https://doi.org/10.48048/tis.2024.8091

# Evaluation of Acute Toxicity of Ethanolic Extract from Fruit of *Cyanometra cauliflora* in ICR Mice

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*Received: 17 February 2024, Revised: 17 March 2024, Accepted: 24 March 2024, Published: 20 July 2024*

**Abstract**

This research aims to investigate the acute toxicity profile of the ethanolic extract derived from *Cyanometra cauliflora* fruit, a plant commonly found in Southeast Asia. Male ICR mice were divided into 5 groups and administered varying oral doses of *C. cauliflora* extract: 100, 200 and 400 mg/kg body weight. Subsequently, the effects on both histological and biochemical parameters were meticulously examined post-experimentation. The results indicated no significant alterations in blood glucose or blood urea nitrogen (BUN) levels attributable to *C. cauliflora* extract administration. Nevertheless, a noteworthy elevation in creatinine, aspartate transaminase (AST), and alkaline phosphatase (ALP) levels was observed across all groups treated with *C. cauliflora* extract in comparison to controls ($p < 0.05$). Intriguingly, the extract consistently elicited a reduction in triglyceride levels across all administered doses, while cholesterol levels exhibited a decline specifically at the 400 mg/kg body weight dose. Notably, the levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) remained unaltered throughout the experimentation. Histological assay suggested signs of hepatocellular damage, portal dilation and infiltration of inflammatory cells across all *C. cauliflora*-treated groups, alongside indications of kidney atrophy and other abnormalities.

These findings collectively suggest structural and functional modifications induced by *C. cauliflora* extract. In summary, while the administration of *C. cauliflora* extract did not exert significant changes in blood glucose and BUN levels, it did lead to heightened levels of creatinine and liver enzyme markers. Furthermore, liver and kidney damage were discerned at doses ranging from 100 to 400 mg/kg body weight, underscoring the imperative need to optimize extract concentrations to mitigate potential cytotoxic effects.

These findings can be further explored to understand the processes behind the observed effects and to assess potential therapeutic applications or safety considerations associated with the use of *C. cauliflora* extract in clinical settings.

**Keywords:** *Cyanometra cauliflora* L., Biochemical parameter, Histopathology, Kidney, Liver

## Introduction

There is a growing trend towards utilizing natural or organic plants and fruits for therapeutic purposes, driven by awareness of the potential adverse effects of synthetic products. Research indicates that therapeutic herbs may provide possible risks [1]. The World Health Organization (WHO) has recognized the significance of plant compounds used for medicinal purposes. Plant-derived secondary metabolites like flavonoids, alkaloids and glycosides are utilized in the treatment of infectious disorders [2]. Furthermore, numerous studies have indicated that certain plants produce harmful secondary compounds and are exposed to air pollution [3-6]. It is crucial to assess the safety of plant and fruit extracts by acute toxicity testing *in vivo* before using them in humans. Chemical hazard evaluation often uses acute toxicity studies to analyze the impact of various concentrations on lab animals and reduce hazards associated with chemical use. Assessment of acute oral toxicity measures the effects of a test material entering the body either once or multiple times during a 24-hour period on acute biological systems. Effects of plant or fruit extracts that lead to anomalies in the body or tissues, resulting in dysfunction of those tissues. Acute or sub-acute toxicity tests are utilized to assess the toxicity of both natural and manufactured compounds. Initial phase of toxicity testing for new drug research involves conducting acute toxicity tests administering either a single dose or several doses and monitoring for acute poisoning symptoms for up to 14 days. Rats, mice or other rodents are commonly used in acute toxicity tests to determine the lethal dose ($LD_{50}$). The lethal dosage is the concentration of a drug or extract that resulted in the death of 50 % of animals in a test [7-10].

*Cyanometra cauliflora* is a member of the Fabaceae family that is widely distributed across Southeast Asia, Africa, America and Australia [11]. *C. cauliflora* is a lofty perennial plant that can reach a height of 3 - 5 m. The plant features robust stems, branches, blooms and kidney-shaped fruits. The bark has a gray-brown hue. The leaves are soft green color elliptical, acute tips, arranged in distinct pairs and resembling the symmetry of butterfly wings but young leaves are white or pink and droop downwards. The flowers are crowded together at the nodes of the stalk, white flower petals, pale pink sepals and brown scales cover the flower stalk. The fruit is flat and kidney-shaped with coarse surface. When immature, it has a brown-green hue, tart flavor akin but the mature fruit possesses a flavor that is both sweet and acidic. There is 1 flat and brown seed inside the fruit similar to a mango seed. Found in Thailand's southern and central regions, this species is commonly known as amphawa, but locally referred to as mapriang, nana, nang-ai, buranum or manga. In Malaysia, it is commonly known as nam-nam or katak puru-puru. The ripe fruit is consumed fresh or used in fruit salads. The bark and roots are utilized in the treatment of blood and cancer ailments. The oil derived from seeds was utilized for the treatment of skin ailments [11,12]. The essential oil (EO) extracted from the leaf, twig and fruit of *C. cauliflora* shown antioxidant and antibacterial properties, as well as action against human breast cancer MCF-7 cells [13]. The Namnam leaf extract exhibited anti-

proliferative action against HeLa cancer cells at a dosage of 25 μg/mL [14]. The leaf extract has demonstrated antiviral activity against HSV-1 and cytotoxic effects on promyelocytic leukemia HL-60 cells, as reported in previous study [15]. Additionally, it demonstrated efficacy against both gram-negative bacteria and gram-positive bacteria, including *Escherichia coli* and *Staphylococcus aureus*. Research has shown that the fruit extract of this plant has antibacterial properties, specifically by inhibiting the growth of bacteria resistant to MRSA. The *C. cauliflora* flower extract showed antioxidant properties and exhibited action against gram-positive bacteria including *Staphylococcus aureus* (TISTR517), *Micrococcus luteus* (TISTR884) and Methicillin-Resistant *Staphylococcus aureus* (MRSA 142) [13,16-18].

