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Phytochemicals and Toxicity of the Extract from *Cosmos Caudatus* Leaves

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The efficacy of traditional medicines is related to the complexity of the chemical properties of the drugs. Cosmos caudatus Kunth is a traditional medicinal plant with therapeutic properties. This study aimed to determine the chemical compounds contained in C. caudatus Kunth leaves and their toxicity. Toxicity tests were conducted on 24 male mice (Mus musculus) divided into one control group consisting of aguadest and five treatment groups consisting of C. caudatus Kunth ethanol extract at doses of 125mg/kg bw, 250mg/kg bw, 500mg/kg bw, 1g/kg bw, and 2g/kg bw. Phytochemical analysis showed that the ethanol extract of C. caudatus Kunth leaves contained alkaloids, tannins, phenols, flavonoids, and saponins. The highest levels of alkaloids, tannins, phenols, and flavonoids were found in the ethanol extract of C. caudatus Kunth leaves fractionated with aqua, while the highest level of saponins was found in the aqua fractionation extract. The highest test dose of 2g/kg bw did not cause poisoning or death in mice. There were no significant differences in liver weight (p=0.14), kidney weight (p=0.44), or creatinine (p=0.21) between the control and treatment groups. Histopathological examination of the liver showed severe hydropic degeneration that was not significantly different between the control and treatment groups. Renal histopathology showed significant differences between the control and treatment groups. Conclusion: ethanol extract of C. caudatus Kunth leaves contains alkaloids, tannins, phenols, flavonoids and saponins. A dose of 2 g/kg bw of ethanol extract of C. caudatus Kunth leaves was not toxic to mice, but most of the mice's livers experienced severe damage.

Keywords: Phytochemicals; toxicity; extract of C. caudatus leaves; invivo.

Around 80% of the world's population is estimated to use traditional medicine[1]. Most of the Indonesian population, especially in rural areas, use traditional herbal medicines[2]. Its efficacy is related to the complexity of the chemical nature of drugs - traditional medicines offer many advantages in terms of efficiency and molecular target selectivity[3].

Phytochemical tests of medicinal plants continue to be developed to develop new medicinal ingredients. Qualitative determinations in phytochemical screening provide information on the presence of certain compounds or groups of compounds, and quantitatively distinguish which are the main components and which are additional components in the mixture. Isolation of secondary metabolite compounds is carried out through an extraction process using organic solvents with increasing polarity sequentially.[4] The polarity of the type of solvent used in extraction must be the same or very close to the polarity of the active ingredient being extracted so that the extraction

runs efficiently because according to the principle like dissolves like not all compounds will dissolve in a liquid solvent.[5]

C.caudatus Kunth originates from Mexico to South Tropical America. This plant is an annual and grows primarily in seasonally dry tropical biomes. *C.caudatus* in Malaysia is called ulam raja[6] and kenikir in Indonesia.[7] one of the traditional medicinal plants with medicinal properties. Its leaves are often consumed by Indonesians. *C. caudatus* leaf extract shows the presence of active compounds such as flavonoids, saponins, alkaloids, tannins, and polyphenols.[8] Traditionally used as antihypertensive, antidiabetic, antioxidant, antiosteoporosis, antifungal, and antibacterial[9]. *C. caudatus* rich in flavonoid glycosides exhibits skin anti-aging effects through inhibiting collagenase, MMP-1 and MMP-3 activities, possibly via the NF-êB pathway.[10] Although traditional medicine has been used for a long time, it is very important to know its potential toxicity.[11] The medicinal-activity plants should have low toxicity because of their long-term use in humans.[12]

Acute toxicity testing is intended to obtain information on poisoning symptoms, causes of death, sequence of death processes and lethal dose range for test animals by a substance.[13] Observations on experimental animals are carried out within 24 hours after giving the test material to the emergence of symptoms of poisonin such as body weight, drowsiness, lacrimation, nasal bleeding, paralysis, piloerection, salivation, skin, utilization of food, water and death and changes in the function of vital organs of the experimental animals. Acute toxicity testing (LD50) can be seen from changes in the structure and function of vital organs such as the liver and kidneys.[14] . The purpose of this study is to determine the chemical compounds contained in *C. caudatus* leaves as well as their toxicity.

MATERIAL AND METHOD

Plant material

The leaves of *C. caudatus* were dried under room temperature with further drying using an oven and ground to fine powder using an electric grinder. 200 g of *C. caudatus* leaf simplicia was macerated with 2000 ml of 96% ethanol for 72 hours at room temperature. The filtrate is obtained by filtering with Whatman No. 1 filter paper. The filtrate obtained was then evaporated at a temperature of 40^0C, to obtain a crude extract in paste form, assumed to be a concentration of 100%.[15] The crude ethanol extract is then fractionated for polar compounds with aqua, semi-polar with ethyl acetate, and non-polar compounds with hexane.[16]

Phytochemical compound test

Qualitative testing of *C. caudatus* extract was carried out according to standard methods to detect the main phytochemicals such as alkaloids, flavonoids, saponins, sterols, sugars, phenols etc. present in the extract.The fractionation results were then examined for the content of alkaloids, tannins, saponins, flavonoids and phenols, steroids and terpenoids.[17] Quantitatif phytochemical test have been carried out on *C. caudatus* leaf extracts using the UV Visible Spectrophotometry method.

