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Subchronic Oral Toxicity of the Ethanol Extract of *Crinum asiaticum* L. Leaves in Male *Rattus norvegicus*: Biochemical and Histopathological Evaluation

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Abstract: Ethanol extract of *Crinum asiaticum L. leaves* (CAL) has been reported to possess pharmacological activities and to be suitable for development into a standardised herbal medicine. However, its safety profile remains unknown. This study aimed to evaluate the effects of administering an ethanol extract of CAL leaves at doses of 200, 400, and 800 mg/kgBW on liver, heart, and kidney functions in male Wistar rats (*Rattus norvegicus*). The test was conducted by repeated oral administration of the ethanol extract over 60 days. The parameters observed included blood biochemistry using a Mindray® BA-88A photometer, gross examination of the heart, and histopathological examination of the liver, heart, and kidney. The conclusion of this study is that repeated oral administration of the ethanol extract of *Crinum asiaticum* leaves for 60 days did not produce marked alterations in several routine biochemical parameters at the lower doses (200 and 400 mg/kgBW). However, dose-related microscopic and macroscopic changes were observed, particularly at 800 mg/kgBW, and early signs of organ alteration were also noted at intermediate dose levels. Therefore, the extract may be considered to show limited tolerability at lower doses under the present experimental conditions, whereas higher doses indicate potential toxicity to the liver, heart, and kidneys.

Keywords: *Crinum asiaticum L*, heart function, kidney function, liver function, subchronic toxicity

1. INTRODUCTION

Indonesia is among the countries with high plant biodiversity, and many of these plants are traditionally used as medicinal plants. One such plant, Crinum asiaticum L. (CAL), is used in several Indonesian communities. Traditionally, the leaves have been applied topically to relieve swelling. CAL leaves can also be consumed orally by making an infusion: boil 3 leaves in 3 cups of water, reduce to 1 cup, and drink [1]. Leaves of *Crinum asiaticum* L., known as "Kobaraun," are green herbs widely used and distributed in China, Hong Kong, India, Sri Lanka, Myanmar, Malaysia, Thailand, the Ryukyu Islands, and mainland Japan. They are used to treat pain, arthritis, polyuria (frequent urination), dyscrasias, throat disorders, and fever [2].

Silalahi [1] reported that CAL leaves exhibit several bioactivities, including antioxidant, anti-inflammatory, antiplasmodial, cytotoxic, anticancer, and antimicrobial effects. According to [3], Cindy, CAL leaves have exhibited antioxidant, antidiabetic, antimicrobial, anticancer, anti-inflammatory, analgesic, and antiurolithic properties. In another study, the ethanol extract of *Crinum asiaticum* L. leaves reduced blood sugar levels in white mice [2]. This plant has the potential to be developed into a standardised herbal medicine.

Developing an ethanol extract of *Crinum asiaticum* L. leaves into a standardised herbal product has not been studied for toxicity in experimental animals. In addition to supporting its safety assessment, preclinical testing is conducted before clinical trials to determine the drug's efficacy and ensure its safety for human use, including both acute and subchronic studies. Toxicity testing in animals is useful for observing biochemical, physiological, and pathological reactions that may occur before use in humans [3]. Toxicity observation included weighing internal organs (heart, liver, kidney) [4].

The level of liver toxicity can be determined by measuring serum ALT and AST levels [5]. In addition to ALT and AST measurements to assess liver function, total serum bilirubin can be measured, as the liver plays a major role in bilirubin metabolism and excretion. If bilirubin excretion is impaired, the total serum bilirubin level will increase [6].

The heart is a vital organ that requires toxicity testing because of its critical role in circulating blood throughout the body. Because the heart continuously contracts, pumping blood that carries nutrients and drug compounds, it is susceptible to adverse effects from medications that may impair its function [7]. The assessed parameters include macroscopic observation of the heart's colour and texture, microscopic measurement of cardiomyocyte diameter, and calculation of the heart-to-body weight ratio.

Kidney failure can be caused by several factors, including hypertension, uric acid and creatinine levels, diabetes, and many others [9]. The presence of urea in the blood is a sign of kidney disease. BUN and serum creatinine are commonly used biochemical markers for assessing renal toxicity. Renal function can be assessed with greater specificity and sensitivity using creatinine clearance. This metric is reliable because creatinine, the final waste product of protein metabolism, is excreted solely by the kidneys. Creatinine clearance can be calculated from serum creatinine levels, urinary creatinine, and urine volume [10].