Chemical analysis has detected saponins, tannins and flavonoids in all parts of the plant except for the leaves and fruits. Terpenoids are exclusively present in the leaves and fruits. Cardiac glycosides can be found in both mature and immature leaves [17]. The fruit of *C. cauliflora* contains many chemical constituents including protein, fat, fiber, carbohydrate, ascorbic acid, calcium, sodium, flavonoid and phenolic compounds. Particularly the phenolic component present in numerous fruits and vegetables. The fruit extract of *C. cauliflora* exhibited a high concentration of phenolic compounds, strong antioxidant properties and demonstrated effectiveness against leukemia [18]. Additionally, *C. cauliflora* fruit extract exhibited anti-lipase properties [19]. Despite the widespread popularity and usage of *C. cauliflora* fruit extract in Narathiwat Province, Thailand, there is a lack of studies assessing its toxicity in folk medicine. This study aims to investigate the acute toxic effects of *C. cauliflora* fruit extract on mice to assess its safety for potential human usage.

## Materials and methods

### Chemical and reagents

Absolute ethanol was purchased from Merck. Hematoxylin, eosin dye and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich while PBS was purchased from Gibco.

### Preparation of *Cyanometra cauliflora* L. extract

This preparation was conducted by following the method described by Somsap *et al.* [16] with slight modification. The fruit of *C. cauliflora* was harvested from Takbai district, Narathiwat province, Thailand, in April 2020 (BKF no. 196498). They were peeled, and the fruit pulp was cut into thin strips before being extracted with 95 % ethanol (ratio 1:4) over 3 days and repeated twice by the maceration procedure. Cotton cloth and Whatman No. 1 paper were used to filter the extract. The filtrate was then evaporated using a rotary evaporator (Heidolph, Hei-VAP Precision, Germany) and allowed to cool for 3 days at room temperature. The *C. cauliflora* crude extract was brown in color and kept at room temperature until use.

### Laboratory animals

Twenty-five male ICR mice, weighing ranges of 25 to 35 g, were purchased from Nomura Siam International Co. Ltd., located in Bangkok, Thailand. All mice were acclimatized for 7 days prior to being housed and normally fed at the animal laboratory, the unit of the research institute for health sciences,

Walailak University. The mice were randomly assigned to 5 groups: Control and test groups. The subjects were accommodated in a chamber that followed a 12-hour cycle of light and darkness, while maintaining a consistent temperature between 22 to 25 °C with relative humidity range of 50 - 55 %. Prior to implementation, all methods carried out in this study obtained approval from the Ethical Committee of Walailak University (WU-AICUC-63007).

**Acute toxicity**

The assessment of acute toxicity accorded the guidelines that was developed by the Organization for Economic Co-operation and Development (OECD) 420 for acute toxicity testing [20]. Mice were categorized into 5 groups using Federer's formula [21] as shown in **Table 1**. Monitoring occurred at hourly intervals for 24 h, daily for 14 days. After a period of experimentation, all surviving mice were anesthetized with sodium pentobarbital. Mice blood samples were collected for biochemical analysis. Both tissue of Liver and kidney samples were dissected, washed with saline and soaked in a 10 % formalin solution and then analyzed by histological method.

**Table 1** Groups of animal tests were categorized following Federer's formula.

| No. | Category | Description of Diet/Diet plus supplement |
|---|---|---|
| 1 | Control | Normal diet |
| 2 | Vehicle | Normal diet supplemented with 2 % DMSO (Dimethyl sulfoxide) in PBS (phosphate buffered saline) |
| 3 | 100 mg/kg BW | Normal diet supplemented with a daily single oral dose of *C. cauliflora* crude extract at 100 mg/kg BW |
| 4 | 200 mg/kg BW | Normal diet supplemented with a daily single oral dose of *C. cauliflora* crude extract at 200 mg/kg BW |
| 5 | 400 mg/kg BW | Normal diet supplemented with a daily single oral dose of *C. cauliflora* crude extract at 400 mg/kg BW |

**Biochemical analysis**

Blood samples from each group were kept in tubes without an anti-coagulant agent. Following collection, the samples were centrifugated at 13,000 g for 30 min, and the resulting serum was stored at −4 °C. A range of biochemical parameters, including lipid profile, blood glucose, liver marker enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine transaminase (ALT), creatinine, and blood urea nitrogen (BUN) were assessed using a clinical chemistry the MINDRY BS-400 fully automated blood chemistry analyzer.

### Histopathological analysis

After being submerged in a 10 % formalin solution, samples of liver and kidney tissue were prepared for embedding using the parafilm technique. Once embedded, the tissue was sliced into thin 5-micron sections using a microtome. These sections were subsequently stained by using hematoxylin and eosin dye and examined under a light microscope.

