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Original Research Article

Toxicological Safety Evaluation of Ethanol Extract of *Artocarpus altilis* Leaves in Wistar Rats (*Rattus norvegicus*)

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ABSTRACT

Safety testing of toxicological properties of a drug could be obtained from preclinical trials using test animals as models designed for a series of toxicity tests. This study was aimed at evaluating the acute toxicity of the ethanol extract of *Artocarpus altilis* leaves in rats. Ethanol extract was prepared from the leaves of *A. altilis*. Twenty rats were randomly divided into four groups of five rats per group: one control group and three treatment groups that were given oral administration of 500, 2,000, and 5,000 mg/kg body weight (BW), respectively of ethanol leaf extract of *A. altilis*. The animals' general toxicological behavior and mortality were observed every 24 hours. The rats were sacrificed on the 15th day and the body weight, relative organ weight, biochemical parameters, and histopathology of the vital organs were analyzed. The results indicated that the extract did not affect the body and relative organ weights of the rats. Conversely, the biochemical parameters showed that alanine aminotransferase (ALT) and creatinine increased in the 5,000 mg/kg treatment group. Meanwhile, there was no significant difference ($P>0.05$) in aspartate transaminase (AST) and urea. In addition, the lethal dose (LD_{50}) of the extract was higher than 5,000 mg/kg BW. There was no evidence of toxicity in the rats after acute exposure to the ethanol extract of *A. altilis* leaves. The findings of this study reveal that oral administration of the ethanol extract of *A. altilis* leaves is considered safe and is not associated with any distinct toxicity or side effects.

Keywords: Acute toxicity, *Artocarpus altilis*, Ethanol extract, Female rats, Toxicology.

Introduction

More research is needed to discover new drugs through a series of preclinical and clinical testing on efficacy and safety in the physiological system. Medicine that is of high quality should be effective, safe, and inexpensive.[1] It is important to test the safety of new drugs to ensure that, in addition to being beneficial, they do not cause additional physiological problems when administered. Furthermore, a safety assessment of a drug's toxicological properties offers information on the cumulative effect of a dose that may cause toxic effects in humans. Preclinical experiments using test animals as models for a series of toxicity tests could provide this information.[2,3]

Artocarpus altilis (Parkinson) Fosberg (Breadfruit) is a plant that is commonly found in Indonesia. It has long been used to cure liver cirrhosis, hypertension, diabetes,[4] gastric ulcers,[5] and other ailments in addition to being a food source. Furthermore, *A. altilis* contains flavonoid compounds, namely; artocarpin, morucine, cycloartobiloxantho, cycloartocarpin A, artoindonesianin V, and artoindonesianin U.[4,6,7] Previous studies have shown that the flavonoids in this plant have anti-inflammatory, antibacterial,[8,9] cytoprotective, and wound healing effects.[10,11] The effectiveness of this plant ought to also be backed up by safety tests to ensure that its use does not create any negative consequences.

To obtain a safe and effective novel drug, this study was, therefore, conducted to provide comprehensive information on the safety of using standardized extracts from the leaves of *A. altilis*.

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Materials and Methods

Plant material

Leaf blades of *A. altilis* were collected from the Pancur Batu area, Deli Serdang, North Sumatera from June to July 2020. The plant leaf specimens were identified at the Herbarium Bogoriense, Indonesian Institute of Sciences (LIPI), Bogor, West Java, Indonesia (Identification number B-24/IV/DI.01/2/2021). Impurities were removed from the fresh leaves, and the tip of the leaf midrib was removed. To obtain simplicia, the leaves were dried at 40°C.

Preparation of plant leaf extract

The maceration method was used to extract 1 kg of powdered leaves in 10 L of 96% ethanol solvent for 5 days, with frequent stirring and protection from sunshine. On the fifth day, the macerated mixture was daubed, and the leaves were squeezed and rinsed using a solvent. The solvent was reintroduced after a two-day break. The macerated mixture was then filtered once more, and all the macerated products were combined and evaporated through a rotary evaporator.