Based on the description above, it is necessary to conduct a subchronic toxicity test of the ethanol extract of *Crinum asiaticum* L. leaves on blood biochemistry and histopathology of the liver, heart, and kidney in male Wistar rats (*Rattus norvegicus*). The extract was administered to male Wistar rats for 60 days at doses of 200, 400, and 800 mg/kgBW. This study aimed to determine the safety of use for 60 days of CAL on biochemical and histopathological parameters to provide preliminary safety data for its further development as a standardised herbal medicine.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were 96% ethanol, dried leaf samples (CAL), Wistar rats, standard rat feed (BP12), 10% formalin solution, paraffin wax, ethanol 70%, ethanol 80%, ethanol 96%, ethanol absolute, xylene, Mayer's albumin, haematoxylin and eosin, control sera, water for analysis (Onelab), and AST, ALT, bilirubin, creatinine, and urea reagent kits (Mindray® and BT-Product). The tools used in this study included an analytical balance (Shimadzu Aw 220), a distillation apparatus (Buchi®), a set of rotary vacuum evaporators (Buchi®), an animal scale (Maxxis MT-322), a hot plate (Toreey Pines), a centrifuge (PLC 05), a micropipette (Eppendorf), microtubes, a photometer (Mindray® BA-88A), a block plate, a water bath, slide preparation equipment, a microscope (Olympus BX-53) with a trinocular lens camera, a rotary microtome, a rotary tissue processor, and paraffin embedding styrofoam.

2.2. Methods

2.2.1. Sampling and Identification of Leaves Crinum asiaticum L.

Crinum asiaticum L is a monocotyledonous plant. It is an annual herb, growing 0.5–1.3 m tall, with a large bulb measuring 5–10 cm in diameter. The leaves are ribbon-shaped or lanceolate, 3–120 cm long and 3–18 cm wide. The flowers are arranged in umbrella-shaped clusters of 10–40 white,

funnel-shaped flowers [11]. The sample used was 15 kg of *Crinum asiaticum* L leaves obtained from the Tanjung area, Kuantan Singgigi Regency, Riau. Plant identification was conducted in the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), University of Riau, Pekanbaru, under licence number 552/UNI9.5.1.1.3-4.1/TU.00.01/2023.

2.2.2. Preparation of Leaf Ethanol Extract Crinum asiaticum L.

CAL fresh leaves are chopped, then air-dried in the shade, and protected from sunlight. They are then macerated three times in ethanol for five days. The resulting macerate is thickened using a rotary evaporator at 60°C under a vacuum of 175 mbar (0.172 atm) to obtain a thick leaf ethanol extract. The ethanol extract obtained was 17,58%. The extract is stored in a refrigerator at 8°C in a dark glass container.

2.2.3. Phytochemical Screening of Fresh Samples and Ethanol Extract of Crinum asiaticum L. Leaves

Secondary metabolite phytochemical tests included: Alkaloid, Flavonoid, Phenolic, Terpenoid, Steroid, and Saponin. Phytochemical screening was performed on fresh samples and on the ethanol extract of leaves, following the method of Harborne (2016) [12].

2.2.4. Subchronic Toxicity Test of Ethanol Extract of CAL.

This research has passed the ethical review of the Medical and Health Research Ethics Unit, Faculty of Medicine, University of Riau (Ethical Clearance No. B./066/UN19.5.1.1.8/UEPKK/2024). The test animals were male Wistar rats, aged 6-8 weeks and weighing 150-200 g. Before the experiment began, the animals were acclimatised for 7 days and housed in standard rat cages (40 x 30 x 20 cm) under a 12–12-hour light-dark cycle, with free access to water and CP 552 pellets given daily. The ambient temperature and humidity were maintained at 22–24 °C and 40-60%. Male Wistar rats were randomly assigned to groups. The animals were segregated into four groups, each comprising 6 male Wistar rats, with one control group and three intervention groups. The control group, comprising the experimental animals, received a 1% NaCMC suspension at a volume of 1% of body weight. The experimental compound was administered orally to the animals at three dose levels: 200, 400, and 800 mg/kgBW. Dosing occurred once daily over a 60-day period.

2.2.5. Test Animal Weight Measurement

Weight changes were monitored twice a week. However, daily weight was still used to determine the volume of the test solution administered.

2.2.6. Urine Sample Collection and Preparation

On day 60, the male Wistar rats were placed in metabolic cages for 24-hour urine collection. They were fasted for 12 hours, and water was provided throughout the collection period. The volume of urine excreted over the 24-hour period was measured.

