https://doi.org/10.48048/tis.2024.7454

The Effect of *Cynometra cauliflora* Linn. Fruit Extract-Induced Congenital Anomalies on Chick Embryos

Manutsanun Santiparadon[1], Kolip Payanglee[1], On-Anong Somsap[2], Halawatee Pengmusaw[3], Kiflanee Seumae[3] and Suriyan Pintarasri[1,*]

[1]Anatomy Division, Faculty of Medicine, Princess of Naradhiwas University, Narathiwat 96000, Thailand
[2]Biochemistry Division, Faculty of Medicine, Princess of Naradhiwas University, Narathiwat 96000, Thailand
[3]Faculty of Medicine, Princess of Naradhiwas University, Narathiwat 96000, Thailand

([*]Corresponding author's e-mail: suriyan.p@pnu.ac.th)

Received: 1 September 2023, Revised: 27 September 2023, Accepted: 3 October 2023, Published: 5 February 2024

Abstract

Background: *Cynometra cauliflora* is a traditional folk medicine that is used to treat a variety of unpleasant symptoms without adverse effects. Previous studies have shown that *C. cauliflora* fruit relieving morning sickness and nausea in pregnant women. However, no research has been performed on the effect of *C. cauliflora* fruit extract on birth anomalies. Objective: In this study, we aim to investigate the effects of *C. cauliflora* fruit extract on developing chick embryos. Materials and Methods: Three hundred fertilized eggs were divided into 2 groups; 1) the experimental groups received various doses of *C. cauliflora* fruit extract at 50, 100, 200, and 400 mg/kg, and 2) the control group received a 0.9 % normal saline solution. One hundred and fifty eggs at embryonic day-3 (ED-3) were observed for LC_{50}, morphology, and histology, while the 150 eggs at embryonic day-6 (ED-6) were investigated for morphology and histology. Results: At a dosage of 291.70 mg/kg, the *C. cauliflora* fruit extract induced mortality in ED-3 by more than 50 %. Moreover, fruit extraction can also result in anomalies in ED-3 and ED-6, such as anterior neuropore openings, heart tubes, liver and kidney tissue defects. Conclusion: As a result, it was determined that tissue damage and organ malformation followed dose-dependent and all-or-none laws. Despite having a wide range of medical properties, *C. cauliflora* fruit extracts have been demonstrated to impair early growth and development and cause birth abnormalities in animal studies. This will allow further determination of regulatory criteria for medical use, particularly for pregnant women.

Keywords: *Cynometra cauliflora*, Birth defects, Chick embryo, Traditional medicine, Pregnant women

Introduction

Medicinal plants have long been used as alternative medicine [1] because they are inexpensive, accessible, haress, widely distributed throughout the tropics, and provide a variety of treatment effects [2,3]. Many natural products contain substances that have been developed to be the principal constituents in many commercially available modern medicines. For example, an extract from the bark of the Cinchona tree is used to produce quinine, a malaria medicine [4].

Cynometra cauliflora Linn., also known as Ma-priang in Thai, is a tropical plant found in Thailand's central and southern areas, eastern Malaysia, Indonesia, and India [3,5]. *C. cauliflora* is a tiny, densely branched perennial tree that grows to 5 m tall and has a rough, greyish-brown, sturdy trunk. The fresh fruit is juicy, sweet and tangy, and can be eaten fresh. It is a popular ingredient in fruit salads and compote, which is high in nutrients such as protein, fat, carbohydrates, fiber, vitamin A, and vitamin C [6,7].

C. cauliflora is a traditional folk medicine that is used to treat a variety of unpleasant symptoms [6]. Because of its phytochemical abilities, it can be utilized effectively in a variety of applications without any adverse effects [2]. Previous research has demonstrated that the extract of the roots, leaves, and fruit can be used to reduce inflammation; function as an antibiotic, antiviral, and antioxidant; eliminate toxins within cells; treat leukemia; and enhance appetite in patients with malnutrition. *C. cauliflora* has historically been used for diabetes by Malay folk, which can be explained by the presence of terpenoids that can control lower blood glucose levels, thereby reducing complications related to diabetes [8]. Furthermore, Indonesians who have diarrhea commonly consume *C. cauliflora* fruit juice. Because fruit juice is favored over other extraction techniques, it is simpler for those in the community to access than techniques like infusion or maceration [9]. Pregnant women also consume compote made from *C. cauliflora* fruits to ease