Also, the macerated mixture was concentrated in a water bath to produce a thick extract. For the toxicity test, the viscous extract was prepared in the form of a suspension dosage using carboxy methyl cellulose (CMC) as a suspending agent.

Experimental grouping and treatments

In this study, healthy non-pregnant female Wistar rats of 8 to 12 weeks, weighing 120 to 140 g were used. The animals were obtained from the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, Indonesia. Before testing, the subjects were acclimatized under laboratory conditions for 7 days and were given a standard diet and drinking water *ad libitum*.

A total of 20 rats were randomly divided into four groups, each of which contained five rats: one control group and three treatment groups, each of which received a 0.5% sodium-CMC suspension at a

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dose of 1% BW and an extract suspension at doses of 500, 2,000, and 5,000 mg/kg, respectively.[12]

Acute toxicity testing

Acute toxicity testing in this study was carried out according to the Organization for Economic Cooperation and Development (OECD) guideline for testing of chemicals, with procedure number 420.[12] The rats were fasted for 18 hours before starting the treatment and had unlimited access to water. The extract suspension was administered to the rats in a single dose, and they were then fed after 4 hours. Furthermore, observations were made for a period of 14 days. The first 30 minutes after providing the test preparation were spent closely observing the behavior and indications of toxicity. It was also made every four hours for the first 24 hours and once a day for the next 14 days. All the rats were sacrificed on day 15 for clinical biochemical and histopathological examinations of vital organs.[12] The Animal Research Ethics Committee (AREC) of Universitas Sumatera Utara, Indonesia approved the testing protocol (Approval number 0638/KEPH-MIPA/2020). Tremor, salivation, diarrhea, limp, sedation, coma, convulsions, walking backward, and walking on the stomach were all the observable toxic signs. From the first day of treatment until 14 days afterward, the number of deaths was recorded. The death data were examined using the LD_{50}, and any rat which was terminally sick was sacrificed and counted as dead.[12]

Measurements of body and relative organ weights

Each week during observation, the animal's body weight was measured before being given the test preparation treatment.[12] The relative organ weight was calculated by dividing the organ weight by the test animal's weight. The heart, liver, kidneys, pancreas, and uterus were among the organs examined. They were washed with saline solution, dried with absorbent paper, then weighed to obtain absolute organ weight.[12]

Clinical biochemical and histopathological analyses

The values of aspartate transaminase (AST), alanine transaminase (ALT), urea, and creatinine were among the clinical biochemical tests determined.[12] The animals were immediately autopsied, and macro pathological observations for each organ were made. The color, surface shape, and consistency of the heart, liver, kidneys, pancreas, and uterus were all visually examined. The organs of the heart, liver, kidneys, pancreas, and uterus were isolated and immersed in 10% formaldehyde. They were paravinated and excised for staining with hematoxylin and eosin. A microscope attached to a computer was used to visualize the colored pieces.[12]

Statistical analysis

The quantitative data obtained were analyzed statistically using the Statistical Package for Social Sciences (SPSS) version 22.0 program. The data were analyzed for normality and homogeneity among the variants. The normal and homogeneous data were also evaluated using the one-way analysis of variance (ANOVA), which was followed by Tukey's post hoc test to determine significant differences. Meanwhile, the non-homogeneous data were analyzed using the Kruskal-Wallis analysis followed by the Mann-Whitney test to determine the significant differences. A difference of $P < 0.05$ was considered significant.