2.2.7. Blood Sampling and Serum Preparation

Blood samples were taken from a male Wistar rat on day 61 via the orbital vein in one eye. The rat was fasted for 12 hours, with water provided. Prior to blood collection, the male Wistar rat was anaesthetised with ether, applied by dropping it onto cotton wool and placing it under the animal's nose. Following collection via the orbital vein, up to 2 mL was obtained using a Pasteur haematocrit tube. The test was performed by trained laboratory personnel, inserting the pipette at a 45° angle. Blood was left to stand at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes. The serum was separated, and blood biochemistry was measured. The male Wistar rat was sacrificed for organ harvesting by neck dislocation.

2.2.8. Measurement of Total Serum Bilirubin Levels

The method used to measure total serum bilirubin was based on the endpoint method. Measurements were made using a photometer (Mindray® BA-88A) at 25°C and a wavelength of 546 nm to determine the total serum bilirubin level (mg/dL).

2.2.9. AST and ALT Activity Measurement

The AST and ALT determination method is based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method and uses the AST/ALT reagent kit (Mindray®). Measurements were performed using a photometer (Mindray® BA-88A) at a wavelength of 340 nm and a temperature of 37°C, yielding AST and ALT activity (U/L). Before measurements, the instrument is calibrated, and a control check is performed. The calibration process uses a multi-calibrator.

2.2.10. Serum Urea Nitrogen Level Measurement

Serum urea nitrogen levels were determined using the enzymatic UV test, Urease-GLDH, with the Urea Kit (BT-Product). The method used was Two Point Kinetic (fixed time), Decreasing Reaction. Measurements were performed using a photometer (Mindray® BA-88A) at a wavelength of 340 nm and a temperature of 37°C, yielding blood urea levels (mg/dL). To obtain serum urea nitrogen (BUN) levels, the urea level must be converted to blood urea nitrogen. The urea level obtained is then converted into a BUN level (Conversion Factor: Urea [mg/dl] x 0.467 = BUN [mg/dl]).

2.2.11. Urine and Serum Creatinine Measurement

Creatinine levels in urine and serum were determined using the Jaffe reaction. Measurements were made using a photometer (Mindray® BA-88A) at 510 nm and 37°C for 2 minutes to determine urine and serum creatinine levels.

2.2.12. Macroscopic Observation of the Liver, Heart, and Kidney Organs

Macroscopic observation of the liver, heart, and kidneys was performed by visual inspection after the experimental animals were sacrificed by neck dislocation. The texture and colour of the liver, heart, and kidneys were observed.

2.2.13. Microscopic Examination of Liver, Heart, and Kidney Histology

Histological organ preparations are carried out in several stages. Each organ is fixed in 10% formalin for 2 days. The organ is sectioned, and dehydration continues using successive solvents (ethanol, xylol, and paraffin) in a Rotary Tissue Processor. The embedding process is carried out by pouring liquid paraffin into a block plate containing the organ. The paraffin block is removed from the mould and stored at 20 °C. The next step is to section the paraffin block containing the organs at a thickness of 4-5 μm using a microtome. After the thin organ sections are removed onto glass slides, they are stained successively with haematoxylin and eosin. The slide is ready to be mounted. Labeled slides can be examined under a microscope at 10 × 10 and 10 × 40 magnification. For quantitative liver histology data, the central veins were counted across five fields of view from one section. For quantitative heart analysis, the diameter of cardiac muscle cells (myocardium) was measured using the ImageJ application across 5 fields of view from a single tissue section. For quantitative data on kidney histology, the number of damaged kidney glomeruli was counted across five fields of view from one section.

Qualitative data for the liver and heart were assessed by counting the number of veins and arteries. In most cases, the central veins are circular or continuous, with densely packed endothelial cells surrounding them. Normal hepatocytes form a series of branching plates or sheets that create a labyrinth or resemble foam rubber. Between the hepatocyte plates are plate-shaped or irregular sinusoids, and the hepatic nucleus may be round, reduced, fragmented, or missing. Between the sinusoids are plate-shaped or irregular spaces. Qualitative data for the kidneys were assessed in Bowman's capsule/urinary space (narrow/non-narrow), proximal tubule (intact/lysed), and distal tubule (intact/lysed). Qualitative observations of the kidneys were made by examining tissue photographs. For the cardiac muscle cell walls and nuclei, the parameter observed was the presence or absence of thickening. Meanwhile, quantitative data were obtained by measuring the diameter of the cardiac muscle cells (myocardium). These measurements were performed using the *ImageJ* application across five fields of view from a single tissue section.