Quantification of total content of alkaloid compounds

Quantitative analysis of alkaloids was carried out using the spectrophotometry method. 1ml of the sample extract was mixed with 1 ml of 0.025 FeCl3 in 0.5M HCL, and then add 1 ml of phenanthroline.The resultant mixture formed was incubated for 30 minutes in a water bath maintained at 70oC. Then, measure the absorbance of the sample using UV-Visible spectrophotometer with a wavelength of 510 nm. The alkaloid content of samples is expressed in mg/100g sample weight.[18]

Quantification of total content of tannin compounds

A total of 0.01 g of extract was diluted in 5 ml of citrate phosphate buffer. The diluted sample was pipetted to 0.25 ml then 0.25 ml of Folin-Denis reagent was added, then vortexed and 2 ml of 5% Na2CO3 was added. The solution was vortexed and then incubated for 30 minutes. Absorbance was read at a wavelength of 725 nm using a UV-Visible spectrophotometer. The reading results were compared with a standard curve using tannic acid. Total tannins in samples were expressed as tannic acid equivalents in mg TAE/g extract.[19]

Quantification of total content of saponin compounds

The mixture containing 250 µL of vanillin reagent, 50 µL of extract and 2.5 mL of 72% sulfuric acid was mixed and incubated at 60 °C for 10 min. At the end of the incubation, the absorbance

of the solution cooled in an ice bath was read at a wavelength of 544 nm. Saponin content was expressed as mg diosgenin equivalent (DE)/g.[20]

Quantification of total content of phenolic compounds

Folin-Ciocalteu method was employed for the quantification of total phenolic content. A total of 0.01 g of diluted extract into 5 ml of citrate phosphate buffer according to treatment. A sample of 0.1 ml was pipetted and 0.3 ml of 70% ethanol was added. After that, 0.4 ml of Folinciaocalteau was added and then incubated for 6 minutes. After the incubation process, 4.2 ml of 5% Na2CO3 was added, then vortexed and incubated for 90 minutes. Absorbance was read at a wavelength of 760 nm with using UV- Visible spectrophotometer. The content of total phenolic compound was denoted as mg of GAE/g of extract.[21]

Quantification of total content of flavonoid compounds

Determination of total flavonoids using a spectrophotometer with the AlCl3 method refers to (Singh et al., 2012). A total of 0.01 g of extract was diluted in 5 ml of phosphate citrate buffer according to the treatment. A total of 1 ml of sample was mixed with 4 ml of distilled water and 0.3 ml of NaNO2 solution (10%) was added. After that, it was incubated for 5 minutes and 0.3 ml of AlCl3 solution (10%) and 2 ml of NaOH solution (1%) were added, then immediately tested with a spectrophotometer at a wavelength of 510 nm. Flavonoid concentrations in the test samples were calculated C x V x FP W 37 from calibration standards prepared using quercetin standards and expressed as quercetin equivalents in mg QE/g extract.[22]

Animal preparation

24 male mice (*Mus musculus*) of the balb/c strain, aged 2.5 to 3 months and weighing between 25 and 30 grams, were used as test animals. Acute toxicity testing of traditional medicine should be performed on at least one rodent species, namely mice or rats.[23] Male mice are not affected by the estrus cycle.[24] The mice used were acclimatized for 10 days at room temperature. The mice were placed in a plastic cage. Every three days, the mouse cage was cleaned and the bedding was changed. The mice were fed chicken pellets once a day in the morning and given water ad libitum.

Experimental groups

The recommended dose is a minimum of four levels.[13] Referring to Herlina et al (2021), the LD50 test dose for this study: 125mg, 250mg, 500mg, 1g and 2g/kg BW of mice.[25] 24 experimental animals were randomly divided into one control group, namely distilled water and five treatment groups, namely *C. caudatus* ethanol extract 125mg/kg bw, 250mg/kg bw, 500mg/kg bw, 1g/kg bw and 2g/kg bw. Before treatment, mice were fasted for 10 hours, to avoid food factors. Each experimental animal was given 1 cc of the extract solution according to the dose. The extract is given in a single dose orally using a gastric probe. Observation of acute toxic effects was carried out within 1 x 24 hours.[24] After 1 x 24 hour observation, the mice were anesthetized with a mixture of ketamine + zylazine intramuscularly in the thigh muscle as much as 0.02 cc. Blood is taken using capillary hematocrit, in the retro orbital area as much as 0.8cc - 1cc. Dead mice were dissected to remove the liver and kidneys, the weighed. The liver and kidneys of mice were washed with 0.9% NaCl. The liver and kidneys were fixed for 1x24 hours in 10% buffered formalin. Next, trimming, tissue dehydration, clearing, impregnation, embedding, cassette is removed from the tissue processor and placed in a paraffin bath, clean the brass blocks, arrange them neatly. Take the specimen from the embedding cassette with tweezers, put it on a brass block, pour the liquid paraffin on the embedding machine into the block, give a marker number, cut the block to a thickness of 4 microns. The preparation was floated in a water bath. The preparations were placed in an incubator for one night. Stain the preparations with Harris-Haematoxyllin-eosin (HE) dye. Histopathological examination of the liver and kidneys was carried out at 400X magnification.[26]