### Statistical analysis

All experimental results were presented as mean ± standard deviation (S.D.) using an analytical software program. Data analysis was conducted utilizing one-way ANOVA, with a significance level set at $p < 0.05$, to compare between the control group and the test group. The statistical analysis was performed using IBM SPSS Statistics Standard Version 29.0.1.0.

## Results and discussion

### Oral acute toxicity

In this study an acute toxicity test was conducted following the standards described by [20]. The criteria for OECD 420 fixed dose of 5, 50, 300 and 2,000 mg/kg but this study is the first time to test the toxicity of *C.cauliflora* fruit extracts in mice. So, concentration of 100, 200 and 400 mg/kg were selected to test an acute toxicity. Acute toxicity is defined as the harmful effects caused by a single dose administered during a 24-hour period. The acute toxicity test was conducted using *C. cauliflora* crude extract at doses of 100, 200 and 400 mg/mL to assess biochemical parameters and histopathology of the liver and kidney. The study did not show any mortality but did disclose alterations in histopathology when examined under a light microscope, as well as a loss of tissue function based on calculated biochemical parameters. These findings indicate that the $LD_{50}$ of *C. cauliflora* crude extract is greater than 400 mg/kg. Following the OECD guidelines for the Globally Harmonized Classification System (GHS), the *C. cauliflora* crude extract can be categorized as non-toxic under class 1 substances. Furthermore, the ethanolic extract from the leaves of *Pericampylus glaucus* (Lam.) Merr at doses of 2,000 and 4,000 mg/kg did not result in mortality but substantial behavioral changes such as drowsiness, sedation and lethargy [22].

Furthermore, the acute toxicity test of *C. fistula* fruit (CFE) extract at a concentration of 5,000 mg/kg did not show any signs of toxicity or fatality. The extract consumed more than 5,000 mg/kg, which is the $LD_{50}$ value. In the sub-acute test, no changes were observed in histology or morphology, and no mortality was reported. The biochemical parameter analysis did not show a significant difference when compared to the control group [23]. Conversely, the methanolic extract of *Carissa edulis* Vahl did not exhibit any detrimental effects on animals during an acute toxicity test, since it did not cause death or show any indicators of toxicity throughout the study period [24]. Our study revealed that the quantities of *C. cauliflora* crude extract did not have a significant effect on lowering blood glucose levels in the experimental group as compared to both the control and vehicle groups (**Figure 1**).

These findings suggest that the concentration employed may be unsuitable for lowering blood glucose levels. Pomelo extract given at doses of 250 - 500 mg/kg BW showed results similar to those seen in the

control group. At a dosage of 1,000 mg/kg BW, a significant reduction in blood glucose levels was noted compared to the control group, with statistical significance at $p < 0.05$ [25]. The methanolic extract of *Ricinus comminus* seeds has demonstrated the ability to reduce blood glucose levels in diabetic rats [26]. Simultaneously, extracts from *Picralima nitida*, *Nauclea latifolia* and *Oxytenanthera abyssinica* have been found to lower blood glucose levels in diabetic pregnant rats [27]. *Becium grandiflorum* extract at doses of 200, 400 and 600 mg/kg, as well as glibenclamide at 5 mg/kg, lowered blood glucose levels compared to the control group [28]. Moreover, *Momordica dioica* roxb. ethanolic fruit extract at concentration of 10 mL/kg BW reduced serum glucose in type2 diabetic rat and phytochemical in this extract presence with saponin, alkaloid, flavonoid, phenol and terpenoid [29]. Besides, *Piliostigma thonningii* ethanolic stem bark extract showed presence of alkaloids, flavonoids, saponins, tannins, glycosides and terpenoids. The extract at concentration of 500 mg/kg BW caused a 55.3 % reduction in blood glucose of the experimental animals over the treatment period compared with glibenclamide [30]. The mechanism of quercetin, bioactive compound or phytochemical in plant increasing insulin sensitivity [31] and many research using quercetin as standard for determine the total flavonoid content in extract.

Additionally, the hydro-methanol extract from *Heteromorpha arborescens* leaves at a dosage of 400 mg/kg reduced blood glucose levels in normoglycemic mice significantly ($p < 0.01$) [32]. A water-based leaf extract of *Rubus erlangeri* Engl (Rosacea) at concentration ranges of 100 - 400 mg/kg decreased blood glucose levels in mice [33]. Furthermore, 2 mL of aqueous extracts from *Stevia rebaudiana* leaves have been shown to reduce blood glucose levels while fasting [34]. In the future, if there is a requirement to research a high concentration dose of 1,000 mg/kg of *C. cauliflora* crude extract following the method in reference [25] to assess its impact on reducing blood glucose levels, it is important to be aware that this dose may have adverse effects that could injure the organ tissue of the test animals. From many researches it concluded that phytochemicals in plant can be extracted by both ethanol and methanol solvents but methanolic extract showed high polar than ethanolic extract, while the different of phytochemicals and biological properties depended on part of plant. Moreover, some research using water and other solvent such as butanol, acetone and chloroform for the extraction process [35,36].

