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Research Article

Combination Bitter, Ginger, Turmeric Extract in Mice: Acute and Sub Acute Toxicity Analysis

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ABSTRACT

Herbal plants are widely used as traditional medicine for generations. Some of them are bitter, in Indonesian called Sambiloto *(Andrographis paniculata),* red ginger in Indonesian called Jahe merah *(Zingiber officinale var rubrum),* and turmeric in Indonesian called Kunyit *(Curcuma domestica)*. This study tested extracts containing a combination of three ingredients, hereinafter named 'SIJAKUN' extract (**S**amb**I**loto, **JA**he, **KUN**yit)". This combination has good benefits for the body, especially as an anti-inflammatory and antioxidant. This study aims to measure the acute and subacute toxic effects of 'SIJAKUN' extract. This is a true experimental study. In the acute toxicity study, the SIJAKUN extract was given to 5 groups of mice a doses of 1000 mg/kg, 2,000 mg/kg, 3,000 mg/kg, 4,000 mg/kg, and 5,000 mg/kg. After being observed for 24 hours, the number of dead mice from each group was counted to calculate the LD50 of the 'SIJAKUN' extract. Whereas in subacute toxicity, 5 groups of mice were given 0.5 ml of CMCNa solution, 'SIJAKUN' extract at doses of 25 mg/kg, 75 mg/kg, 150 mg/kg, and 25 mg/kg ibuprofen, the test substance was given orally once daily for 28 days. Then an examination was carried out: SGOT, SGPT, BUN, and serum creatinine. the results of subsequent examinations were analyzed statistically. To assess the safety of the SIJAKUN combination, it is necessary to conduct research to determine the acute and sub acut toxicity of SIJAKUN to the liver and kidneys. Based on the results of acut toxixity study conducted, no experimental animals died at one dose of 1000, 2000, 3000, 4000 and 5000 mg/kgbb 'SIJAKUN' extract. Based on these results, the LD50 of 'SIJAKUN' extract was above 5000 mg/kgbb. Therefore, it can be said that 'SIJAKUN' extract is a non-toxic compound. In the subacute toxicity study, examination result of SGOT, SGPT, BUN, and serum creatinin was not change significantly, with P value > 0.05. This result indicating no significant difference between the control group and the treatment group. It can be said that the sub-acute toxicity test found that 'SIJAKUN' extract did not toxic for liver and kidney. In acute and subacute toxicity studies that has been carried out, 'SIJAKUN' extract was classified as a non-toxic compound, so it is safe to use as herbal medicine.

Keywords: SIJAKUN (bitter, ginger, turmeric), Acute and Sub Acute Toxicity, LD50.

INTRODUCTION

Indonesia is a tropical country which is rich in medicinal plants. In various regions in Indonesia, medicinal plants have been used as traditional medicine for various diseases. According to Basic Health Research in 2010, it was stated that consumption of traditional medicines reached 59.12% from various regions in Indonesia

Indonesia is a tropical country which is rich in medicinal plants. In various regions in Indonesia, medicinal plants have been used as traditional medicine for various diseases. According to Basic Health Research in 2010, it was stated that consumption of traditional medicines reached 59.12% from various regions in Indonesia.[1-3]

Bitter, in Indonesian called **Sambiloto** *(Andrographis paniculata L)* is one of the medicinal plants which is a top priority for development in Indonesia with several benefits such as antioxidants and anti-inflammatories.[4-5] Sambiloto contains diterpene lactones which have many uses for health and have the potential as an alternative to increase body immunity.[6-7] Some of the main components of the diterpene lactones in Sambiloto identified in the leaves are andrographolide, neoandrographolide, deoxyandrographolide, deoxyandrographolide-19-β-D-glucose and dehydroandrographolide.[8-9] This bitter plant is easy to find in Indonesia, usually growing around yards, gardens, yards, rice fields, fields, shrubs, even on the side of the road.[10-11]