Results and Discussion

Plant extracts' safety could be predicted using toxicological studies on animals such as rats. As a result, the acute toxicity of the ethanol extract of *A. altilis* was qualitatively and quantitatively detected by toxic symptoms. Qualitative symptoms in the rats were observed through the influence of the extract on behavior in the form of tremors, diarrhea, salivation, limp, sedation, walking backward, and with the stomach. Acute toxicity was observed quantitatively through the death of the rats, and the biochemical parameters which included AST, ALT, and creatinine levels.[13–16]

General toxicological observations

The behavioral responses and general appearance of the rats treated with a single dosage of ethanol extract of *A. altilis* leaves revealed that no toxic symptoms were affecting the digestive and central nervous systems and that there were no tremors or diarrhea (Table 1). To determine the cause of death, the acute toxicity assessment included not only the LD_{50}, but also the rats' abnormal behavior, stimulation, and motor activity. The results of the 14-day quantitative test observations showed that none of the test animals died in either of the treatment groups as presented in Table 2. As a result, the LD_{50} was greater than 5,000 mg/kg BW. Furthermore, when acute toxicity is low, the LD_{50} does not need to be calculated, and an estimate may be more useful. Based on the ratio of body surface area, the maximum dose translation in humans to rats is 5,000 mg/kg BW.

Effects of Artocarpus altilis ethanol extract *on the body and relative organ weights*

After being administered a single dose of *A. altilis* ethanol extract, the average body weight of each group showed no significant difference between the control and treatment groups (500, 2,000, and 5,000 mg/kg BW). This indicates that the rats did not experience changes in body weight in the form of reduction, which is one of the important parameters in toxicological testing (Figure 1). The relative organ weight (Table 3) at the end of the treatment demonstrated that the ratio parameter had no significant ($p>0.05$) change between the control and treatment groups in the heart, liver, kidney, pancreas, and uterine. This suggests that a single dose of *A. altilis* ethanol extract does not influence the organ-to-body weight ratio.

Table 1: Behavioral response and general appearance of rats after treatment with a single dose of *Artocarpus altilis* ethanol extract.

Observation	Groups of treatment			
	Control	500 mg/kg	2000 mg/kg	5000 mg/kg
Tremor	Not present	Not present	Not present	Not present
Seizure	Not present	Not present	Not present	Not present
Salivation	Not present	Not present	Not present	Not present
Diarrhea	Not present	Not present	Not present	Not present
Limp	Not present	Not present	Not present	Not present
Sedation	No effect	No effect	No effect	No effect
Coma	Not present	Not present	Not present	Not present
Walking backwards	Not observed	Not observed	Not observed	Not observed
Walking with the stomach	Not observed	Not observed	Not observed	Not observed

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Table 2: Number of dead rats after treatment with a single dose of *Artocarpus altilis* ethanol extract.

Treatment	Number of rats	Number of death in 24 hours	in 7 days	in 14 days
Control	5	-	-	-
500 mg/kg BW	5	-	-	-
2,000 mg/kg BW	5	-	-	-
5,000 mg/kg BW	5	-	-	-

Effects of Artocarpus altilis ethanol extract *on clinical biochemical parameters*

When compared to the control group, the AST and urea biochemical parameters of all the treatment groups (500, 2000, and 5000 mg/kg BW) were not significantly different (P>0.05). Meanwhile, ALT and creatinine levels in the treatment group at the dose of 5,000 mg/kg BW showed a statistically significant (p>0.05) increase compared to the control group (Table 4). A common liver function test to detect liver disorders is the amount of SGPT (*Serum Glutamic Pyruvic Transaminase*) or also known as ALT. It is a transaminase enzyme made in the liver cells (hepatocytes), therefore, it is more specific for liver diseases than other enzymes. Furthermore, ALT is often found in the liver, whereas AST is more abundant in the heart, skeletal muscle, brain, and kidneys than in the liver. In comparison to AST, ALT gives more specific results, and serum levels are higher during liver damage. When the hepatocyte cell membrane is damaged, the permeability of the hepatic cells increases, allowing enzymes normally found in the cell's cytoplasm to leak into the bloodstream. The presence of an increase in the SGPT (ALT) enzyme (Table 4) that was accompanied by an increase in the AST (AST) enzyme is an indicator of long-lasting liver damage that will result in a decrease in the enzyme's level. This is caused by damage to the hepatocyte cell membrane, and some enzymes may be released through the cell membrane as a result.[17–20] According to the results obtained for the biochemical analysis, there was no significant difference in AST levels between the control and treatment groups. AST is an enzyme that is usually located in the liver cells. It is released into the blood when the liver or heart is damaged. AST levels in the blood are relevant to high levels of liver damage or heart damage (eg heart attack). This enzyme is found in small amounts in the heart muscle, kidney, and skeletal muscle. Measurement of liver function parameters in the form of circulating chemicals generated by injured or necrotic liver cells could be used to identify the extent of ongoing damage to liver cells. In many cases, enzyme assays are often the only indication of early or localized liver disease.[21–24]