2.2.14. Data Analysis

The research data were analysed using a one-way Analysis of Variance (ANOVA) for total serum bilirubin and relative organ weights, and by examining histological images of the heart in the control and treatment groups. Body weight was analysed using two-way ANOVA. If the results differed significantly, further analysis was performed using the Tukey multiple range test.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening of Fresh Samples and Ethanol Extract

The phytochemical profile of CAL leaves was characterized using screening tests. Fresh leaves tested positive for alkaloids, flavonoids, and terpenoids, and the ethanol extract contained these metabolites as well as saponins [13]. From 15 kg of fresh CAL, 1.2 kg of dry leaves were obtained, which were then extracted to yield a thick ethanol extract of 211 grams, representing a yield of 17.58% from the dry leaves.

3.2. *Subchronic Toxicity Test of Ethanol Extract*

The selected experimental animals were healthy, of approximately the same age and weight, and were randomly divided into four groups with a body weight variation of no more than 20%. The selected animals were acclimatized for 7 days. Afterward, they were grouped according to the extract administered in three dosage variations: 200, 400, and 800 mg/kgBW. One negative control group received only a 1% Na CMC suspension orally. Orally administered drugs undergo enterohepatic circulation, so significant results are observed if liver damage occurs. Liver damage can be caused by hepatotoxic compounds.

Table 1. Results of measurements of total bilirubin, AST and ALT levels, urine and serum creatinine levels, creatinine clearance value and BUN level in male Wistar rats after administration of the ethanol extract of CAL leaves for 60 days

Inspection	Negative control	Dose 200 mg/kgBw	Dose 400 mg/kgBw	Dose 800 mg/kgBw
Total Bilirubin level (mg/dL)	0.188 ± 0.1971	0.174 ± 0.1326	0.190 ± 1.471	0.194 ± 0.1796
AST (U/L)	59.605 ± 8.900	61.729 ± 7.24	68.771 ± 8.26	73.388 ± 8.07
ALT (U/L)	26.933 ± 6.280	21.196 ± 3.49	23.339 ± 6.50	24.990 ± 5.46
24 Hour urine volume (mL)	1.220 ± 0.080	1.340 ± 0.060	1.520 ± 0.080	1.990 ± 0.160
Urine creatinine level (mg/dL)	69.200 ± 0.494	69.383 ± 0.527	69.550 ± 0.562	70.193 ± 0.661
Serum creatinine level (mg/dL)	0.324 ± 0.010	0.326 ± 0.011	0.330 ± 0.010	0.332 ± 0.010
Creatinine clearance value (mL/menit)	0.180 ± 0.009	0.198 ± 0.013	0.221 ± 0.006	0.293 ± 0.027
BUN level (mg/dL)	15.380±9,84	19.462±2.051	20.785±1.266	20.033±1.994

Based on the results of the measurements carried out, the serum AST levels of white rats (*Rattus norvegicus*) in the average negative control group and at doses of 200, 400, and 800 mg/kgBW were 59.615, 61.729, 68.771, and 73.388 U/L, with a normal range of 45.7-80 U/L [14]. Standard deviations were 8.9, 7.2, 8.2, and 8. Serum ALT levels were 26.933, 21.196, 23.339, and 24.990 U/L, with a normal range of 17-30.2 U/L [14]. Standard deviations were 6.2, 3.4, 6.5, and 5. Furthermore, the data were analysed using one-way ANOVA, showing no significant difference ($p>0.05$) between the administration of the ethanol extract in the treatment group and the negative control. The data obtained showed that AST and ALT levels were within the normal range and were considered non-toxic. The standard deviations in each group were quite variable, presumably due to the differing

physiological conditions of the experimental animals. Statistical analysis via one-way ANOVA showed that the administration of the leaf ethanol extract did not significantly alter serum bilirubin levels. There was no significant main effect of any dose group (200, 400, 800 mg/kg BW) compared with the control, or between the dose groups ($p > 0.05$). This finding suggests the absence of treatment-related toxic effects on bilirubin metabolism over the 60-day period.

Based on the results of the one-way ANOVA, Blood Urea Nitrogen (BUN) levels after administration of the ethanol extract differed significantly between the treatment and negative control groups ($p<0.05$), so Tukey's post hoc test was applied. However, there was no significant difference ($p>0.05$) in BUN levels among the 200, 400, and 800 mg/kgBW dose groups. The mean BUN levels in the negative control group and the 200, 400, and 800 mg/kgBW dose groups were 15.380, 19.462, 20.785, and 20.003 mg/dL, respectively, all within the normal range for male Wistar rats, namely 15-21 mg/dL [14].