Statistical analysis

The data were statistically tested with *One Way Anova* and *Kruskal Wallis* in SPSS statistic. Statistical test results are significant if $p < 0.05$.

RESULTS AND DISCUSSION

Extracts of *C. caudatus* leaves were fractionated into three categories: water, ethyl acetate, and hexane. All three categories were found to contain alkaloids, tannins, saponins, flavonoids, and phenols, but none of them contained steroids

and terpenoids. This suggests that the compounds present in the extract are polar, semi-polar, and non-polar. Further studies by Vikneswari Perumal et al. in 2014 also supported these findings, as they discovered that *C. caudatus* leaves contain active compounds such as flavonoids, saponins, alkaloids, tannins, and polyphenols.[9] However, Muhamad Dea Firdaus et al. in 2021 found that the aqua extract of *C. caudatus* leaves only contained alkaloids and tannins, while the 50% ethanol extract contained alkaloids, flavonoids and tannins, and the 96% ethanol extract contained alkaloids, tannins, saponins, and terpenoids. This could be due to variations in harvest time, weather conditions, and environmental temperature.[27]

The results of the Kruskal-Wallis test indicate a significant difference in the mean levels of alkaloids, tannins, saponins, phenols, and flavonoids in various plant extracts. The obtained p-value is 0.000. The highest levels of alkaloids, tannins, phenols, and flavonoids were found in the ethanol extract of C. caudatus leaves with water, while the highest level of saponin was found in the water extract fractioned with water. According

Table 1. Qualitative Phytochemical Test of Ethanol Extract *C. caudatus* Leaves

	Test	Aqua	Aqua Ethyl Acetate	Hexane	Aqua	Ethanol Ethyl Acetate	Hexane
Alkaloid	Drangendroff	+	+	+	+	+	+
Tannin	$FeCl_3$	+	+	+	+	+	+
Saponin	Foam	+	+	+	+	+	+
Flavonoid	HCl	+	+	+	+	+	+
Fenol	Folin-Ciocalteau	+	+	+	+	+	+
Steroid	Chloroform, acetic acid, concentrated sulfuric acid	-	-	-	+	+	-
Terpenoid	Chloroform, acetic acid concentrated sulfuric acid	-	-	-	-	-	-

Table 2. Quantitative Phytochemical Means of *C. caudatus* Leaf Extracts

Extract	Alkaloid (mg/100g)	Tannin (mgTAE/g)	Saponin (mgDE/g)	Phenol (mgGAE/g)	Flavonoid (mgQE/g)
Aqua fractionated aqua	14.837 ± 0.985	626.885 ± 9.273	19.615 ± 7.117	615.172 ± 2.438	252.031 ± 2.209
Aqua fractionated ethyl acetate	13.568 ± 0.150	9.410 ± 0.231	11.957 ±1 4.959	24.678 ± 0.162	13.484 ± 0.441
Aqua fractionated hexane	6.036±0.892	2.114 ± 2.434	2.221 ± 1.196	17.552 ± 0.487	3.797 ± 0.000
Ethanol fractionated aqua	18.881 ± 2.457	1162.798 ±13.772	18.430 ± 6.644	1073.973 ±25.751	1019.957 ± 2.187
Ethanol fractionated ethyl acetate	13.381 ± 1.074	95.991 ± 2.066	9.700 ± 2.256	71.549 ± 1.448	34.390 ± 0.000
Ethanol fractionated heksana	6.914 ± 0.696	31.634 ± 0.306	5.026 ± 4.635	26.693 ±0 .710	10.285 ± 0.965
p value	0.000	0.000	0.000	0.000	0.000

to Stevens GW et al.[28], ethanol is a versatile solvent and is excellent for preliminary extraction, extracting bioactive compounds faster. Ethanol and water are polar solvents. The research results show that 96% ethanol solvent is better than water for extracting polar compounds such as alkaloids, tannins, phenols, and flavonoids.