Ginger, in Indonesia called **Jahe merah** *(Zingiber officinale, var rubrum)* is used in traditional medicine in Asia. It is widely used in food and beverages due to its hypolipidemic, antiemetic, antiviral, anti-inflammatory, anti-inflammatory, and anti-inflammatory properties.[12] Previous studies have shown that the addition of ginger extract increases antioxidant activity.[13] Ginger plants grow a lot in tropical and subtropical areas, and can be found in Southeast Asia, including Indonesia.[14-15] Ginger contains polyphenol components, including zingiberene, zingerone, shogaol, and gingerol which are antioxidant agents.[16] Ginger with a high water content is very susceptible to damage through the growth of microorganisms carried by moisture.[4,17]

Tumeric, in Indonesia called **Kunyit** (*Curcuma domesctica*) has been used as herbal medicine, coloring, seasoning and food additive for thousands of years in various parts of the world, especially in Asian countries. Turmeric is one of the most studied for its antioxidant, anti-inflammatory, antibacterial and anticancer properties.[18-19]

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This study aims to analyze the acute and sub acute toxicity test of the compound 'SIJAKUN' which contains a combination of Bitter (**S**amb**I**loto), Ginger (**JA**he) and Turmeric (**KUN**yit), by calculating the lethal dose of 50% (LD50). A sub-acute toxicity test was also carried out by evaluating the occurrence of toxic effects on the liver and kidneys after administration of SIJAKUN extract for 28 days. If the acute toxicity and subacute toxicity tests prove that the combination of 'SIJAKUN' extract is safe and and does not cause toxic effects on the liver and kidneys, then the SIJAKUN extract is said to be safe for consumption, so further research can be carried out with clinical trials on humans.[20]

METHODS

This is a experimental research conducted using mice as experimental animals. Research aims to do Acute and subacute toxicity test of 'SIJAKUN' extract. The study was conducted at the Laboratory of the Faculty of Medicine, University of Nahdlatul Ulama, Surabaya. The acute toxicity test was evaluated using the LD50 value, while the subacute toxicity test by giving a combination of sijakun extract for 2 weeks and check whether it causes interference with the liver and kidneys function.[21-22]

Acute Toxicity

Twelve white mice samples were subjected to the 'SIJAKUN' extract test treatment with five treatment groups randomly, namely in the first group as the first treatment group with a dose of Sambiloto-Ginger-Turmeric extract dose of 1000 mg/kgBW, the second treatment group with a concentration of dose of 'SIJAKUN' extract dose of 2000 mg/kgbw, the third treatment group with a dose of 'SIJAKUN' extract dose of 3000 mg/kgbw, the fourth treatment group with a dose dose of 4000 mg/kgbw, the fifth treatment group with 5000mg/kgBW of Sambiloto-Ginger-Turmeric extract. JAhe merah, and KUNyit extract in Indonesian and other substances such as CMCNa and Ibuprofen as a comparison.[21,23]

In this study, white mice were subjected to experimental treatment using bitter or bitter (*Andrographis paniculate*), red ginger or red ginger (*Zingiber officinale* var Rubrum Rhizoma), and turmeric or turmeric (*Curcuma domesctica*), and also, we use a combination of those three extracts called 'SIJAKUN' stand for SambIloto, red JAhe, and KUNyit extract in Indonesian. In addition, the creatinine, BUN, SGOT, and SGPT were examined. The examination was then carried out after 28 days by testing related levels of creatinine, BUN, SGPT, and SGOT to determine the stories in the test.[24-26]

Subacute Toxicity

Twenty eight samples of white mice were subjected to the test of Sambiloto-Ginger-Turmeric extract with 5 treatment groups randomly, namely the first group as the control group or the group that was only given CMCNa 05 ml, the second, third, and fourth treatment groups with dose levels of 'SIJAKUN' 25 mg/kgbw extract 75 mg/kgbw, extract 150 mg/kgbw and Ibuprofen 25 mg/kg BW. "SIJAKUN" Extract is given through oral administration once per day for 28 days, then the following examinations are carried out per treatment group: SGOT, SGPT, BUN and SC.[21-22]