The mean 24-hour urine volumes for male Wistar rats in the negative control group, the 200 mg/kgBW, 400 mg/kgBW, and 800 mg/kgBW groups were 1.22 ml, 1.34 ml, 1.52 ml, and 1.99 ml, respectively. Statistical analysis showed that the 200 mg/kgBW dose did not significantly alter 24-hour urine volume relative to the control ($p>0.05$). However, significant diuretic effects were observed at the higher doses of 400 and 800 mg/kgBW ($p<0.05$).

Flavonoids in plants confer diuretic activity by increasing urination and the glomerular filtration rate [13]. A higher glomerular filtration rate accelerates renal excretion of toxins, as increased urinary flow facilitates their removal. Supporting this, research by Wientarsih [15] indicates that flavonoids contribute to this protective effect by both raising the GFR and suppressing urinary crystallin formation [16]. The observed increase in urine volume may be related to phytochemicals present in the extract, but this mechanism remains speculative because the phytochemical analysis was only qualitative.

The average urine creatinine levels in male Wistar rats from the negative control group and from the 200 mg/kgBW, 400 mg/kgBW, and 800 mg/kgBW treatment groups were 69.200 mg/dL, 69.383 mg/dL, 69.550 mg/dL, and 70.193 mg/dL, respectively. Based on one-way ANOVA, the urine creatinine levels in male Wistar rats after administration of the ethanol extract of CAL leaves in the treatment group were not significantly different ($p>0.05$) from those in the negative control. The average urine creatinine levels in white rats remained within the normal range, namely 60.39-75.97 mg/dL [14].

Creatinine is a metabolic waste product derived from protein breakdown, produced in muscle tissue and released into the circulation at a constant rate. It is filtered from the blood and removed from the body solely by the kidneys via glomerular filtration. It is not reabsorbed by the tubules but is secreted in the proximal tubules [17]. Under normal conditions, tubular secretion is negligible and can be ignored, but with kidney damage, the amount of secretion increases [18]. Each individual excretes creatinine at a constant rate, making it an ideal measure of kidney function [19].

The mean serum creatinine levels in male Wistar rats from the negative control group and the 200, 400, and 800 mg/kgBW dose groups were 0.324, 0.326, 0.330, and 0.332 mg/dL, respectively. Based on one-way ANOVA analysis, the serum creatinine levels in male Wistar rats (*Rattus norvegicus)* after administration of the CAL leaf ethanol extract in the treatment group were not significantly different ($p>0.05$) from those in the negative control. Serum creatinine is a robust biomarker of renal function. As noted by [20], a doubling of the serum creatinine concentration from baseline corresponds to an approximate 50% reduction in functional capacity, while a threefold elevation signifies a decline of roughly 75%.

The average creatinine clearance values in male Wistar rats from the negative control group at doses of 200, 400, and 800 mg/kgBW were 0.180, 0.198, 0.221, and 0.293 ml/minute, respectively. Based on one-way ANOVA, creatinine clearance values in male Wistar rats after administration of the ethanol extract of leaves at 200 mg/kgBW were not significantly different ($p>0.05$) from the negative control, whereas the doses of 400 and 800 mg/kgBW were significantly different ($p<0.05$) from the negative control. Rat creatinine clearance data remain within the normal range, namely

0.1649-0.2959 ml/minute [14]. Based on kidney biochemical parameters, including urine creatinine, serum creatinine clearance, and BUN, no changes were observed. The significant increase in urine volume could also be due to other factors, such as increased water intake.

In general, changes in body weight in experimental animals reflect toxicity following exposure to toxic substances or toxicants. Changes in body weight are an indicator of the side effects of a drug or compound, and a decrease of 10% from the initial weight is considered significant. Toxic effects can be observed through mortality, weight loss, and changes in relative liver and kidney weights. The increase in body weight of male Wistar rats is proportional to their age, indicating that their growth is proceeding normally.

Changes in rat body weight were assessed using a two-way ANOVA. The results of the two-way ANOVA showed a significant difference between the negative control and the administration of ethanol extract of CAL leaves at doses of 200, 400, and 800 mg/kgBW ($p<0.05$). Tukey test results indicated that all doses of the CAL leaf ethanol extract (200, 400, and 800 mg/kgBW) produced effects that were statistically distinct from the negative control ($p<0.05$). However, no significant differences were observed between the three dose levels themselves ($p>0.05$). The absence of significant changes between treatment doses indicates that the ethanol extract of CAL leaves did not significantly affect weight gain or loss. Based on the average body weight data of male Wistar rats for 60 days, the negative control group and the doses of 200, 400, and 800 mg/kgBW showed 199.563 g, 212.711 g, 231.317 g, and 213.865 g, respectively.