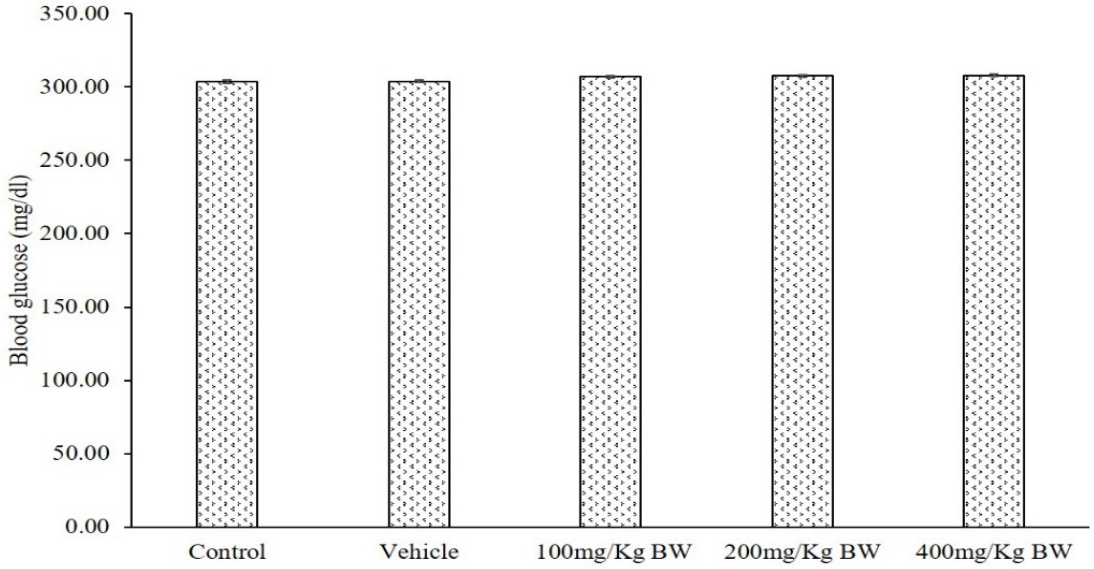


**Figure 1** The impact of *C. cauliflora* crude extract on the blood glucose levels in ICR male mice. Results are presented as mean ± standard deviation (n = 5).

When analyzing the BUN and creatinine levels in *C. cauliflora* crude extract at concentrations between 100 - 400 mg/kg BW, it was noted that all concentrations did not show any noticeable impact on lowering BUN levels compared to the control and vehicle groups (**Figure 2**). *C. cauliflora* crude extract at all concentrations caused a substantially different response in serum creatinine levels compared to the control and vehicle groups. Impaired kidney function was observed after exposure to *C. cauliflora* crude extract, leading to increased levels of creatinine in the blood compared to the control and vehicle groups (**Figure 3**). The results indicate that the concentration of *C. cauliflora* crude extract used in this investigation was unsuitable as it had adverse effects on the kidneys, as seen in **Figure 7**. The methanolic extracts from pomelo fruit at 1,000 mg/kg and ethanolic extract of *Moringa oleifera* leaf at 250 and 250 mg/kg were discovered to notably reduce BUN and creatinine levels when compared to the control group. The positive result is due to the non-toxic properties of pomelo fruit and *M. oleifera* leaf extracts on liver and kidney cells, enabling optimal functioning of these organs and their systems [25,37]. Additionally, the whole mushroom ethanolic extract of *Boletus griseipurpureus* Corner at 2,000 mg/kg showed no toxicity to mice as indicated by the similar BUN and creatinine values compared to the control group [38].

Additionally, the aqueous extract of Moroccan *Ferula communis* fruit at concentration dose of 500 mg/kg had no impact on the kidneys, and there was no significant change in creatinine levels compared to the control group [39]. A study on the toxicity of natural plant extracts found that analyzing biochemical parameters can reveal normal or abnormal kidney function. Elevated levels of urea or creatinine in the serum may indicate a decrease in the ability of renal tubules and glomeruli to excrete these substances [40]. The biochemical parameters for renal function tests, such as BUN and creatinine levels in the serum, show the efficiency of the glomerular filtration rate [41]. Furthermore, in a sub-acute test, the ethanolic extract

from the leaves of *P. glaucus* (Lam.) Merr was found to be non-toxic to the kidneys at doses of 600 and 1,000 mg/kg as there were no changes in biochemical markers [22].

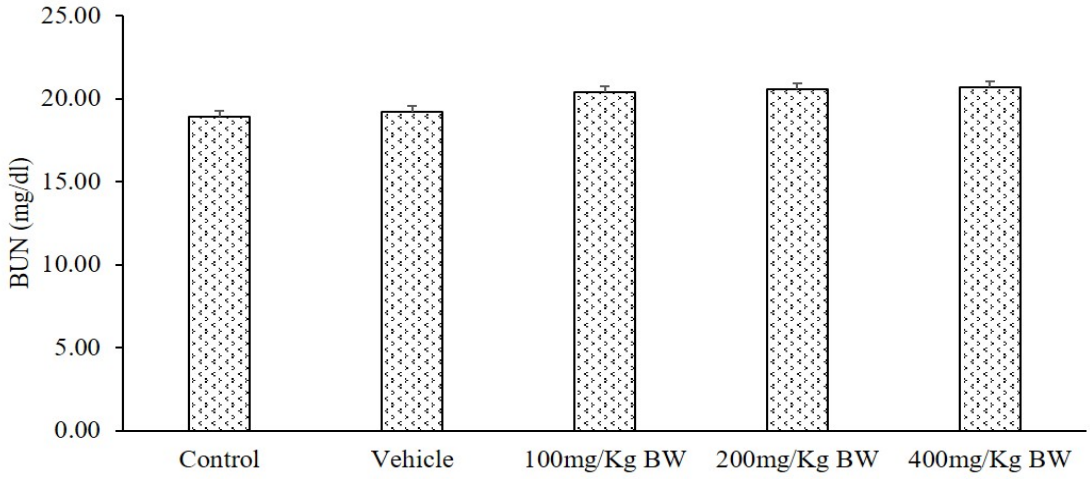


**Figure 2** The impact of *C. cauliflora* crude extract on the BUN level in ICR male mice. Results are represented as mean ± S.D. (n = 5).