morning sickness and nausea in region along the Thai-Malaysia border. Wahab *et al.* (2018) reported that oil extracted from *C. cauliflora* seeds can be utilized in the treatment of skin disorders [2]. Furthermore, *C. cauliflora* fruit extract combined with vitexin, a phenolic glycoside medication, has been demonstrated to reduce adipose tissue and fatty liver development in rats [10]. Tannins, which are antibacterial and antiviral; terpenoids, which considerably stimulate wound healing; and flavonoids, which function as antioxidants to eliminate intracellular toxins, are all found in the phytochemical purity obtained from *C. cauliflora* fruit extract [2,11].

Despite having desirable antiviral activity against virus-infected cells, *C. cauliflora* fruit extract is considered to be averse to normal cells [12]. It has been reported that a concentration of 2.19 mg/mL of fruit extract can cause more than 50 % cell death (*EC50*) [2]. Furthermore, Anliza *et al.* (2002) reported that 100 ppm of *C. cauliflora* leaf extract caused more than 50 % of the shrimp embryos in their study to die [13]. However, even though compote is a common remedy for nausea and morning sickness in pregnant women, there has not been any study of the possible adverse effects of *C. cauliflora* during pregnancy. In order to determine whether *C. cauliflora* fruit extract has an impact on embryonic development, we investigated our hypothesis that it might cause embryonic abnormalities and increase the risk of mortality. As a result, this study was performed on chick embryos at various stages of development and organ formation in 3- and 6-day-old chick embryos exposed to *C. cauliflora* fruit extract in order to provide information on the usage of *C. cauliflora* fruit for alleviating uncomfortable pregnancy symptoms.

Materials and methods

Ethics statement

This research has been approved by Walailak University Institutional Animal Care and Use Committee (WU-IACUC), number WU-ACUC-65019.

Plant materials and sample preparation

The *C. cauliflora* fruit utilized in this study was obtained from a local market in Tak Bai District, Narathiwat Province, Thailand. The ethanol extraction protocol was modified from the protocol used in the study by Ong *et al.* [12]. The total of 1 kg of fruit is used; one fruit is average 28.26 ± 0.27 g. Fresh fruits with orange-yellow skin were washed in distilled water. The fruit pulp was then completely crushed after the seeds were removed. The crushed pulp was then dried for 5 days at 40 °C in an oven. The samples were soaked in 95 % ethanol for an additional 3 days, before being covered and stored in a shaded place until they had dried fully. The extracted sediment was then collected for further analysis after the 95 % ethanol solvent had been eliminated using a rotary evaporator set to 40 °C. In order to formulate the stock solution, 0.40 g of the extracted sediment was weighed, and then dissolved in 1 liter of 0.9 % saline. The *C. cauliflora* fruit extract was then further diluted with a 0.9 saline solution to make the working solution.

Chick embryo experiments

The freshly laid, fertilized Rhode Thai (*Gallus gallus domesticus*) eggs with an average weight of 0.45 ± 0.5 g, were obtained from Ban Rai Hatchery, Ban Phru Sub-district, Hat Yai District, Songkhla Province, Thailand. All eggs were wiped clean with distilled water and then incubated at a temperature of 37 ± 0.5 °C with 70 - 80 % humidity for 21 h. They were turned 180 degrees 3 times per day to prevent the embryos from attaching to the eggshell. The experiments involved delivering a single injection of 100 µL soluble agent into the fertilized eggs after 21 h of incubation using the ovo-injection method [14]. Three hundred fertilized eggs were divided into 2 groups. The aim of using the ED-3 (n = 150) was to study the LC_{50}, morphology, and histology, while the aim of using the ED-6 (n = 150) was to study the morphology and histology (**Figure 1**).

Determining fifty percent lethal concentrations (LC_{50})

In this study, the LC_{50} of the *C. cauliflora* fruit extract was determined by observing the mortality rate of 3-day-old embryos. After 0.9 % normal saline solution was injected into the control groups, and various dosages of *C. cauliflora* fruit extract (50, 100, 200, and 400 mg/kg) were injected into the experiment groups, all of the eggs were incubated at 38 °C for 3 days, and turned 180 degrees 3 times per day to prevent the embryos from attaching to the eggshell. Then the eggs were opened, and the chick embryos were examined and the mortality rate recorded. An embryo was recorded as having died if a heartbeat could not be observed by the naked eye, while surviving embryos were harvested for assessment in other experiments.