3.3. Macroscopic examination of the liver, heart, and kidneys

Macroscopic examination of the liver in the control group and the 200 mg/kgBW dose group still appeared normal, with a deep red colour and a smooth texture. However, at doses of 400 and 800 mg/kgBW, the colour changed to pale and spots appeared. This observation indicates changes in the liver's macroscopic appearance. Macroscopic examination of the heart and kidney showed a deep red colour and a smooth texture, indicating no macroscopic differences between the control group and the 200, 400, and 800 mg/kgBW groups. Therefore, macroscopic examination of the heart and kidney showed no changes between the control and treatment groups.

Table 2. Ratios of liver, heart, right kidney, and left kidney in male Wistar rats after 60 days of administration of the ethanol extract of *Crinum asiaticum L.*

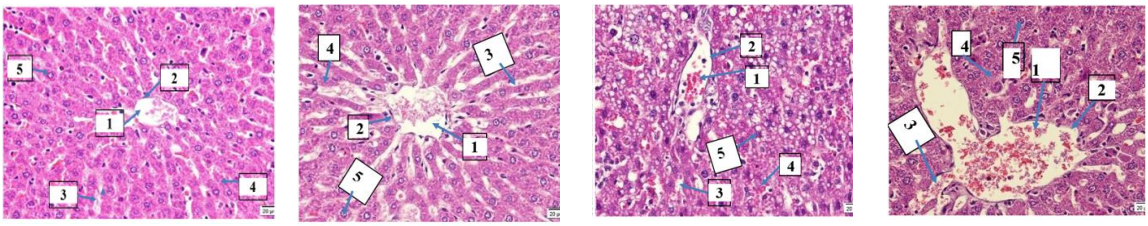
Group	Liver Weight Ratio (g)	Heart Weight Ratio (g)	Right Kidney Ratio (g)	Left Kidney Ratio (g)
Negative Control	0.0222 ± 0.0032	0.005± 0.0009	0.0023 ± 0.0003	0.0028 ± 0.0003
Dose 200 mg/kgBW	0.0269 ± 0.0030	0.005 ± 0.001	0.0020 ± 0.0007	0.0025 ± 0.0003
Dose 400 mg/kgBW	0.0236 ± 0.0026	0.005 ± 0.0006	0.0023 ± 0.0003	0.0026 ± 0.0003
Dose 800 mg/kgBW	0.0237 ± 0.0076	0.005 ± 0.0008	0.0028 ± 0.0003	0.0030 ± 0.0003

Based on the one-way ANOVA results, the liver, heart, right kidney, and left kidney organ weight ratios for the ethanol extract of CAL leaves at doses of 200, 400, and 800 mg/kgBW did not differ significantly ($p>0.05$). This may be due to the presence of flavonoids and alkaloids in CAL leaves, which have antioxidant activity and act as hepatoprotectors. The test results are not intended to prove its safety for human use, but they can nevertheless provide an overview of a compound's relative toxicity and help identify potential toxic effects in humans. For this reason, it is necessary to conduct organ examinations functionally and cellularly for the toxic level of the chemical compound given [20].

3.4. Microscopic Liver Histology Examination

The damage observed in the hepatocytes of male Wistar rats given the ethanol extract of CAL leaves at 200 mg/kgBW was clearly visible, whereas at doses of 400 and 800 mg/kgBW, necrosis was observed. Necrosis can occur due to toxins or acute oxygen deprivation within the cells. Bouzidi *et al.* [21] reported that alkaloids can cause histological signs of centrilobular hepatocyte necrosis and dilation of the central veins.

According to Vairetti [22], alkaloids can affect cellular metabolic pathways, including glutathione pathways and detoxification enzymes. This can reduce the liver's capacity to clear toxins, increasing the toxic burden on hepatocytes. Saponins can affect mitochondrial function, with mitochondria being essential organelles for energy production and metabolism in hepatocytes. Mitochondrial disruption can lead to cell death and impaired overall liver function. In the liver tissue of control male Wistar rats, several central veins were intact, with endothelial cells still arranged in an orderly manner. At a dose of 200 mg/kgBW, some central veins began to be damaged, while at doses of 400 and 800 mg/kgBW, some were already inflamed.