The results of the LD50 test showed that all mice moved agilely, behaved in kissing/ smelling each other, showed no signs of poisoning and no deaths were found. According to Gil, M.I., et al. (2002), the absence of death in experimental animals means that the LD_{50} value does not need to be determined.[16] The most severe toxicity categories (LD_{50} d" 0.005 g/kg bw), relatively low toxicity (LD50 > 2"5 g/kg bw), very low toxicity (LD50 > 5 g/kg bw).[29] The highest dose tested was 2 g /kg BW so a larger dose needs to be tested on mice to determine the LD50.

The weight of the liver and kidneys does not show a tendency to increase or decrease with the dose given. The one-way Anova test results for the difference in the weight of the mouse liver had a p-value of 0.14 (> 0.05). This means that there is no significant difference in the weight of the mouse liver between treatments and control groups. The one-way Anova test results for the difference in the weight of the mouse kidney had a p-value of 0.44 (> 0.05). This means that there is no significant difference in the weight of the mouse kidney between treatment and control groups. Most liver cells are composed of parenchymal cells such as hepatocytes, as well as other non-parenchymal cells present in sufficient numbers.. Changes in liver and other organ weights may be temporary. According Michael et al., in toxicity studies, organ weight changes are sensitive indicators of toxicity, effects on enzymes, physiologic disturbances

Table 3. Liver and Kidney Weight Mice After LD_{50}

Groups	Liver weight (gr)	Kidney weight (gr)
Control	1.257 ± 0.155	0.682 ± 0.557
P1	1.540 ± 0.144	0.235 ± 0.034
P2	1.295 ± 0.124	0.185 ± 0.031
P3	1.542 ± 0.227	0.232 ± 0.037
P4	1.637 ± 0.372	0.165 ± 0.036
P5	1.470 ± 0.208	0.215 ± 0.046
p value	0.14	0.44

Table 4. Creatinine Serum Means (mg/dl)

Groups	Creatinine Serum
P1	0.50 ± 0 .000
P2	0.55 ± 0.057
P3	0.55 ± 0.057
P4	0.50 ± 0.000
P5	0.55 ± 0.057
Control	0.50 ± 0.000
p value	0.21

Table 5. Histopathology of Mouse Liver

Mouse Number	Groups Control	P1	P2	P3	P4	P5	p value
1	3	3	3	0	3	3	0.331
2	3	0	3	3	3	3	
3	0	3	3	3	3	3	
4	0	3	3	3	3	3	

Table 6. Histopathology of Mouse Kidney

Mouse Number	Groups Control	P1	P2	P3	P4	P5	p value
1	0	0	0	0	2	2	0.002
2	0	0	1	2	2	2	
3	0	0	1	1	2	2	
4	0	1	1	1	2	2	

and target organ injury. Teo et al. stated that an increase in organ weight suggests the occurrence of hypertrophy while a decrease suggests necrosis in the target organ.[30] The liver has the extraordinary ability to regenerate to its original size after injury. The stability of liver function and size is essential for whole body homeostasis. [31,32]

One way to determine kidney function is by determining serum creatinine and urine creatinine clearance which is commonly used and highly sensitive[16,31] The one way Anova test for mean creatinine differences yielded a p-value of 0.21 (> 0.05) indicating no significant difference in serum creatinine levels between treatment groups and the control group. Normal creatinine levels in mice range from 0.2 – 0.8 mg/dl.[33] Ethanol extract of *C. caudatus* leaves had no effect on the kidney function of mice, as indicated by the

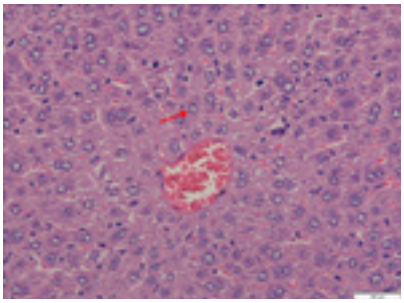

Fig. 1. Liver of Control Mice number 3, normal hepatocytes

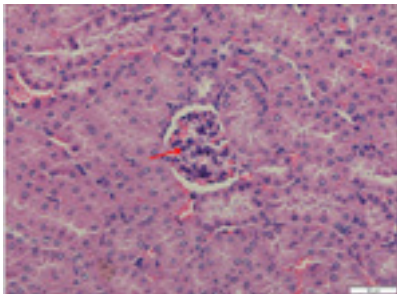

Fig 2. Control Mice Kidney number 3, normal kidney, no glomerular reduction

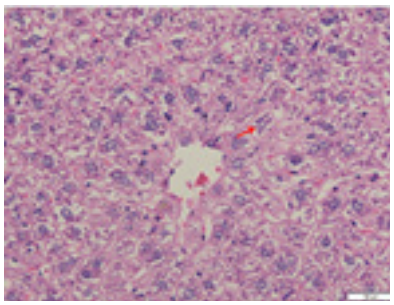

Fig 3. Liver of mice treated with 125 mg/kg bw, severe damage occurred in the form of hydrophic degeneration of the liver in all places (diffuse)