The morphological study

The whole bodies of 5 randomly-selected ED-3 from each group were stained with Mayer's Carmine for 30 min, dehydrated with a graded series of alcohol for 20 min for each change, cleared with xylene for 30 min, mounted by using Canada balsam (Panreac, Spain), and then observed under a light microscope (Olympus BX53, Japan) and photographed. In addition, the whole bodies of 5 randomly-selected ED-6 were photographed.

The histological study

The ED-3 and ED-6 were fixed with Dietrich's FAA fixative, processed, and embedded in paraffin wax. The paraffin block was sectioned at 4 - 6 µM. The tissue sections of each group were stained with hematoxylin and eosin (H&E), observed under a light microscope (Olympus BX53, Japan), and photographed.

Statistical analysis

The data were analyzed using Prism software version 8. All data were expressed as the mean ± SD. Differences in the measured parameters in the different studied groups were tested using the One-Way ANOVA test. Significance was considered at $p < 0.05$.

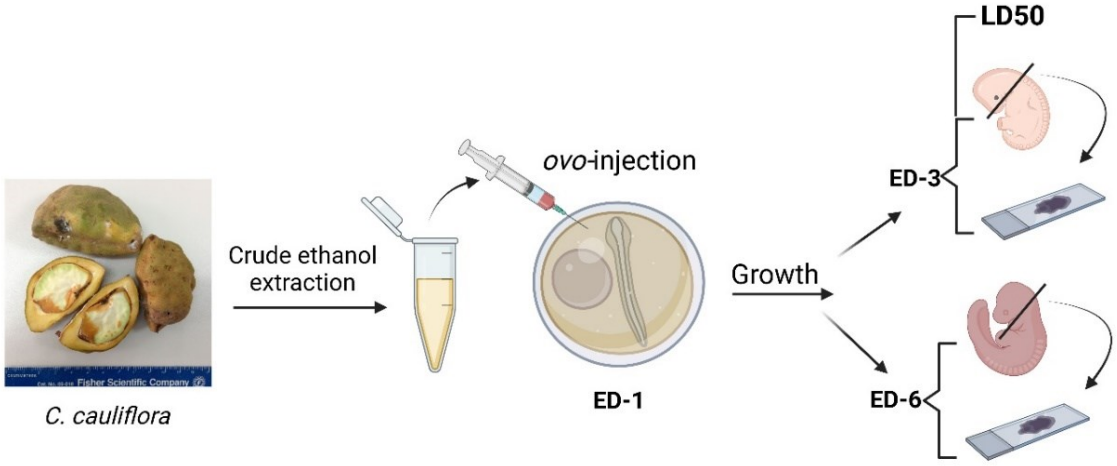

Figure 1 The schismatic diagram of the experimental design shows the details of the experiments. The diagram shows the raw material, the chemical agent that was injected into each group, and how the aim of using ED-3 and ED-6 was to study the morphology and histology. (Created with BioRender.com).

Results and discussion

Determining fifty-percent lethal concentrations (LC_{50}) of the *C. cauliflora* fruit extract

In this study, the toxicity of *C. cauliflora* fruit extract was investigated on 3-day-old embryos. The control group had a 0.00 % mortality rate, whilthe lowest *C. cauliflora* fruit extract concentration, at 50 mg/kg, resulted in a 5.94 ± 0.15 % mortality rate, whereas the 100 mg/kg concentration led to a 25.77 ± 1.01 % mortality rate, and the 200 mg/kg concentration resulted in a 32.31 ± 0.84 % mortality rate. The highest dose used in the study was 400 mg/kg, which led to a mortality rate of over 50 % (67.57 ± 1.54), as shown in **Figure 2**. These results showed a significant difference between groups at $p < 0.0001$.