Meanwhile, as indicated in Table 4, the results of the rats' creatinine levels were shown to differ significantly between the control and 5,000 mg/kg BW treatment groups. Blood creatinine levels are influenced by certain factors in the blood such as non-creatinine

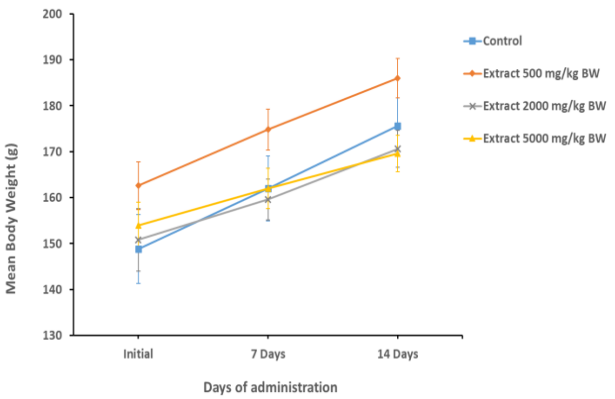

Figure 1: Changes in body weight of rats after treatment with a single dose of *Artocarpus altilis* ethanol extract.

chromogen, changes in muscle mass, and inflammatory processes. Therefore, a more sensitive and specific examination is required to describe kidney function. The kidneys are the main organs responsible for eliminating metabolic waste products that the body no longer requires. It is a component of the urinary system that filters metabolic waste from the blood, particularly urea, and excretes it along with water in the form of urine. Creatinine is also the end product of creatine. When the kidneys are damaged or interfered with, the levels of this chemical may rise.[25–29] The results of measuring urea levels in the experimental animals revealed no significant differences between the control and treatment groups given doses of 500, 2,000, and 5,000 mg/kg BW. Furthermore, the mean urea level was within normal limits (Table 4).

Organ histopathological observations

Figure 2 shows the histopathological features observed in the various organs examined, which include the heart, kidney, liver, pancreas, and uterus. The heart histopathology results in the control and 500 mg/kg BW treatment groups were both normal, with no apparent bleeding, hyperemia, degeneration, or necrosis. However, the 2,000 mg/kg BW dose began to cause hemorrhage and hyperemia in the heart muscle in the treatment group. The treatment group which received a dose of 5,000 mg/kg BW resulted in increased heart muscle bleeding and hyperemia. Furthermore, increasing the treatment dose caused increased histological damage to the liver. In the kidney, the histological figure showed a normal condition; no visible mesangial cell proliferation in the glomerulus, and Bowman's capsule was still clearly visible in the control group. Furthermore, at a dose of 5,000 mg/kg BW, the treatment group began to show mesangial cell proliferation, but no glomerular tissue hypertrophy. The histopathological features of the liver in the control and 500 mg/kg BW treatment groups were still in normal condition, as the hepatocytes were arranged radially and there was no visible hydropic degeneration or necrosis.

Table 3: Relative organ weight of rats at 14 days after treatment with a single dose of *Artocarpus altilis* ethanol extract.