(**a**) (**b**) (**c**) (**d**)

Figure 1. Liver histopathology (a) Control group, (b) 200-dose group, (c) 400-dose group, and (d) 800 mg/kgBW dose group (H&E staining, 400x). (1) Central vein (2) Endothelium (3) Sinusoid (4) Hepatocyte (4) Cell nucleus

Based on observations of the percentage of central vein damage, including widening and endothelial damage, the percentage increased with higher doses of CAL leaf ethanol extract. In the control group, 200, 400, and 800 mg/kgBB, the percentages were 40.51; 53.89; 56.48; 60.16%. One-way ANOVA of the % central vein damage variable showed no significant difference ($p>0.05$) between the 200, 400, and 800 mg/kgBB dose groups and the control. According to two-way ANOVA with Tukey's post hoc test, administration of CAL leaf ethanol extract at all tested doses (200, 400, and 800 mg/kgBW) resulted in statistically significant changes in body weight compared with the control group ($p<0.05$).

The endothelium observed was the central venous endothelium. In the control group, the endothelium remained regularly arranged. At doses of 200, 400, and 800 mg/kgBW, the endothelium became irregular and thickened. In the liver tissue, the sinusoids of control male Wistar rats and those given a dose of 200 mg/kgBW still appeared neat and normal, whereas at doses of 400 and 800 mg/kgBW, the sinusoids were damaged and dilated.

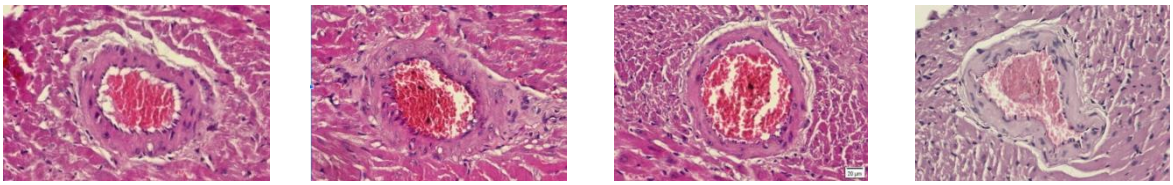
Sinusoidal damage at doses of 200, 400, and 800 mg/kgBW can also occur due to fatty degeneration, resulting in the formation of fat vacuoles that create empty spaces in the sinusoids and cause them to widen. Another possible cause is pressure on the sinusoidal walls due to blood clots in the veins caused by toxic substances. In general, the hepatic artery branches to form arterioles in the portal area, some of which terminate directly into the sinusoids at a certain distance from the portal fissure, allowing oxygen-rich arterial blood to mix with portal venous blood in the sinusoids [23]. The liver tissue in control male Wistar rats still had normal, round nuclei, with clearly visible cell boundaries. At a dose of 200 mg/kgBW, the nuclei were also normal. However, in male Wistar rats given doses of 400 and 800 mg/kgBW, the nuclei were damaged, resulting in fatty degeneration.

Fatty degeneration is the accumulation of fat in the cytoplasm of cells. It typically occurs in parenchymatous cells, such as liver cells (fatty liver), kidney tubules, myocardium, and others. Hematoxylin-eosin (HE) staining reveals the loss of fat due to alcohol-induced dehydration,

forming vacuoles, often referred to as vacuolar degeneration. Fat in the cytoplasm can push the cell nucleus to the edge, which is visible on microscopic examination, resulting in small, clear spots of fat. Fatty degeneration can occur in conditions such as ischaemia, anaemia, toxic substances, and excessive consumption of fat and protein. Another possible cause is excessive consumption of pellets, which leads to the accumulation of fat and protein, a contributing factor to fatty degeneration. If fatty degeneration continues, hepatocytes may experience necrosis [24].

3.5. Microscopic Heart Histology Examination

Histological examination of cardiac muscle cells (myocardium) revealed changes following administration of increasing doses of white bakung leaf extract, particularly at a dose of 800 mg/kgBW administered for 60 days.



(**a**) (**b**) (**c**) (**d**)

Figure 2. Heart (arterial, endothelial, and nucleic) histopathology (a) Control Group, (b) Dose 200mg/Kg, (c) Dose 400mg/Kg, (d) Dose 800 mg/Kg (H-E Staining 400x).

Microscopic observations of cardiac muscle cells in this study showed changes characterised by thickening of the myocardium, with myocardial diameters exceeding 15 µm, whereas the normal value is less than 15 µm [23]. This thickening of the heart muscle is commonly referred to as hypertrophy. Cardiac hypertrophy is defined as the thickening or increase in mass of the heart muscle, or myocardium.