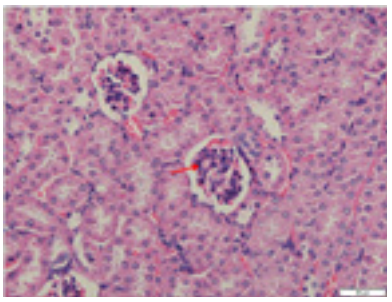

Fig. 4. The kidneys of mice treated with 125 mg/kg bw looked normal, there was no glomerular reduction

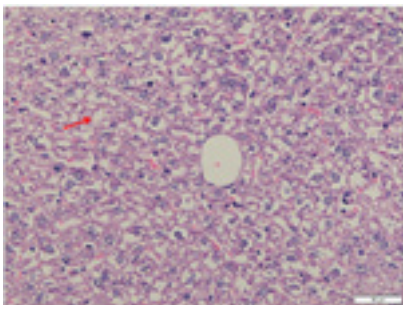

Fig. 5. Liver of mice treated with 250 mg/kg bw, severe damage occurred in the form of hydrophic degeneration in the liver in all places (diffuse)

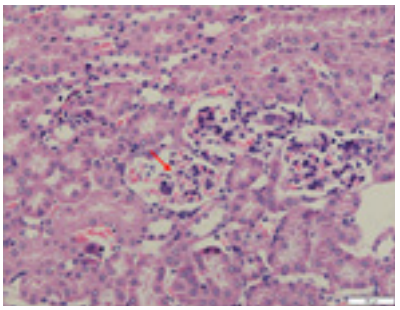

Fig. 6. Mice Kidneys Treated with 250 mg/kg bw, damage occurred in the form of mild glomerular reduction in the kidneys in one place (focal)

normal serum creatinine levels in all mice. Creatine levels can increase if there is renal or glomerular dysfunction.[34] Since this study used mice, the amount of serum obtained was limited. If the study wereconducted using rats, more serum could be obtained, which could be used to examine liver function through AST and ALT tests.

The Kruskal Wallis Liver Test results obtained a value of p = 0.331 (> 0.05), indicating that there was no significant difference in liver damage in mice between the treatment and control groups. The histopathological picture of all treatment and control groups showed that hepatocyte cells experienced a severe degree of hydrophic degeneration. The liver is an organ that has the potential to experience damage due to various chemicals and the environment because of its function in the metabolic process and as a

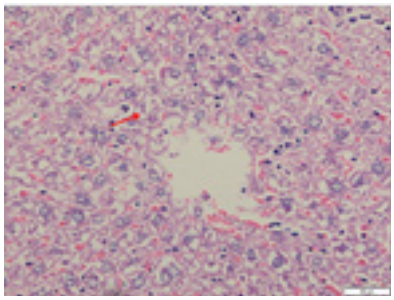

Fig. 7. Liver of mice treated with 500 mg/kg bw, severe damage occurred in the form of hydrophic degeneration of the liver in all places (diffuse)

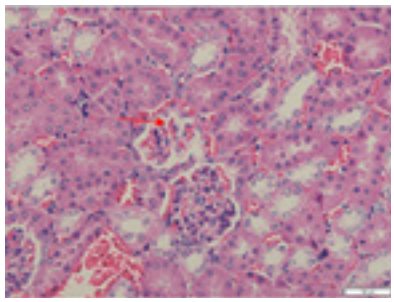

Fig. 8. Kidneys of mice treated with 500mg/kg bw, damage occurred in the form of mild glomerular reduction in the kidneys in one place (focal)

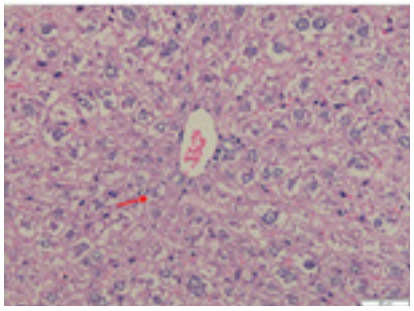

Fig. 9. Liver of mice treated with 1g/kg bw, severe damage occurred in the form of hydrophic degeneration in the liver in all places (diffuse)

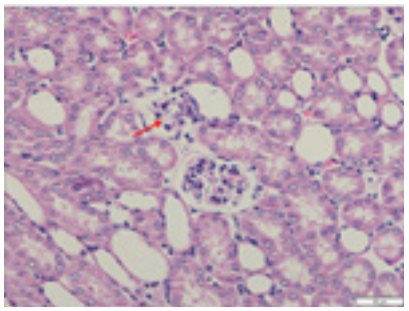

Fig. 10. Kidneys of mice treated with 1g/kg bw, damage occurred in the form of moderate glomerular shrinkage in the kidneys in several places (multifocal)