Data analysis

Data analysis was carried out in this study using descriptive analysis methods, normality analysis then testing with the Kolmogorov-Smirnov test technique. Then the analysis continued with the non-parametric Kruskal-Wallis method.[27-28]

RESULTS

The combination "SIJAKUN" is a perfect combination, all three are traditional herbs used by Indonesian people for generations. Given the use in the community in the form of aqueous extracts, it is necessary to research to test the safety of a material, one of which is a toxicity test. Toxicity is the ability of a toxicant to cause damage or abnormalities in the function of a biological system.[29-30]

Acute systemic toxicity evaluates the adverse effects that occur following exposure of organisms to a single or multiple doses of a test substance within 24 hours by a known route (oral, dermal or inhalation).[31-32] Acute toxicity tests are used to determine the lethal dose/concentration of a substance that causes death in 50% of the test population (LD/LC50) during short-term exposure. They are the only standardised toxicity tests where death is the intended endpoint.[33-34] One part of the long-term toxicity test is the subacute toxicity test.

This aims to obtain data on drug poisoning or (chemical) substances used intentionally or accidentally enter the body repeatedly over a long period.[35] This test determines if the substance under study causes lesions or injuries to the body, the nature of the lesions (reversible or irreversible), and the onset of toxic effects.

a. Acute Toxicity Test

Before testing, the test animals were adapted for 7 days. After undergoing the adaptation process, the mice were given "SIJAKUN" extract orally to each group according to calculations, for 24 hours (table 1). Observations were made and the dead mice seen. Based on observations, there was no death of mice in all groups.

Based on the above data it can be seen that "SIJAKUN" extract at doses of 1000 mg, 2000 mg, 3000 mg, 4000 mg and 5000 mg/Kg BW can be said to be relatively safe because for 72 hours it did not show the death of the test animals.

Toxic behavior and symptoms after treatment were observed to see any toxic effects resulting from the administration of "SIJAKUN" extract. The results of observing toxic symptoms and behavior after administration of Sambiloto-Ginger-Turmeric extract from the five doses, look in table 2.

Table 2 shows that in the treatment of the five test groups giving "SIJAKUN" no toxic symptoms were found that attack the central nervous system and digestion, as indicated by the absence of tremors and diarrhea.

The determination of the LD50 value is obtained using the Thompson and Weil formula. This method was chosen because it has a reasonably high confidence level and is the most frequently used method. This method also uses an LD50 calculation list to make the results more accurate. However, the LD50 calculation shows no R-value in the Weil table because there was no dead mouse, so the R values obtained are 0, 0, 0, 0. Therefore, these results are ignored because no dead mice (does not reach 50%) .

If the maximum dose does not cause the death of the experimental animal, then the LD50 is expressed by the apparent LD50 by taking the full dose. So that in this study the LD50 is known as the apparent LD50, which is 5000 mg/kgBW. This result cannot be included in the Loomis criteria, because the LD50 obtained is not the real LD50.

In this study at the maximum dose there was no death in experimental animals as shown in table 2, so it is clear that the compound is included in the "Practically Non-Toxic" criteria. So that the maximum dose in humans converted to 1000 mg/kgbw, 2000 mg/kgbw, 3000 mg/kgbw, 4000 mg/kgbw, 5000 mg/kgbw in mice, where these doses did not cause death in experimental animals.

b. Sub Acute Toxicity Test

As part of a long-term toxicity test, the subacute toxicity test aims to obtain data on drug poisoning or (chemical) substances that are used

Table 1. Acute Toxicity Test in Mice.