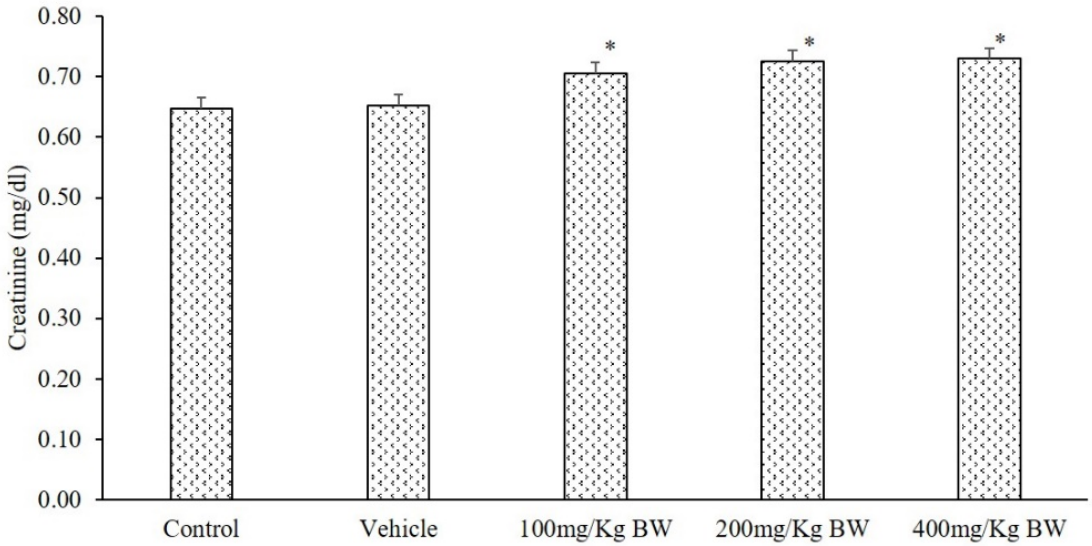


**Figure 3** The influence of *C. cauliflora* crude extract on the creatinine level in ICR male mice. Results are presented as mean ± standard deviation (n = 5). *Indicates significant difference compared to the control group, determined by 1-way ANOVA at $p < 0.05$.

The liver marker enzyme assay showed that the levels of AST, ALT and ALP were affected by the *C. cauliflora* crude extract (**Figure 4**). Concentrations between 100 and 400 mg/kg BW caused an increase in the enzyme levels of AST and ALP, which were statistically different from the control group at $p < 0.05$. Furthermore, *C. cauliflora* crude extract at dosages of 200 - 400 mg/kg BW caused a notable increase in

ALT levels compared to the control group, with statistical significance at $p < 0.05$. The study indicates that the crude extract of *C. cauliflora* affected the animal test by increasing liver enzyme levels, which is a result of liver cell destruction. This result corresponds to the histopathological findings of the liver shown in **Figure 6**.

According to biochemical parameters, higher levels of ALP, AST and ALT can indicate impaired liver function. The root bark aqueous extract of *Cassia sieberiana* D.C. at dose of 5,000 mg/kg had an impact on rats by raising ALP levels in comparison to the control group [42]. Moreover, stem bark ethanolic extract of *Dichaetanthera africana* demonstrated notable alterations in biochemical markers when administered at doses of 500 and 1,000 mg/kg BW [37,38]. Male mice that received whole mushroom ethanolic extract of *Boletus griseipurpureus* Corner at a dosage of 2,000 mg/kg BW showed elevated AST levels compared to the control group. Elevated levels of liver enzymes can indicate liver injury, leading to the production of these enzymes [38].

The previous study in aqueous root extract of *Rauwolfia vomitoria* Afzel at dose of 200 mg/kg indicates that the enzyme ALT is more sensitive to liver cell injury compared to AST [43]. The aqueous fruit extract of Moroccan *Ferula communis* at dose of 500 mg/kg was shown to be non-toxic to liver cells as there was no change in AST and ALT levels compared to the control group, indicating that this extract is not harmful to the liver [39]. On the one hand, the methanolic extracts from pomelo fruit at dose of 1,000 mg/kg, effectively lowered ALT and ALP enzyme levels [25].

Nevertheless, ethanolic extract of *M. oleifera* leaf at dose of 250 and 250 mg/kg led to reduced levels of ALT, AST and ALP [37]. Moreover, aqueous extract of aerial parts of *Haloxylon scoparium* Pomel at doses of 1,000 and 2,000 mg/kg BW led to an increase in ALP levels [1]. Conversely, mice that were given a diet high in fat and fructose along with Aloe vera showed reduced ALT levels in comparison to the control group. Furthermore, in a sub-acute test, the ethanolic extract from the leaves of *P. glaucus* (Lam.) Merr. was found to be toxic to the liver at doses of 600 and 1,000 mg/kg as there were changes in ALT, liver marker enzyme rose when compared to the control group [22].