The LC_{50} in this experiment is 291.70 mg/kg, which can be calculated from the linear regression equation by probit analysis using MS-Excel as shown in the graph in **Figure 2**. These results indicate that *C. cauliflora* fruit extract induced toxicity, in a dose-dependent manner, leading to a high rate of mortality when the dose of *C. cauliflora* fruit extract concentration was increased on day 3 after administration.

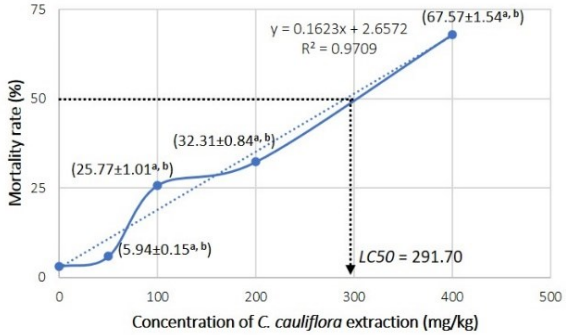

Figure 2 The graph shows the LC_{50} of *C. cauliflora* fruit extract on ED-3 of the chick embryos experiment. The numbers in the graph demonstrate the percentage of chick embryonic deaths and an SD of n = 30. [a]There was a significant difference between the *C. cauliflora* fruit extract group and the control group ($p < 0.0001$). [b]There was a significant difference between the *C. cauliflora* fruit extract group ($p < 0.0001$).

***C. cauliflora* fruit extract induced the risk of retardation and organ malformation in ED-3**

We further explored the effects of *C. cauliflora* fruit extract on growth retardation and organ malformation. The results show that *C. cauliflora* fruit extract induced congenital malformation by delaying growth and organ formation, which was observed in both external morphology and histology. The chick embryos showed developmental disruptions followed by growth retardation, which reduced the development of the eyes and delayed the formation of the limb buds (**Figure 3(A)**). The head malformation was caused by microcephaly and the anterior neuropore opening; this was found in embryos which had received doses of 200 and 400 mg/kg (13.34 ± 0.2, and 20.00 ± 0.4 %, respectively), while the absence of eye primordia was found in embryos which had received doses of 400 mg/kg (20.00 ± 0.4 %). The heart tube was irregular and U-shaped with a dilated lumen, as found in embryos which had received doses of 100, 200, and 400 mg/kg (13.34 ± 0.1, 13.34 ± 0.2 and 20.00 ± 0.4 %, respectively). Overall, *C. cauliflora* fruit extract showed statistically significant adverse effects leading to embryo retardation and malformation when compared to the control group ($p < 0.05$) (**Figures 3(A) - 3(B)**).

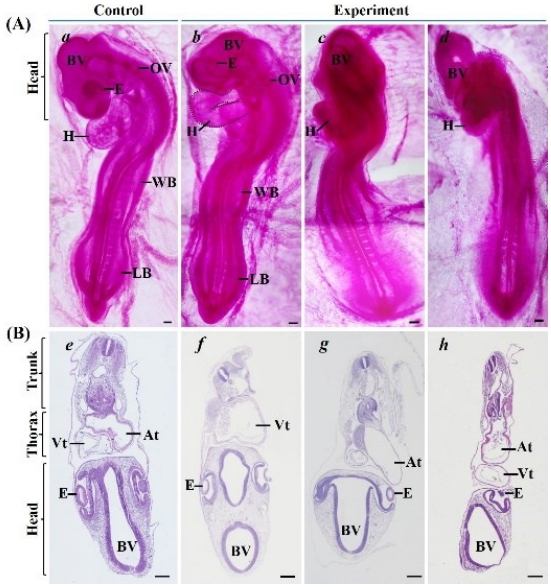

Figure 3 Photographs showing the whole body of surviving ED-3 in the dextrodorsal view. The pictures include Mayer camalum staining (A), and the micrograph tissue section stained with H&E (B). The normal embryos, in both whole body and sections, are indicated in photos *a* and *e* as follows: Brain vesicles prominence (BV), eye (E), otic vesicle (OV), heart (H) and heart chamber; atrium (At) and ventricle (Vt), and extremity; wing buds (WB) and leg buds (LB). The embryos from the experiment group showed microphthalmia on photos (*b*) and (*f* - *h*). An absence of extremities can be seen in photos (*c* - *d*). Moreover, heart loop dilation is visible in photos (*f* - *h*). The pictures were captured under a light microscope at 1.25x magnification, scale bar = 200 µM.