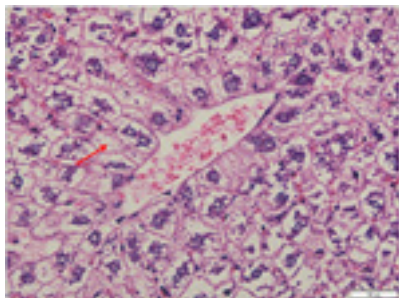

Fig. 11. Liver of mice treated with 2g/kg bw, severe damage occurred in the form of hydrophic degeneration in the liver in all places (diffuse)

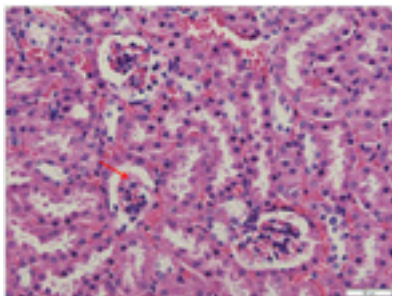

Fig. 12. Kidneys of mice treated with 2 gr/kg bw, damage occurred in the form of moderate glomerular reduction in the kidneys in several places (multifocal)

detoxification center for toxic substances in the body. Administration of toxic compounds can cause changes such as hemorrhage, congestion, degeneration and necrosis.[24]

Degeneration is divided into two types, namely parenchymatous degeneration and hydropic degeneration. Hydropic degeneration, often called ballooning degeneration, is degeneration that occurs in the liver which has the characteristics of liver cells swelling up to twice normal, and is reversible[28]. The liver plays a central role in nutrient metabolism, glucose and lipid synthesis, and detoxification of drugs and xenobiotics.[35] The liver is often the target organ that is injured by chemicals. Many compounds can cause liver cell damage. Oxidative stress results in excess pro-oxidants causing damage to cells, often resulting in cell death.[14] This research shows that after the LD_{50} test , the degree of damage to the liver is more severe than the kidneys in mice

On the other hand, the test for differences in kidney damage obtained a sig. value: 0.002 (< 0.05), meaning there was a significant difference in kidney damage in mice between the treatment group and the control group. The kidney histopathology seen in the control group was all normal, whereas in the treatment group there was multifocal damage. Kidney is an organ of the body that is susceptible to the influence of chemical substances because the kidneys work in the rest of the metabolic products of the blood so that the possibility of pathological changes is very high.[36] The susceptibility of the kidney to toxic injury is related to the complexity of renal anatomy and physiology. Nephrotoxicity can be a serious complication of drug therapy or chemical exposure.[14] Glomerular function can be observed by measuring urea and creatinine levels, but histopathological testing remains the gold standard in identifying nephrotoxicity.[37]

Many plant families contain potentially toxic alkaloids.[38] Secondary metabolite compounds in plants include alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, and others which are basically toxic to plants and animals. In some plants secondary metabolite compounds are used to defend themselves from enemies, but at certain doses they can be used as medicine.[39] Some agents can cause hypersensitivity or allergic reaction which manifests as inflammatory infiltrate in the interstitial and tubular compartment. Several plant toxicity studies show that increasing doses of herbal medicines causes cell degeneration activity which worsens the condition of kidney function. Likewise, the results of this study show that kidney damage increases with increasing dose of the test substance.[40]Possible damage to the liver and kidneys of mice after the LD50 test is due to secondary metabolites contained in the ethanol extract of *C. caudatus* leaves.

Description[41]

Score 0: normal or no damage

Score 1: there is focal (mild) damage, damage in one place

Score 2: there is multifocal (moderate) damage, damage in several places

Score 3: there is diffuse (severe) damage, damage all over the place

Damage in the form of hydrophic degeneration in the liver and glomerular shrinkage in the kidneys

CONCLUSION

The ethanol extract of *C. caudatus* leaves contains alkaloids, tannins, phenols, flavonoids and saponins. A dose of 2g/kg BW extract is not toxic to mice. After being given ethanol extract of *C. caudatus* leaves, most of the mice's livers experienced severe damage and most of the mice's kidneys were in normal condition. It is recommended to use larger experimental animals such as white mice, so that the amount of serum obtained is greater, so that AST and ALT can be checked as parameters of liver function.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper. We certify that the submission is original work and is not under review at any other publication.

