings might indicate that embelin is not an embryo-disrupting agent in rats, especially at low and middle doses. Yet, the decrement in crown-ramp length of embryos from the high dose group (1000 mg/kg) might be attributed to a transient effect of embelin in the stature of the embryo since such a difference was not appreciated in the CRL of near term rat fetuses. This might be due to the fact that outcomes from embelin treatment usually return to normal after withdrawing the treatment [12,13].

The current study revealed that there is a significant dose-dependent difference between embelin-treated animals and controls with respect to mean maternal food intake and weight gain in pregnant rats. The mean maternal food intake of all treatment groups was significantly higher than that of the control animals. This might be explained by the anthelmintic effect of embelin, which possibly harmonizes the gastrointestinal environment to increase their appetite [13,55,56]. On the other hand, rats in the treatment groups gained significantly less weight than those in the control group despite their high food intake. This might be attributed to the fact that animals in the treatment group had fewer pregnancies than those in the control group, which directly influenced the amount of weight gain measured since the more pregnancies, the more weight gained. Furthermore, embelin is reported to have a weight reduction effect in rats to the extent that it contributes to regulating metabolic disorders at a low dose of 50 mg/kg [57]. Correspondingly, the current study depicts that the number of implantations is significantly lower in the experimental groups than in the controls during the late pregnancy experiments. This could be due to embelin's effect on lowering sex hormone concentrations as well as the implantation process, which can increase the risk of resorption [58–60].

According to the current study, there are no significant treatment-related changes in the number of skeletal ossifications or external or visceral morphological alterations in near-term fetuses. This finding might be attributed to the resolved effect of embelin after the treatment period since the treatment period was only during the period of organogenesis [12].

9. Conclusions

Embelin is predicted to have a high probability of immunotoxicity potential and affect drug metabolism by inhibiting CYP2D6. In addition, it affects food intake, weight gain and the number of implantations in pregnant rats. Therefore, it is highly recommended not to take embelin and embelin-rich plants during pregnancy. Further *in vitro* and *in vivo* studies need to be conducted to understand the mechanism behind the toxicity of embelin.

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CRediT authorship contribution statement

Zelalem Animaw: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft. **Girma Seyoum**: Conceptualization, Supervision, Methodology, Validation, Writing – review & editing. **Kaleab Asres**: Supervision, Methodology, Resources, Validation, Writing – review & editing. **Abiy Abebe** and **Samson Taye**: Investigation, Methodology, Resources, Validation, Writing & review & editing.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2023.06.006.

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