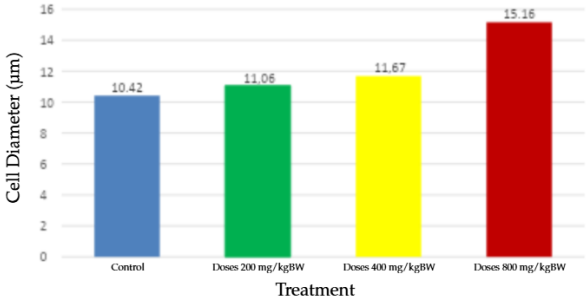


Figure 3. Average diameter of cardiac muscle cells in male white rats (*Rattus norvegicus*) across treatment groups

The one-way ANOVA indicated a significant difference ($p<0.05$) in cardiac muscle cell diameter among the groups of rats treated with the test preparation at doses of 200, 400, and 800 mg/kgBW. This indicates that the ethanol extract of *Crinum asiaticum* increases cardiac muscle cell diameter at a dose of 800 mg/kgBW. There was a significant difference ($p<0.05$) in cardiac muscle cell diameter at 800 mg/kgBW compared with the control group, with an increase in diameter associated with cardiac muscle hypertrophy. Microscopic observations also showed broken veins and arteries.

3.6 Microscopic Kidney Histology Examination

The percentage of glomerular damage in male Wistar rats increased with dose. The control group had an average glomerular damage of 10.32% (right kidney) and 12.88% (left kidney). The 200 mg/kgBW dose had an average glomerular damage of 13.88% (right kidney) and 15.13% (left kidney). The 400 mg/kgBW dose resulted in 16.07% (right kidney) and 19.03% (left kidney). The 800 mg/kgBW dose resulted in 22.64% (right kidney) and 26.17% (left kidney).

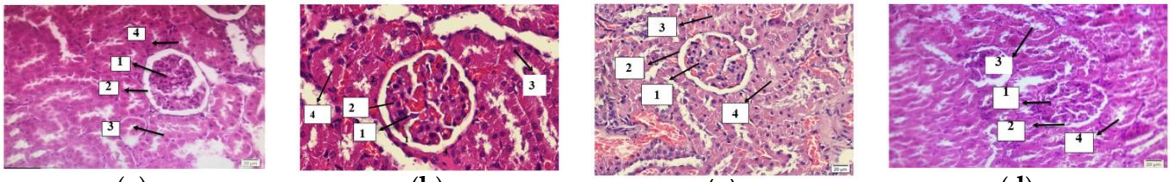


Figure 4. Kidney histopathology (**a**) Control Group, (**b**) Dose 200mg/Kg, (**c**) Dose 400mg/Kg, (**d**) Dose 800 mg/kg (H-E Staining 400x). (1) Enlarged Glomerulus, (2) Narrowed Bowman's Capsule, (3) Normal Proximal Convoluted Tubule, (4) Normal Distal Convoluted Tubule

Histological examination of male Wistar rats kidney tissues revealed dose-dependent changes in the glomeruli but not in the tubules. In the control and 200 mg/kgBW groups, the glomeruli and Bowman's capsules appeared normal. At 400 mg/kgBW, the percentage of glomerular damage began to increase. However, at 800 mg/kgBW, glomerular enlargement was observed, resulting in narrowing of the Bowman's space. In contrast, the architecture of both proximal and distal tubules remained unchanged across all treatment groups compared with the control.

Table 3. Percentage of Central Vein Damage to the Liver and of Glomerular Damage in the Right and Left Kidneys in male Wistar rats after Administration of Ethanol Extract of *Crinum asiaticum* L.

Groups	% Central Venous Damage	% Right Renal Glomerular Demage	% Left Renal Glomerular Demage
Negative control	40.51 ± 9.468	10.32 ± 4.539	12.88 ± 3.576
Dose 200 mg/KgBw	53.899 ± 23.149	13.88 ± 2.504	15.13 ± 3.649
Dose 400 mg/KgBw	56.485 ± 16.114	16.07 ± 3.310	19.03 ± 2.504
Dose 800 mg/KgBw	60.165 ± 23.603	22.64 ± 3.696	26.17 ± 5.062

4. CONCLUSION

Based on the present study, repeated oral administration of the ethanol extract of *Crinum asiaticum* leaves for 60 days did not produce marked alterations in several routine biochemical parameters at lower doses (200 and 400 mg/kgBW). However, dose-related microscopic and macroscopic changes were observed, particularly at 800 mg/kgBW, and some early signs of organ alteration were also noted at intermediate doses. Therefore, the extract may be considered to show limited tolerability at lower doses under the present experimental conditions, whereas higher doses indicate potential toxicity to the liver, heart, and kidneys. Further studies with more rigorous toxicological design are required before claiming its safety.

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