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REFERENCES

1. WHO.WHO establishes the Global Centre for Traditional Medicine in India. 25 March 2022. https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india. Accessed 20 September 2023
2. Isnawati A, Gitawati R, Raini M, Alegantina S, Setiawaty V. Indonesia basic health survey: self-medication profile for diarrhea with traditional medicine. *Afr Health Sci*. 2019;19(3):2365-2371. doi: 10.4314/ahs.v19i3.9.
3. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*. 2016;21(5):559. Published 2016 Apr 29. doi:10.3390/molecules21050559
4. Ojah EO. Medicinal plants : Prospective drug candidates against the dreaded Coronavirus. *Iberoamerican Journal of Medicine*. 2020;Vol.2, num.4, pp 314-321
5. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. *Chin Med*.2018;13, 20. https://doi.org/10.1186/s13020-018-0177-x
6. Yusoff NAH, Sanuan FM, Rukayadi Y. *Cosmos Caudatus* Kunth. Extract Reduced Number of Microflora in Oyster Mushroom (Pleurotus Ostreatus). *International Food Research Journal*. 2015;22(5):1837-1842
7. Tandi J, Claresta JA, Ayu G, Irwan I. Effect of Ethanol Extract of Kenikir (*Cosmos Caudatus* Kunth.) Leaves in Blood Glucose, Cholesterol and Histopathology Pancreas of Male White Rats (Rattus Norvegicus). Indonesian *Journal of Pharmaceutical Science and Technolog*. 2018;5(1). https://jurnal.unpad.ac.id/ijpst/article/view/17247
8. Moshawih SFS, Cheema MS, Ahmad Z, Zakaria ZA, Abdullah MNH. A Comprehensive review on Cosmos caudatus (Ulam Raja): Pharmacology, ethnopharmacology, and phytochemistry. *Intl Res J Educ Sci* 1. 2017; 1(1): 14-31.
9. Ahda M, Jaswir I, Khatib A, Ahmed, QU, Syed Mohamad SNA. *A review on Cosmos caudatus* as A potential medicinal plant based on pharmacognosy, phytochemistry, and pharmacological activities. *International Journal of Food Properties*. 2023; *26*(1), 344–358. https://doi.org/10.1080/10942912.2022.2158862
10. Loo YC, Hu HC, Yu SY, et al. Development on potential skin anti-aging agents of Cosmos caudatus Kunth via inhibition of collagenase, MMP-1 and MMP-3 activities. *Phytomedicine*. 2023;110:154643. doi:10.1016/j.phymed.2023.154643
11. Eriadi A, Arifin H, Nirwanto N. Uji Toksisitas Akut Ekstrak Etanol Daun Kirinyuh (Chromolaena (L) R.M King & H.Rob) Pada Mencit Putih Jantan. *Jurnal Farmasi Higea*. 2016; 8(2)
12. Pandhare RB, Belhekar SN, Balakrishnan S, Mohite PB, Khanage SG, Deshmukh VK. Assessment of Acute and 28-Day Sub-Acute Oral Toxicity of a Polyherbal Formulation in Rats. *Int J Pharmacogn Chinese Medicine*. 2020; 4(1). DOI: 10.23880/ipcm-16000200
13. Paul A, Anandabaskar N, Mathaiyan J, Raj GM. Introduction To Basics Of Pharmacology And Toxicology: Essentials Of Systemic Pharmacology : From Principles To Practice. Springer. 2021. https://www.researchgate.net/publication/350046199
14. Ghauri AO, Mohiuddin E, Rehman T, Siddiqui HSM. Acute and subacute toxicity studies of a poly herbal formulation used for diabetes. *Pak J Med Sci*. 2022;38(6):1668-1673. doi: 10.12669/pjms.38.6.5928
15. Mohamed ES. Propolis Harvesting and Extraction. Egypt. *J. Chem*. 2023;66(1):313 - 321
16. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *J Exp Pharmacol*. 2023;15:51-62. Published 2023 Feb 8. doi:10.2147/JEP.S379805
17. Solanki SL, Modi CM, Patel HB, Patel UD. Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh, Gujarat, India. *J Pharmacogn Phytochem*.2019;8(4):3122–3126.
18. Mahani M, Wulandari E, Lembong E, Adela LHR. Correlation of Alkaloid Content and Taste of Honey from Various Provinces in Indonesia. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2022;4(12):16-21
19. Habibah N, Ratih GAM. Phytochemical Profile and Bioactive Compounds of Pineapple Infused Arak Bali. *International Journal of Natural Science and Engineering*. 2023; *7*(1):84–94. https://doi.org/10.23887/ijnse.v7i1.58776
20. Akinseye OR, Morayo AE, Olawumi AS. Qualitative and quantitative evaluation of the phytochemicals in dry, wet and oil extracts of the leaf of *Morinda lucida*. *J. Biol. Agric. Healt.,* 2017;7(7):22–25
21. Molina-Cortés A, Sánchez-Motta T, Tobar-Tosse

F, et al. Spectrophotometric Estimation of Total Phenolic Content and Antioxidant Capacity of Molasses and Vinasses Generated from the Sugarcane Industry. *Waste Biomass Valor*. 2020;11:3453–3463. https://doi.org/10.1007/s12649-019-00690-1