***C. cauliflora* fruit extract induced the risk of retardation and organ malformation in ED-6**

Retardation and organ malformation in ED-6 was observed in both external morphology and histology. As a result, the *C. cauliflora* fruit extract group had incidences of brain malformation, which was found in embryos which had received doses of 200 and 400 mg/kg (6.67 ± 0.2, and 6.67 ± 0.2 %, respectively), as shown in **Figures 4(c) - 4(d)**. Overall, *C. cauliflora* fruit extract showed statistically significant adverse effects leading to embryo retardation and malformation when compared to the control group ($p < 0.05$). The tissue sections from the 200 and 400 mg/kg experiment groups confirmed that *C. cauliflora* fruit extract induced brain malformation in the form of a narrowing of the brain vesicle as well as irregularities in the shape and unidentified portions, as seen in **Figures 5(b) - 5(c)**. The heart tissue showed dilation of the heart chamber, especially on the atrium, and the heart walls were thinner, as seen in **Figures 5(e) - 5(f)**. Moreover, the liver tissues were slightly dilated with a large venous space and sinusoidal lumen, and reduced hepatic cord generation, as seen in **Figures 6(b) - 6(c)** and **6(e) - 6(f)**. The kidney tissues showed tubular and vascular dilation, the malformation of glomerulus, and an increase in the Bowman's space, as seen in **Figures 6(h) - 6(i)**.

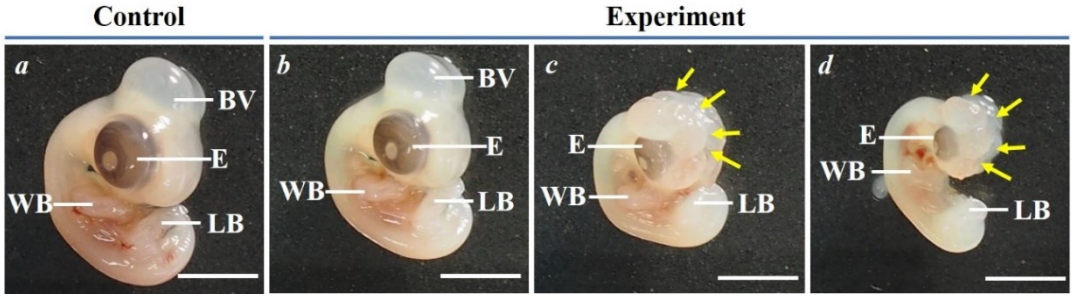

Figure 4 Photographs showing the whole body of ED-6 in control *a* and the experimental groups with concentrations of *C. cauliflora* fruit extract at 100 (*b*), 200 (*c*) and 400 mg/kg (*d*). Normal embryos from the control group showed brain vesicles (BV), a vesicular eye (E) and extremity; wing buds (WB) and leg buds (LB). The experimental group showed brain vesicles opened (yellow arrow) with microphthalmia and retardation (*c* - *d*). The pictures were captured under a digital camara, scale bar = 5 mm.