Group	Dose	Number of Death
Group 1	1000 mg/kgbw	0
Group 2	2000 mg/kgbw	0
Group 3	3000 mg/kgbw	0
Group 4	4000 mg/kgbw	0
Group 5	5000 mg/kgbw	0

Table 2. Observations of toxic symptoms and behavior in mice after administration of "SIJAKUN" extract 1000 mg/kgbw , 2000 mg/kgbw , 3000 mg/kgbw, 4000 mg/kgbw, 5000 mg/kgbw.

Group	Observation result
Dose 1	-No toxic symptoms that affect the central nervous system - No diarrhea and normal stool color (black)
Dose 2	-No toxic symptoms that affect the central nervous system - No diarrhea and normal stool color (black)
Dose 3	-No toxic symptoms that affect the central nervous system - No diarrhea and normal stool color (black)
Dose 4	- No toxic symptoms were seen affecting the central nervous system - No diarrhea and normal stool color (black)
Dose 5	- No toxic symptoms were seen affecting the central nervous system - No diarrhea and normal stool color (black)

Table 3. Statistical test results and levels of BUN, SGPT, SGOT, creatinine in groups 1, 2, 3, 4 and 5 after administration of SIJAKUN extract.

Treatment	Creatinin	BUN	SGPT	SGOT
Group 1	0.5	23.64	74.8	332.8
Group 2	0.36	23.74	71.4	391.9
Group 3	0.44	20.27	119	341.6
Group 4	0.36	28.05	97.6	410
Group 5	0.44	24.11	74.6	350
P value	0.431	0.604	0.702	0.317

Table 4. Levels of creatinine, BUN, SGOT & SGPT in administration of sub-acute SIJAKUN extract.

Treatment	Creatinin	BUN	SGPT	SGOT
K	0.47 ± 0.59	27.33 ± 37.03	43.8 ± 79.1	100.3 ± 546.2
P1	0.52 ± 0.56	21.92 ± 37.65	33.9 ± 54.9	116.5 ± 407.2
P2	0.52 ± 0.55	26.59 ± 32.15	22.2 ± 51.5	98.3 ± 302.6
P3	0.46 ± 0.54	28.52 ± 44.23	33.7 ± 62.9	147.7 ± 559.9
P4	0.47 ± 0.54	24.17 ± 30.69	33.5 ± 72.7	114.6 ± 399.1
P Value	0.136	0.208	0.201	0.700

Table 5. Criteria for Classification of Test Preparations.

Toxicity Level	LD_{50} oral	Classification
1	≤ 5 mg/kg	Super Toxic
2	5-50 mg/kg	Very Toxic
3	>50-500 mg/kg	Toxic
4	>500-2000 mg/ kg	Moderate Toxic
5	>2000-5000 mg/kg	Mild Toxic
6	>5000 mg/kg	Non Toxic

intentionally or accidentally enter the body repeatedly, over a long period.

Table 3, data analysis with Kruskal-Wallis obtained by group shows that the administration of Sambiloto-Ginger-Turmeric extract to mice in the levels of creatinine, BUN, SGOT, SGPT there was no significant difference between treatment groups 1 to 5 because P> 0, 05 which means there is no toxicity in all treatments performed (Figures 1 and 2).

Serum creatinine, BUN, SGPT, and SGOT levels were obtained from the control group (K) or group 1 given CMCNa, group 2 was given "SIJAKUN" extract at a dose of 25 mg/kgbw, group 3 was given a dose of SIJAKUN extract 75 mg/kgbw, group 4 with SIJAKUN dose of 150 mg/kg weight group 5 given ibuprofen at a dose of 25 mg/kgbw are presented in table 4. a combination of sijakun extract given for 2 weeks.

Table 4, analysis of data using the non-parametric Kruskal-Wallis obtained by group shows that the administration of SIJAKUN extract to mice with creatinine, SGOT, SGPT levels did not show a significant difference between the control group and the treatment group because P> 0.05, which means There was no toxicity in all treatments performed.