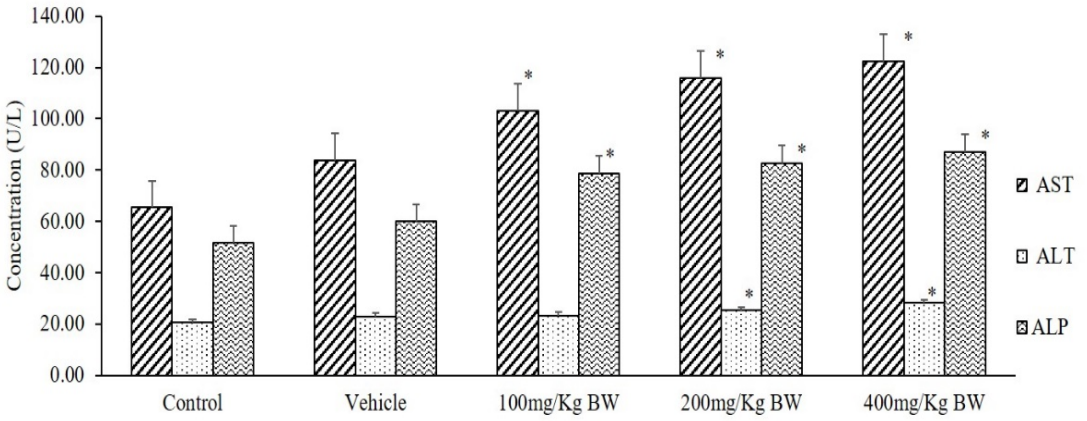


**Figure 4** The impact of *C. cauliflora* crude extract on the serum levels of AST, ALT and ALP in ICR male mice. Results are presented as mean ± standard deviation (n = 5). * Indicates significant difference compared to the control group, determined by 1-way ANOVA at a significance level of $p < 0.05$.

The investigation on the impact of *C. cauliflora* crude extract on lipid profile revealed that concentrations between 100 to 400 mg/kg BW notably reduced triglyceride levels, while showing no significant influence on LDL or HDL levels. The previous study found that whole mushroom ethanolic extract of *Boletus griseipurpureus* Corner reduced triglyceride levels at concentrations of 300 and 2,000 mg/kg BW [38]. At a dose of 400 mg/kg BW, the cholesterol level was significantly lower than that of the control group (**Figure 5**). The methanolic extracts from pomelo fruit showed significant decreases in triglyceride levels at doses ranging from 250 to 1,000 mg/kg BW. At a concentration of 1,000 mg/kg BW, cholesterol levels were dramatically reduced while HDL levels were increased compared to the control group [24]. In addition, administering ethanolic leaves extract of *Manilkara zapoda* L. at doses of 100 and 300 mg/kg resulted in an improved lipid profile [44]. Studies have demonstrated that the methanolic extract from *R. comminus* seeds at dose 100, 200 and 400 mg/kg can lower cholesterol and LDL levels in diabetic rats [26]. Additionally, 2 mL of aqueous extracts from *S. rebaudiana* leaves have been shown to reduce LDL-C and total cholesterol levels while increasing HDL-C levels [34].

Additional botanical preparations, including *Allium sativum* tuber, *Allium ascalonicum* tuber and *Salvia officinalis* leaves, have demonstrated effectiveness in lowering lipid levels [17]. *Sideritis hyssopifolia* extracts were found to effectively lower fat levels in rabbits when administered at a dose of 15.73 mg/kg BW. *Manikara zapota* L. extract had a positive impact on the lipid profile of diabetic mice by decreasing total cholesterol, total triglyceride and LDL levels, while increasing HDL levels. Similarly, *Cordia Africana* stem bark extract reduced lipid profile level that induced by Acetaminophen (APAP) caused a rise in lipid profile in rats [36]. These findings indicate that a high concentration of *C. cauliflora* crude extract decreased cholesterol and triglyceride levels but caused considerable toxicity to the liver and kidney when examined histologically under a light microscope.

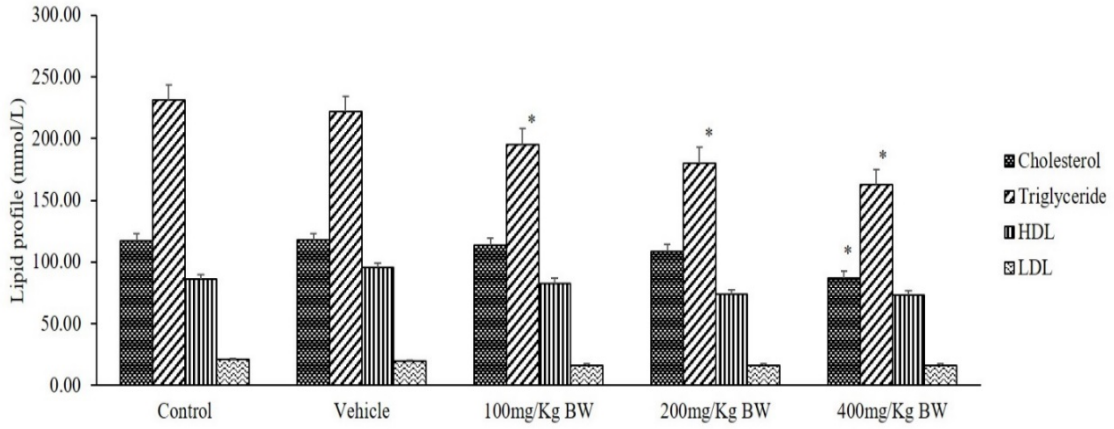


**Figure 5** The impact of *C. cauliflora* crude extract on the serum lipid profile in ICR male mice. Results are presented as mean ± standard deviation (n = 5). * Indicates significant difference compared to the control group, determined by 1-way ANOVA at a significance level of $p < 0.05$.