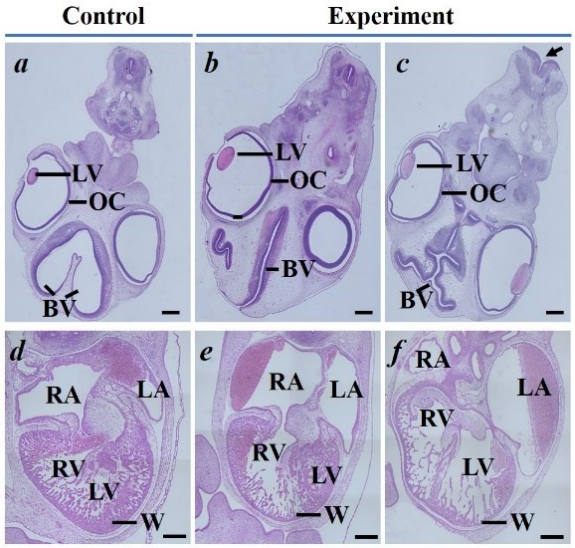

Figure 5 Photographs of the head and heart tissue sections with H&E staining of ED-6. Tissues from the control group (*a*) showed the formation of brain vesicles (BV) and a vesicular eye composed of a lens vesicle (LV) and optic cup (OC). Photograph (*d*) is a normal heart formation including the 4 heart chambers, right and left atrium (RA and LA) and right and left ventricle (RV and LV), and the thickening of the heart wall (W). The tissue sections from the experiment groups (*b* - *c*) show brain malformation such as narrowed brain vesicles, irregularities in the shaped and unidentified portions as well as neural tube unclosed (black arrow). The heart tissues (*e* - *f*) show dilation of the heart chamber, especially of the atrium, and a thinning of the heart walls. The pictures were captured under a light microscope, scale bar = 500 μM.

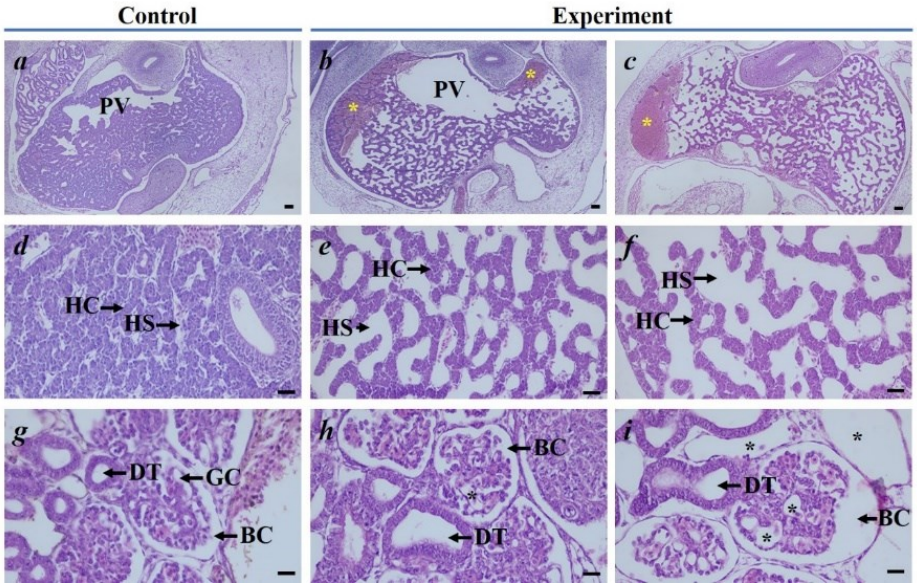

Figure 6 Photographs showing the liver and kidney tissue section with H&E staining of ED-6 in the control and experiment groups. The liver tissues from the control group (*a* and *d*) demonstrate the thickening of liver tissues, acini and lobules. The hepatic cords (HC) are mostly one or 2 layers thick and are divided by sinusoid (HS). Furthermore, on the liver lobe, the large space of the venosus system called portal vein (PV) can be observed. The experiment groups (*b* - *c* and *e* - *f*) demonstrated a severity of vascular dilatation; it presented a large space on PV, dilated a sinusoidal lumen, and reduced HC generation and blood congestion (yellow asterisk). The kidney tissues from the control group (*g*) demonstrated the normal formation of renal corpuscles; glomerular capillaries (GC) and Bowman's spaces (BS), and distal convoluted tubules (DT). Conversely, the experiment groups (*h* and *i*) demonstrated the dilation and malformation of tubular and vascular structures. The renal corpuscle showed GC dilating (black asterisk) and increases in the Bowman's space (BS). Moreover, the afferent and efferent vessels around the renal corpuscle showed dilation in a lumen as well (black asterisk). The pictures were captured under a light microscope, scale bar = 100 µm.

Discussions

C. cauliflora has been used as a medicinal plant to treat illness because it has a wide range of medicinal properties and can treat a variety of symptoms with no side effects [2]. The treatment effects depend significantly on the parts of the plant used, such as the root, leaf, fruit, or seeds. Previous reports revealed that the fruit extracts mainly consist of terpenoids (sesquiterpenoids and monoterpenoids) and fatty acids [3], which can eliminate intracellular toxins [2,15]. However, no research has been carried out on the adverse effects of *C. cauliflora* extract that may occur when it is used to treat illness among pregnant women.

