# Acute Toxicity and Gross Pathology Effect of Leaf Extract *Tephrosia vogelii* on White Rat (*Rattus norvegicus albinos*)

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**Abstract.** Leaf extract of *Tephrosia vogelii* has been extensively tested and proven its effectiveness as a pesticide against some species of insect pests. Botanical insecticides that can be used as integrated pest management in the field are not only effective against the target pest but also meet the safety requirements of non-target organisms. The objective of this study was to test the acute toxicity and gross pathology effect of *T. vogelii* leaf extract on white rats in the laboratory. Four doses (25, 50, 100, and 200 ppm of body weight) of plant extracts were tested according to rat body weight. Five white rats were used in each treatment. The extract was given in a single dose using a blunt tip syringe. Observations were carried out five times after application and were also carried out every day for fourteen days. The variable observed were mortality of rats, consumption of un-hulled rice, and symptoms of poisoning in the internal organs of rats. The mortality data were analyzed to determine the $LD_{50}$ of the leaf extract. The result indicates that $LD_{50}$ of *T. vogelii* extract is 92.90 ppm of body weight and very toxic to treated rats. The body weights of the rats decreased on the first day after treatment. Extract of leaf *T. vogelii* reduced the consumption of rats to un-hulled rice at the three highest doses. The highest dose (200 ppm) of the extract caused a hundred percent of mortality, congestion in the liver and lungs, and inflammation in the intestines and stomach.

## 1. Introduction

The white rat (*Rattus norvegicus albinos*) was the first species to be domesticated for scientific purposes. White rats have good adaptability, so they are suitable as animal models for various types of research, including toxicology tests [1]. This type of rat is often used as an experimental animal for testing human drugs and the level of toxicity of pest poisons to humans [2]. Rats are mammals; therefore, their impact on treatment may not be much different from that of other mammals [3].

White rats are experimental animals that have various advantages. These animals are easy to handle and give fairly reliable replay values. The benefit of white rats as experimental animals is that their life cycle is relatively short, and they can reproduce quickly [1]. The anatomical structure of rats has advantages over other experimental animals, which causes rats to be unable to vomit [3]. In addition, white rats are also susceptible to plant toxicants [4]. The toxic effects of pest poison can be seen in the damage to internal organs. One method that can be done is by observing anatomical pathology.

*Tephrosia vogelii* is one of the plants that is used as a botanical insecticide. The leaves of *T. vogelii* are green and can also be used as green manure. The active ingredient components in the leaves of *T. vogelii* are tephrosin and deguelin, which are isomeric compounds of rotenone [5, 6]. The rotenone

content in the leaves of *T. vogelii* was higher than in other parts such as petioles, stems, and roots [7]. The ethyl acetate extract of purple flower *T. vogelii* leaves contains more non-polar compounds, so it is more active than white flowers [8]. *T. vogelii* plants are self-pollinating and are easily cultivated at various altitudes, and do not require special maintenance [9]. Several studies showed that *T.vogelii* leaf extract was effective in controlling *Bemisia tabaci* [10] and *Myzus persicae* on chili plants [11], *Plutella xylostella* larvae on cabbage plants [12], *Crocidolomia pavonana* larvae on Brassicaceae vegetables [13], and the nymph *Nilapavarta lugens* on rice plants [14].

Based on Indonesian Government Regulation No. 6 of 1995, Article 3 states that plant protection must be carried out with an integrated pest management (IPM) system. Insecticides that can be used in IPM, and effective against target pests, must also meet safety requirements against non-target organisms, so that they can maintain the biodiversity of organisms in an agricultural ecosystem and are safe for users. One class of insecticides that meet these requirements is botanical insecticides. However, the risk of poisoning may occur if a substance is intentionally or not entered into the body in specific doses [15]. Therefore, information about the toxicity of the leaf extract of *T. vogelii* is needed to be present.

One method to obtain toxicity information is compulsory toxicity testing using appropriate test animals. In this study, the acute toxicity test of *T. vogelii* leaf extract in white rats was carried out to determine the $LD_{50}$ value and range, and to determine the effect of *T. vogelii* leaf extract on mortality, consumption rate, body weight, and organ changes in white rats. The results of this study provide preliminary information about the toxicity of *T. vogelii* leaves for the safety of non-target organisms.

## 2. Materials and Methods

### *2.1. Research Time and Place*

This research was conducted at the Vertebrate Pest and Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture and Laboratory of Histopathology, Department of Clinic, Reproduction, and Pathology, Faculty of Veterinary Medicine, Institut Pertanian Bogor (IPB University). This research was conducted from September 2013 to January 2014.