After 14 days of experimentation, the liver and kidney tissues were examined using hematoxylin and eosin staining for histopathological analysis. Subsequent examination using a light microscope showed alterations in the cellular composition and disease characteristics of liver and renal tissues in mice given *C. cauliflora* crude extract at doses of 100, 200 and 400 mg/kg BW, as opposed to the control group. Evidence of liver cell damage, portal vein dilation, inflammatory cell infiltration, and hepatic sinusoid dilation were observed in comparison to the control and vehicle groups (**Figure 6**).

The findings suggest that the crude extract of *C. cauliflora* caused toxicity. Administering ethanolic extract of *Lychnophora pinaster* at dosages of 125, 250 and 500 mg/kg to adult Swiss mice resulted in liver cell destruction, degeneration and tubular malignant cell dilatation, along with significant infiltrative inflammation [45]. The root bark aqueous extract of *C. sieberiana* D.C. caused centrilobular necrosis in the liver micrographs of female Sprague-Dawley rats at a dose of 750 mg/kg BW [42].

In addition, ethanol extract of *Dichaetanthera Africana* caused changes in the liver's structure at doses of 500 and 1,000 mg/kg BW in Wistar rats [46]. Administration of aqueous extract of *H. scoparium* Pomel at doses of 1,000 and 2,000 mg/kg BW in rats resulted in histological alterations in the liver [1]. Pomelo extract did not negatively impact the liver's structure, pathology or function [25]. On the other hand, the aqueous extract of *Zingiber officinale* showed hepatoprotective effects by lowering hepatic enzyme levels in albino rats compared to the control group [47]. *Melissa officinals* extract was linked to decreased liver cell damage, widening of portal veins and accumulation of white blood cells in mice [48]. The methanolic root extract of *Rotula aquatica* did not have any hepatotoxic effects. It did not affect liver function, morphology, or show any indicators of damage such as inflammation or necrosis [49]. Additionally, whole mushroom ethanol extract of *B. griseipurpureus* Corner caused liver toxicity characterized by moderate inflammation, central venous congestion and pyknotic nuclei. Research has shown that free radicals can disturb the structure and function of cell membranes, leading to the inactivation of enzymes and causing cell damage [38]. Plant secondary metabolites such as phenolic compounds, flavonoids, saponins and alkaloids have shown antioxidant activity by acting as scavengers of free radicals.

The kidney's cellular structure and pathology showed abnormalities, indicating tubular atrophy and modifications in the renal glomerulus compared to the control group (**Figure 7**). These data suggest that prolonged consumption of the extract from *C. cauliflora* fruit may have adverse effects on the kidneys. The *M. officinals* extract administration impacted the kidney's structure and disease in rats, resulting in tubular and glomerular atrophy [48]. *L. pinaster* extract at concentrations of 125, 250 and 500 mg/kg caused kidney cell and tubular damage, resulting in dilated tubules and moderate inflammation [45].

Moreover, *H. scoparium* Pomel extract at doses of 1,000 and 2,000 mg/kg BW caused minor histological alterations in the kidneys [1], whereas pomelo extract did not affect the structure or pathology of kidney cells [7,25]. In addition, the aqueous extract of *Z. officinale* showed protective effects on kidney cells in comparison to the control group [47]. In addition, male mice treated with whole mushroom ethanol extract of *B. griseipurpureus* at concentrations of 50, 300 and 2,000 mg/kg showed histopathological changes, including tubular damage and glomerular distortion [38].