We subsequently evaluated the *C. cauliflora* fruit extract in accordance with the toxicity of the ethanol extraction solvent, and it revealed no toxicity to either exogenous or tissue samples. However, the study's findings demonstrate that when delivered at a concentration of 291.70 mg/kg, the *C. cauliflora* fruit extract induced mortality in more than 50 % of ED-3 cases. This is consistent with the research by Wahab *et al.* (2018), who investigated the toxicity of *C. cauliflora* fruit extract on Hela cells, and the work by Anliza *et al.* (2021), who reported that *C. cauliflora* fruit extract at a concentration of 100 ppm was hazardous to shrimp larvae [2,13]. Our results indicate that even the least fatal dose of *C. cauliflora* fruit extract of 50 mg/kg was still hazardous to chick embryos, while increasing the extract concentration led to a dose-dependent phenomenon and more intense embryo mortality. According to the study mentioned above, it can be concluded that *C. cauliflora* fruit extract is harmful to the embryos even at doses lower than the LD_{50} value.

The findings also revealed that even in the embryos that survived, there were abnormalities, which were manifest in a variety of time- and dose-dependent results in an all-or-non-law fashion during the early stages of embryonic growth and development. As a result, the embryos developed abnormally in their adulthood. The defects that can be observed in the nervous system and cranial structure, such as anterior neuropore openings, are evident during ED-3. As a result, the brain vesicles opened, and the brain tissues

were exposed to the outside, as clearly seen in ED-6. Additionally, these defects have been associated with aberrant eyeball development, leading the eyeball to become smaller as the embryo develops. Heart failure is another typical abnormality. It was discovered that the heart tube had dilated during the ED-3 stage. It was also observed during the ED-6 stage and was clearly presented along with thinned heart walls. *C. cauliflora* fruit extract induced abnormalities in the liver and kidney tissues. The results of this study show that the extract from the *C. cauliflora* fruit caused the dilation of blood vessels in the liver and kidney tissues. There was congestion of blood in the tissues and vascular lumen, along with a significant decrease in the number of hepatic cells.

Terpenoids, which were exhibited in the *C. cauliflora* fruit extracts, are the most likely cause of anomalies at the organ and tissue level which led to organs like the liver, kidney, and neurological tissues being more severely impacted than other organs. The induction of toxicity and oxidative stress in the tissues and cells by terpenoids results in plasma membrane disruption, which causes an increase in lipid peroxidation, ROS generation, and mitochondrial impairment in the developing embryos during maturation and cell division. This is consistent with other herbal extracts such as ginger [16], essential oils [17], cranberry, chamomile, and garlic [18]. This also causes the embryos to grow more slowly than usual.

Additionally, the existence of the vast majority of congenital defects during embryogenesis is also not fully understood. There are no appropriate animal models, nor have there been any human embryos that demonstrate specific abnormalities at different developmental stages [19]. In our study of *C. cauliflora*-treated chick embryos, all congenital malformations that occurred were consistent with teratogenesis. The study has an advantage because there are few studies that discuss the adverse effects of *C. cauliflora in vivo*. Furthermore, chick embryos treated according to our experimental protocol might therefore be good models for these abnormalities.

Conclusions

Although the *C. cauliflora* has a wide range of medicinal properties and there are no reports of adverse effects from therapeutic use in humans, the results of this study demonstrate that *C. cauliflora* fruit extract was able to induce disorientation in animal models. The induction of birth defects depends not only on the constituents of the extract used, but also on varying time-and dose-dependent effects. Despite the absence of evidence for a study on the induction of birth defects in humans, the findings from studies on animals that closely resemble humans demonstrate that *C. cauliflora* fruit extract can cause birth defects. Therefore, more studies on the mode of action and metabolism are needed. We suggest studying the toxicity in the offspring of mammal models to determine the organ and cellular toxicology, which is closely related to humans. This will allow further determination of regulatory criteria for medical use, especially among pregnant women.

Acknowledgements

We would like to thank the Ban Rai Hatchery for the freshly laid Rhode Thai fertilized egg. The facilities are supported by the Research Unit, Faculty of Medicine, Princess of Naradhiwas University, Thailand.

Acknowledgments

This research was funded by the Medical Research Fund 2022 from the Faculty of Medicine, Princess of Naradhiwas University, Thailand.