DISCUSSION

In the acute toxicity test of SIJAKUN extract, mice had no deaths. So that in the LD50 assessment it is stated that it can take the maximum dose, then the SIJAKUN Extract compound is "Not Toxic". Based on the LD50 table 5 of the Regulation of the Food and Drug Supervisory Agency Number 10 of 2022 concerning Guidelines for In Vivo Preclinical Toxicity Tests that an acute study has no toxicity, if the LD50 reaches 5000 mg/kgbw.[36] In this study, at a dose of 5000 mg/kgbw, no experimental animals died.[21-23]

SGOT and SGPT serum levels were not significantly different between the first to fifth treatment groups of mice given Sambiloto-Ginger-Turmeric extract (SGOT: p=0.70 ; SGPT: p=0.201), the statistical test results indicated that the SIJAKUN extract had no effect toxic, and does

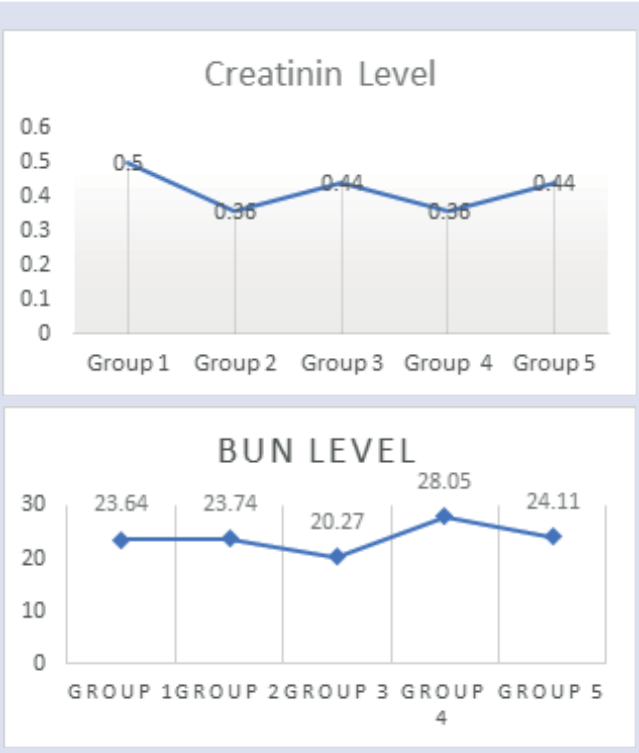

Figure 1. Creatinine values and BUN values to describe kidney function.

Note:
Group 1: only given CMCNa 05 ml
Group 2: a dose of 'SIJAKUN' extract 25 mg/kgbw
Group 3: a dose of 'SIJAKUN' extract 75 mg/kgbw
Group 4: a dose of 'SIJAKUN' extract 150 mg/kgbw
Group 5: given Ibuprofen 25 mg/kg BW

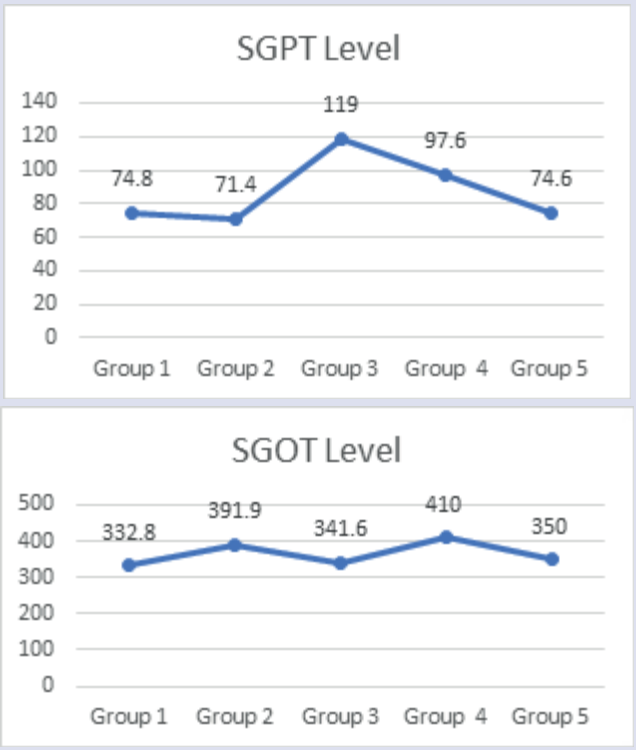

Figure 2. SGPT values and SGOT values to describe liver function.