22. Sankhalkar S, Vernekar V. Quantitative and Qualitative Analysis of Phenolic and Flavonoid Content in Moringa oleifera Lam and Ocimum tenuiflorum L. *Pharmacognosy Res*. 2016;8(1):16-21. doi: 10.4103/0974-8490.171095
23. Mega A, Marzi L, Kob M, Piccin A, Floreani A. Food and Nutrition in the Pathogenesis of Liver Damage. *Nutrients*. 2021;13(4):1326. Published 2021 Apr 16. doi:10.3390/nu13041326
24. Zeng PY, Tsai YH, Lee CL, Ma YK, Kuo TH. Minimal influence of estrous cycle on studies of female mouse behaviors. *Front Mol Neurosci*. 2023;16:1146109. Published 2023 Jul 4. doi:10.3389/fnmol.2023.1146109.
25. Herlina H, Amriani A, Sari IP, Apriani EF. Acute toxicity test of kenikir leaf (*Cosmos caudatus* H.B.K) ethanolic extract on Wistar white male rats with fixed dose procedure method and its effect on histopathology of pancreatic cells. *J Adv Pharm Technol Res*. 2021;12(2):157-161. doi:10.4103/japtr.JAPTR_90_20
26. Singla K, Sandhu SV, Pal RAGK, Bansal H, Bhullar RK, Kaur P. Comparative evaluation of different histoprocessing methods. *Int J Health Sci (Qassim)*. 2017;11(2):28-34.
27. Firdaus MD, Artanti N, Hanafi M, Rosmalena. Phytochemical Constituents, and In vitro Antidiabetic and Antioxidant Properties of Various Extracts of Kenikir (Cosmos caudatus) Leaves. *Pharmacogn J.* 2021;13(4):890-895. https://www.phcogj.com/sites/default/files/PharmacognJ-13-4-890.pdf
28. Ariel DG, Winarsih S, Putri FF, Erwan NE, et al. BN Optimation of Combination of N-Hexane Solution and Ethyle Acetate on Secondary Metabolite Compounds Profile of Streptomyces hygroscopicus. *Jurnal Kedokteran Brawijaya*. 2021;31(3):186-192. file:///C:/Users/admin/Downloads/Optimation_of_Combination_of_N-Hexane_Solution_and%20(1).pdf
29. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdiscip Toxicol*. 2018;11(1):5-12. doi:10.2478/intox-2018-0001
30. Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, et al. Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. *Heliyon*. 2019;5(1):e01179. Published 2019 Jan 29. doi:10.1016/j.heliyon.2019.e01179.
31. Groeneveld D, Pereyra D, Veldhuis Z, et al. Intrahepatic fibrin(ogen) deposition drives liver regeneration after partial hepatectomy in mice and humans. *Blood*. 2019;133(11):1245–56. doi: 10.1182/blood-2018-08-869057
32. Michalopoulos GK, Bhushan B. Liver regeneration: biological and pathological mechanisms and implications. *Nat Rev Gastroenterol Hepatol*. 2021;18(1):40–55. doi: 10.1038/s41575-020-0342-4
33. McClure DE. Clinical pathology and sample collection in the laboratory rodent. *Vet Clin North Am Exot Anim Pract*. 1999;2(3):565-vi. doi:10.1016/s1094-9194(17)30111-1
34. Gassler N, Press A, Rauchfuß F, Theis B, Kaemmerer E. Etiology–histomorphology–entity correlation in liver pathology: a narrative review. *Ame Medical Journal*. 2022;7. https://amj.amegroups.org/article/view/6758/html
35. Chiang J. *Liver Physiology: Metabolism and Detoxification*. In: Linda M. McManus, Richard N. Mitchell, editors. Pathobiology of Human Disease. San Diego: Elsevier. 2014:1770-1782.
36. Radi ZA. Kidney Pathophysiology, Toxicology, and Drug-Induced Injury in Drug Development. *Int J Toxicol*. 2019;38(3):215-227. doi:10.1177/1091581819831701
37. Faria J, Ahmed S, Gerritsen KGF, et al. Kidney-based in vitro models for drug-induced toxicity testing. *Arch Toxicol*. 2019;93, 3397–3418. https://doi.org/10.1007/s00204-019-02598-0
38. Griffiths, M.R., Strobel, B.W., Hama, J.R. *et al.* Toxicity and risk of plant-produced alkaloids to *Daphnia magna*. *Environ Sci Eur.* 2021; 33(10). https://doi.org/10.1186/s12302-020-00452-
39. Simorangkir M, Subakti R, Barus T, Simanjutak P. Analisis fitokimia metabolit sekunder ekstrak daun dan buah Solanum blumei Nees ex Blume lokal. *Jurnal Pendidikan Kimia*. 2017;9(1):244–248.
40. Kiliœ-Pstrusiñska K, Wiela-Hojeñska A. Nephrotoxicity of Herbal Products in Europe-A Review of an Underestimated Problem. *Int J Mol Sci*. 2021;22(8):4132. Published 2021 Apr 16. doi:10.3390/ijms22084132.
41. Zotti A, Banzato T, Gelain ME, Centelleghe C, Vaccaro C, Aresu L. Correlation of renal histopathology with renal echogenicity in dogs and cats: an ex-vivo quantitative study. *BMC Vet Res*. 2015;11:99. Published 2015 Apr 25. doi:10.1186/s12917-015-0415-8