References

[1] BB Petrovska. Historical review of medicinal plants' usage. *Phcog. Rev.* 2012; **6**, 1-5.
[2] NZA Wahab, A Azizul, N Badya and N Ibrahim. Antiviral activity of an extract from leaves of the tropical plant Cynometra cauliflora. *Phcog. J.* 2021; **13**, 752-7.
[3] S Sabiha, R Serrano, K Hasan, IBMD Silva, J Rocha, N Islam and O Silva. The genus cynometra: A review of ethnomedicine, chemical, and biological data. *Plants* 2022; **11**, 3504.
[4] G Gachelin, P Garner, E Ferroni, U Tröhler and I Chalmers. Evaluating Cinchona bark and quinine for treating and preventing malaria. *J. Roy. Soc. Med.* 2017; **110**, 31-40.
[5] BA Samling, Z Assim, WY Tong, CR Leong, SA Rashid, NNSNM Kamal and WN Tan. Cynometra cauliflora L.: An indigenous tropical fruit tree in Malaysia bearing essential oils and their biological activities. *Arabian J. Chem.* 2021; **14**, 103302.

[6] SAT T-Johari, N Mat, AB Siti-Aishah, AAM Yusran, A Alwi and AM Ali. Cytotoxicity, antiproliferative effects, and apoptosis induction of methanolic extract of Cynometra cauliflora Linn. whole fruit on human promyelocytic leukemia HL-60 cells. *Evid. Base. Compl. Alternative Med.* 2012; **2012**, 127373.
[7] TK Lim. *Edible medicinal and non-medicinal plants*. Springer Dordrecht, Dordrecht, Netherlands, 2012.
[8] A Aziz, A Farina and I Mohammad. Antioxidant activity and phytochemical composition of Cynometra cauliflora. *J. Exp. Integr. Med.* 2013; **3**, 337-41.
[9] Nurbidayah, Nafila, N Amalia. Antibacterial activity of Namnam fruit juice from South Kalimantan against staphylococcus aureus and Escherichia coli. *J. Vocat. Health Stud.* 2023; **7**, 48-54.
[10] A Seyedan, Z Mohamed, MA Alshagga, S Koosha and MA Alshawsh. Cynometra cauliflora Linn. Attenuates metabolic abnormalities in high-fat diet-induced obese mice. *J. Ethnopharmacol.* 2019; **236**, 173-82.
[11] MA Ado, A Mediani, ISM Ismail, HM Ghazali and F Abas. Flavonoids from Cynometra cauliflora and their antioxidant, α-glucosidase, and cholinesterase inhibitory activities. *Chem. Nat.* 2019; **55**, 112-4.
[12] CW Ong, YS Chan, KS Khoo, HC Ong and NW Sit. Antifungal and cytotoxic activities of extracts obtained from underutilised edible tropical fruits. *Asian Pac. J. Trop. Biomed.* 2018; **8**, 313.
[13] S Anliza and N Rachmawati. Cytotoxic activity of ethanol extract in namnam leaves (cynometra cauliflora l.) to hela cell. *Walisongo J. Chem.* 2021; **4**, 107-12.
[14] J Roongruangchai, Y Viravud, V Plakornkul, K Sripaoraya, W Boonmark and K Roongruangchai. The teratogenic effects of monosodium glutamate (MSG) on the development of chick embryos. *Siriraj Med. J.* 2018; **70**, 514-22.
[15] KA Wojtunik-Kulesza. Toxicity of selected monoterpenes and essential oils rich in these compounds. *Molecules* 2022; **27**, 1716.
[16] N Bernstein, M Akram, Z Yaniv-Bachrach and M Daniyal. Is it safe to consume traditional medicinal plants during pregnancy? *Phytother. Res.* 2021; **35**, 1908-24.
[17] NS Dosoky and WN Setzer. Maternal reproductive toxicity of some essential oils and their constituents. *Int. J. Mol. Sci.* 2021; **22**, 2380.
[18] B Sarecka-Hujar and B Szulc-Musioł. Herbal medicines-are they effective and safe during pregnancy. *Pharmaceutics* 2022; **14**, 171.
[19] J Männer, W Seidl, F Heinicke and H Hesse. Teratogenic effects of suramin on the chick embryo. *Anat. Embryol.* 2003; **206**, 229-37.