Note:
Group 1: only given CMCNa 05 ml
Group 2: a dose of 'SIJAKUN' extract 25 mg/kgbw
Group 3: a dose of 'SIJAKUN' extract 75 mg/kgbw
Group 4: a dose of 'SIJAKUN' extract 150 mg/kgbw
Group 5: given Ibuprofen 25 mg/kg BW

not cause damage to hepatocyte cells. If there is severe cell damage, there will be an increase in SGPT and SGOT levels simultaneously, up to two times, even up to 20-100 times the normal levels.[37-38]

A very high increase in SGPT enzyme levels accompanied by an increase in SGOT enzymes is an indicator that indicates severe liver damage. Conversely, in cases of long-lasting liver damage, it will cause a decrease in the level of this enzyme. This is due to damage to the hepatocyte cell membrane so some enzymes can exit through the cell membrane.[37,39]

Toxicity tests on experimental animals have also shown convertible results in humans. According to Jaffri et al (2007), an increase in SGPT enzyme levels up to 1.5 times normal in male and female patients indicates acute hepatitis. Twofold increase in SGPT or SGOT enzyme levels indicated liver disease.[40-42]

Statistical test results (p=0.431) showed that doses one to five did not affect creatinine levels. Therefore, creatinine levels for research rats are still included in the normal category. Recent studies support this finding, with standard creatinine levels in Sprague Dawley rats being 0.3-0.8 mg/dl.[43-44]

Saka et al (2012) stated that creatinine level is a calculation of the concentration of creatinine in urine, blood serum, and urine flow rate on urea disposal. Creatinine levels determine the glomerular filtration rate of the kidney and kidney function, so plasma creatinine and urea concentrations can be used as indicators of nephrotoxicity.

While the results of statistical tests (p= 0.208) on BUN levels also showed that doses one to five did not affect BUN levels. Increased creatinine and BUN simultaneously can indicate impaired kidney filtration. Blood urea nitrogen (BUN) is the end product of protein catabolism.[45] Creatinine is the end product of creatine metabolism in muscles. BUN and creatinine levels may increase if renal or glomerular disorders occur.

In the sub-acute toxicity test in the administration of the SIJAKUN extract, death occurred in one of the mice treated with the extraction of the SIJAKUN extract at a dose of 150 mg/kgbw. mice stress, cage conditions that are less than ideal, and the mice's endurance is lacking.

In the analytical test, there was no significance of sub-acute toxicity, so the sub-acute toxicity test in mice with SIJAKUN extract did not have a toxic effect or cause damage to kidney and liver function. Death under 50% of the total sample was still classified as non-toxic.

The limitations in this study could be since before sampling the mice livers were not examined, so there is a possibility that when the mice were taken as samples, they had experienced previous damage. In addition, limitations in this study can also occur due to factors that can affect research results such as feeding and drinking that are not according to standard and lack variety, less than ideal cage conditions, stress factors for mice, the influence of other substances or diseases, as well as other internal factors such as resistance and susceptibility of mice. Furthermore, some docking experimental using *in silico* approach needed to predict and add the data of this research.[46-48] Therefore, the research will be more accurate and can be useful for all person.