Organ	Groups of treatment			
	Control	500 mg/kg	2,000 mg/kg	5,000 mg/kg
Heart	0.32 ± 0.01	0.31 ± 0.01	0.35 ± 0.01	0.38 ± 0.02
Liver	3.77 ± 0.05	3.45 ± 0.15	3.15 ± 0.15	3.58 ± 0.26
Kidney	0.33 ± 0.01	0.34 ± 0.01	0.35 ± 0.02	0.35 ± 0.01
Pancreas	0.30 ± 0.04	0.24 ± 0.03	0.21 ± 0.02	0.33 ± 0.05
Uterus	0.19 ± 0.02	0.25 ± 0.02	0.24 ± 0.03	0.22 ± 0.03

All values were expressed as mean ± SEM; n:5 animals/group; *: $p < 0.05$ (ANOVA / Tukey test compared to control group)

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Table 4: Biochemical estimation of rats' blood serum at 14 days after treatment with a single dose of *Artocarpus altilis* ethanol extract.

Parameters	Groups of treatment			
	Control	500 mg/kg	2000 mg/kg	5000 mg/kg
AST (U/L)	213.67 ± 27.64	157.33 ± 28.26	156.67 ± 23.51	234.00 ± 22.72
ALT (U/L)	99.00 ± 10.02	97.67 ± 2.85	131.67 ± 5.21	135.67 ± 10.68*
Urea (mg/mL)	27.33 ± 1.86	36.00 ± 5.29	37.67 ± 4.67	34.00 ± 3.21
Creatinine (mg/mL)	0.62 ± 0.10	0.60 ± 0.03	0.98 ± 0.06	1.28 ± 0.23*

All values were expressed as mean ± SEM; n: 3 animals/group; *: $p<0.05$ (ANOVA/Tukey test compared to control group).