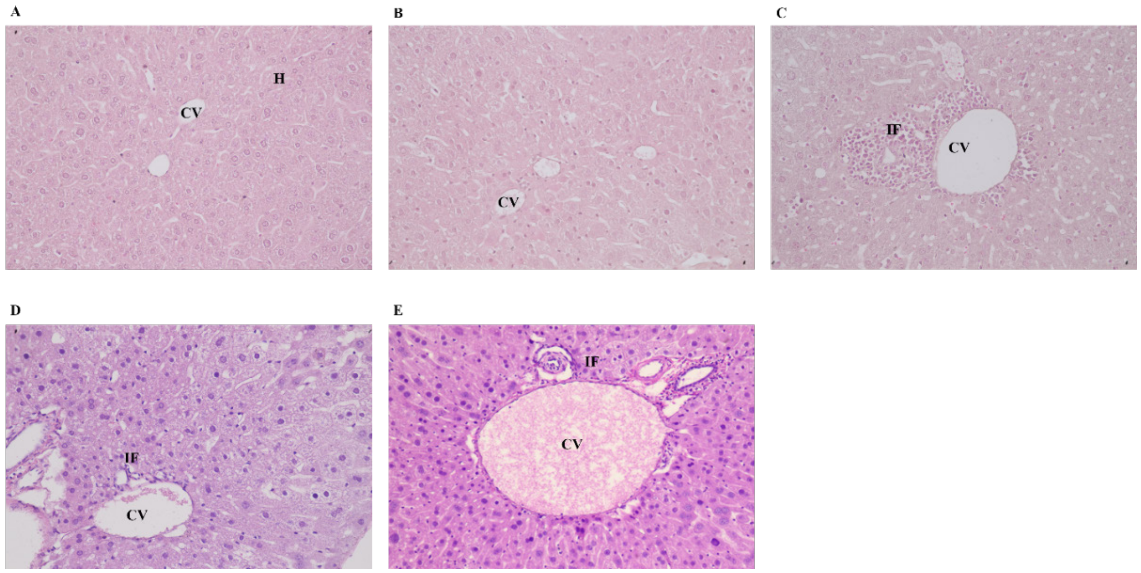


**Figure 6** The impact of *C. cauliflora* crude extract on liver morphology analysis in ICR male mice (40×, H & E). (A) Control group and (B) Vehicle group displayed normal structure and typical pathology of hepatocytes (H). (C) *C. cauliflora* crude extract at 100 mg/kg BW exhibited dilation of the central vein. (D) *C. cauliflora* crude extract at 200 mg/kg BW displayed dilation of the central vein (CV) and infiltration of inflammatory cells (IF). (E) *C. cauliflora* crude extract at 400 mg/kg BW revealed dilation of the central vein (CV), infiltration of inflammatory cells (IF) and dilation of hepatic sinusoids.

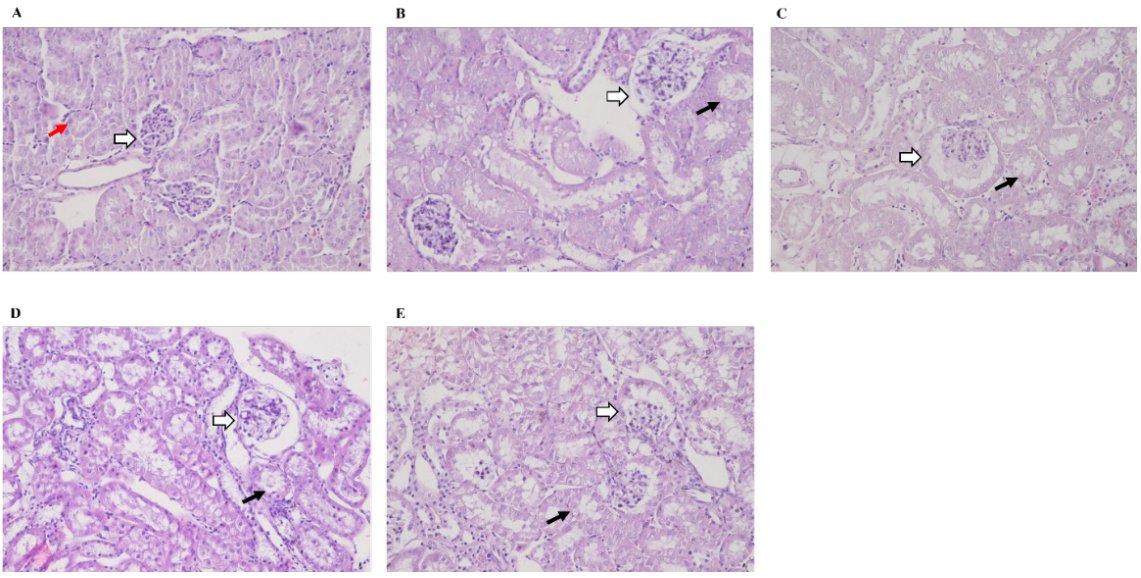


**Figure 7** The impact of *C. cauliflora* crude extract on kidney morphology analysis in ICR male mice (40×, H & E). (A) Control group displayed a normal structure and pathology of tubules (red arrow) and glomeruli (light arrow). (B) Vehicle group exhibited mild atrophy of tubules (dark arrow) and glomeruli (light arrow). (C) *C. cauliflora* crude extract at 100 mg/kg BW group, (D) *C. cauliflora* crude extract at 200 mg/kg BW group and (E) *C. cauliflora* crude extract at 400 mg/kg BW group showed atrophy of tubules (dark arrow) and glomeruli (light arrow).

## Conclusions

The findings from this study shed light on the effects of administering *C. cauliflora* crude extract on various biochemical parameters in mice. The *in vivo* study indicates that the fruit extract of *C. cauliflora* is toxic, although it did not cause death. Because the histopathology analysis exhibited alteration of liver and kidney after treatment with fruit extract of *C. cauliflora*. The alteration was appeared such as hepatocellular damage resulting elevated of AST and ALP. Moreover, kidney atrophy and other abnormalities revealed increasing of BUN and creatinine levels in serum.

The observed liver and kidney damage may be attributed to the prolonged administration of the extract at concentrations within this range, which potentially exceeded the tolerable limits for mice. This underscores the critical need for dose optimization to mitigate cytotoxic effects on vital organs. It is recommended to conduct sub-acute toxicity tests to further investigate the impact of *C. cauliflora* extract on additional physiological parameters, such as body weight, blood hematological parameters, food consumption and water intake in animal models. Such comprehensive assessments will provide a more holistic understanding of the extract's overall safety profile and potential health implications.

Furthermore, the insights obtained from this study could serve as a valuable foundation for further research, especially in the context of human studies. These findings can be further explored to understand the processes behind the observed effects and to assess potential therapeutic applications or safety considerations associated with the use of *C. cauliflora* extract in clinical settings.

## Acknowledgement

The authors would like to thank the Faculty of Medicine, Princess of Naradhiwas University and the Animal House Unit at Walailak University. This research received a fund from the Faculty of Medicine, Princess of Naradhiwas University, Thailand (Grant number 03/2563).

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