CONCLUSION

From the results of the research that has been done, it can be concluded as follows:

1. In Acute toxicity test, LD50 value of the SIJAKUN extract is known to have an LD50 of 5000 mg/kgbb so that it is included in the practically non-toxic criteria, and does not show clinical symptoms of significant acute toxicity that occurs in all experimental animals.
2. Subacute tests related to the administration of "SIJAKUN" extract to mice in the levels of creatinine, BUN, SGOT, SGPT there was no significant difference between the control group and the treatment group because P> 0.05, which means there was no toxicity in all treatments given.

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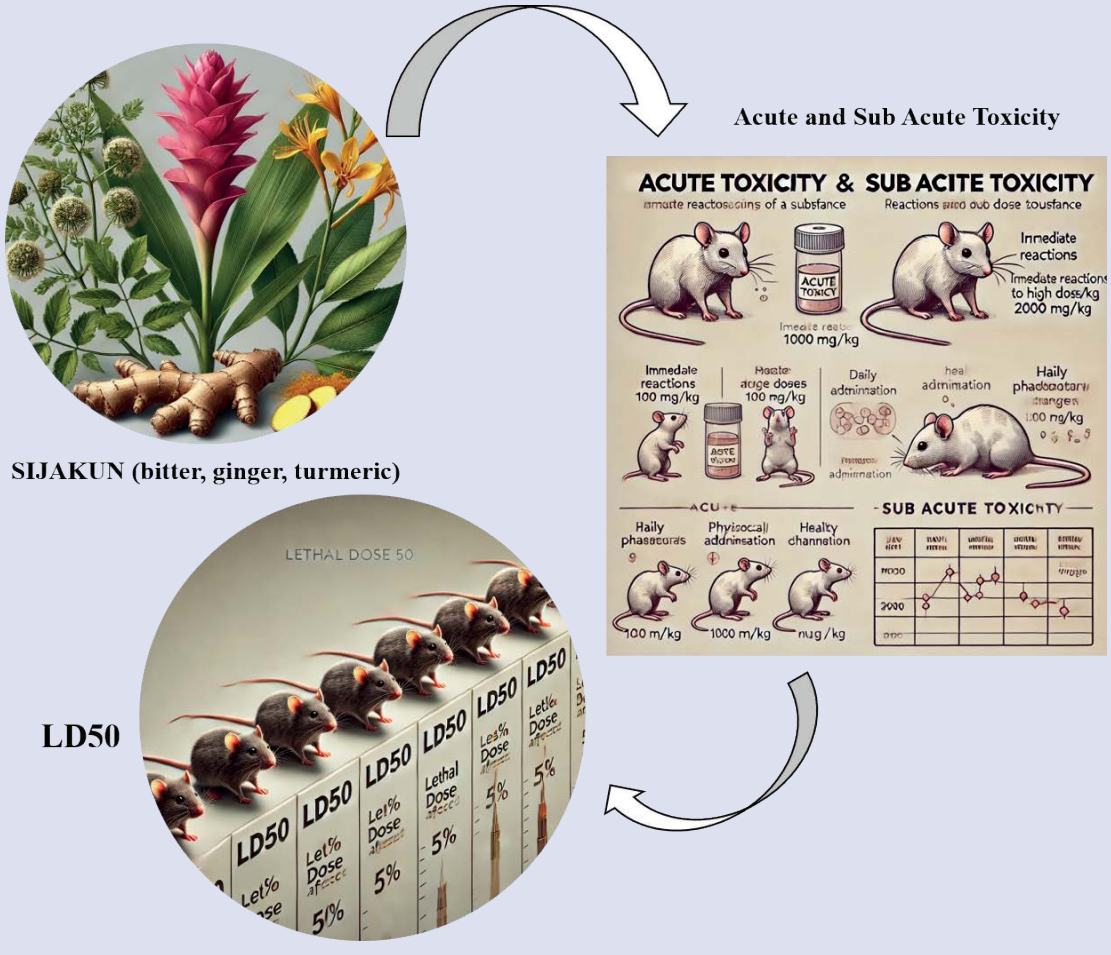
GRAPHICAL ABSTRACT



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