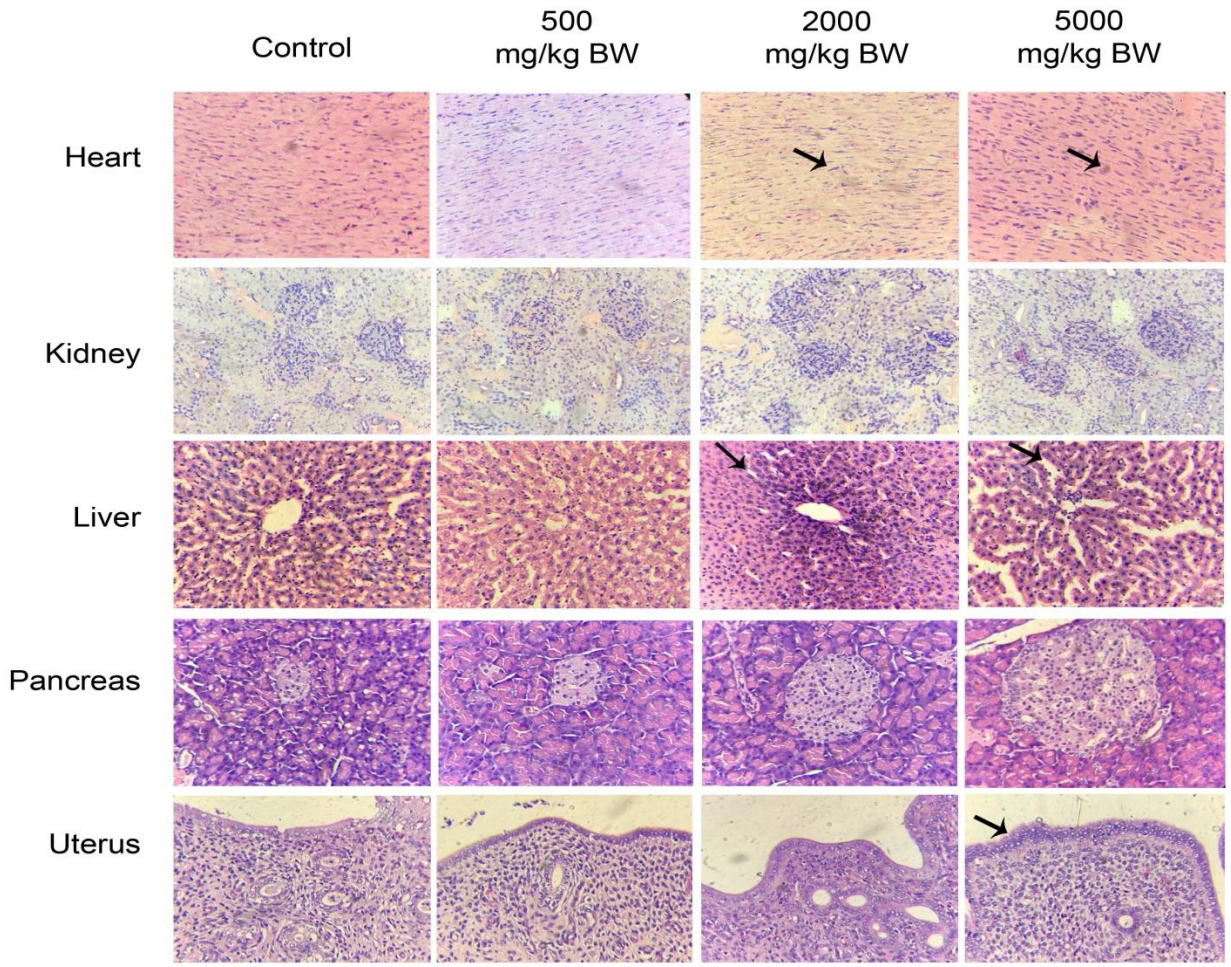

Figure 2: Histopathology of rats's organs of the treatment groups after treatment with a single dose of *Artocarpus altilis* ethanol extract.

Meanwhile, in the treatment groups, the liver began to change with the administration of the 2,000 and 5,000 mg/kg BW doses, with an irregular sinusoidal arrangement and cell expansion, and it began to cover the sinusoids, but the hepatocytes had not yet undergone necrosis. The term necrosis refers to the death of cells or tissues in living organisms. The chromatin and reticular fiber of the dead cell core may be more modest, whereas the chromatin and reticular fiber of the living cell core could be more varied. The core becomes denser, and sections of it may have separated. Necrotized liver cells might cover a large or small area.[30]

On the Langerhans islands, the histology of the pancreas in the control group looked normal. However, dosages of 500, 2,000, and 5,000 mg/kg BW in the treatment groups showed that the glands around the Langerhans islets had started to swell and had not yet experienced cell necrosis. Meanwhile, the Langerhans islet cells showed normal conditions. The reduction of Langerhans islands in the number of *β* cells and degranulation, and cell vacuolization are all changes in the cells caused by cytotoxic agents. Furthermore, in diabetes mellitus patients, some β cells show complete degranulation and empty cytoplasm.[31] The treatment groups at the doses of 500 and 2,000 mg/kg BW still showed normal epithelial luminal layers in the uterus. Meanwhile, the luminal epithelial layer in the treatment group was hypertrophied at a dose of 5,000 mg/kg BW.

Although the rats in the acute toxicity study of the ethanol extract of *A. altilis* leaves did not die, there was an increase in ALT and creatinine levels, as well as changes in the histology of their organs. The changes in biochemical properties could be an indication of changes or damage to some tissues and organs. Microscopically, there are histological changes in the liver, heart, kidney, and uterus at a dose of 5,000 mg/kg BW. Furthermore, the results of the acute toxicity testing should be followed up by sub-chronic toxicity testing to gain more insight into the extracts' long-term effects on vital organs.

Conclusion

The findings of this study suggest that oral administration of ethanol extract of *Artocarpus altilis* leaves is considered safe and showed no distinct toxicity or side effects.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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