### 2.2. Method

#### *2.2.1. T. vogelii Leaf Extraction.*

The plant material used as the extract source was the purple flower *T. vogelii* leaf obtained from the Bina Sarana Bakti Foundation, Cisarua, Bogor Regency. The leaves of *T. vogelii* were cut into pieces using scissors to get smaller sizes. The leaf pieces are placed on a tray lined with newspapers and air-dried indoors so as not to be exposed to direct sunlight. Drying is done until the leaves are dehydrated and have a crumb texture. The dried leaves were mashed with a blender until they became powder, then sieved using a 0.5 mm mesh sieve. A total of 500 g of leaves were soaked in ethyl acetate with a ratio of 1:8 (w/v) and repeated three times [13]. The immersion liquid was filtered using a glass funnel lined with filter paper and accommodated in an evaporation flask, then evaporated using a rotary evaporator with a pressure of 240 bar at a temperature of 50°C. The extract obtained was in the form of a thick, sticky, and dark green liquid. The extract was put into an extract bottle and stored in a refrigerator at ± four Celsius degree, until it tested on the rats.

#### *2.2.2. Acute Toxicity Testing in Rats.*

Toxicity describes the potential of a substance to cause direct death in high-level animals [16]. Toxicity is expressed in $LD_{50}$, a lethal dose of 50% of tested animals (rats), calculated in mg/kg (ppm) of body weight. In this study, acute toxicity testing was carried out on white rats (*Rattus norvegicus albinos*). The tested animals were obtained from the Faculty of Animal Science, IPB. The sampled rats were 2 to 3 months old, with a weight range of 150 to 210 g [17]. In this test, the first step that was prepared was to put 25 rats into 25 test cages. Every cage was ready with a bowl for food containers and a glass as a place to drink rats during the test.

Data collection in this test was carried out in two phases, namely pre-treatment and post-treatment. In both stages of the trial, the rat was fed 20 g of grain and weighed every 24 hours. Data collection on feed consumption in pre-treatment was carried out for five days. The weight of the rats before and after the pre-treatment data collection was observed. Data collection on pre-treatment consumption was to determine the average amount of grain feed consumption before application and to ensure that grain feed consumption in pre-treatment was not statistically significantly different at each treatment level. The consumption rate was converted to 100 g of rat weight using the formula [18]:

$$\text{Consumption rate (g)} = \text{Consumption of feed (g)/Body weight of rat (g)}$$

The treatment of acute toxicity testing on white rats was carried out in five replications with four levels of stratified testing [17]. The doses selected in this test were 25, 50, 100, and 200 ppm of body weight. The four treatment doses-were equivalent to extract concentrations, respectively of 0.5%, 1%, 2%, and 4% for 1 ml fed volume given to rats weighing 200 g. However, in this test, the 4% concentration cannot be tested because at that concentration, the extract cannot be dissolved evenly with solvents, emulsifiers, and aquadest. Therefore, a concentration of 3% was used for a dose of 200 ppm of body weight, so that the volume given was 4/3 times the concentration of 4%. Extract preparation for acute toxicity testing was carried out by weighing the extract as needed, then adding solvent tween 80 and methanol (1.2%). The extract that has been mixed with solvent and emulsifier is then shaken using an ultrasonic shaker. After being evenly mixed, distilled water is added to the mark. Several research results on various pests showed that the extract of *T. vogelii* was effective in causing insect mortality at concentrations of 0.1% to 2%, so the test dose was selected which, when converted to an engagement, the value was between and higher than that concentration. The control was given distilled water mixed with tween 80 and methanol (1.2%). The extract was shown once (a single dose) for each treatment. Treatment on rats was carried out orally or by mouth with a gastric probe, a special needle with a length of about 5 cm, and a blunt tip intragastrical or directly into the stomach. The extract was given once (a single dose) for each treatment. Observations were made at 15 and 30 minutes, and 1, 2, and 4 hours after treatment. The data observed were mortality and symptoms before death. The dead rats were then weighed.

After the treatment, the rat mortality was monitored, and the feed was weighed every day for 14 days [17]. Data collection of post-treatment consumption is helpful to know differences in rat feed consumption after application at all treatment levels. Rats were weighed every day for 14 days to determine the pattern of changes in body weight after application which was used as a parameter for rat health. This observation was done in determining whether there was a change in the body's resistance or the health of the rats after the extract was administered.

Rat mortality data were used to calculate the $LD_{50}$. In this study, the calculation of the $LD_{50}$ value and range uses the Thompson and Weil formula [19]. The $LD_{50}$ value is calculated by the following formula:

$$\text{Log m} = \text{Log D} + \text{d (f+1)}$$

m: $LD_{50}$ value (ppm of body weight). D: the smallest dose. d: log multiples of dose. f: factor in Weil's table.

The $LD_{50}$ range is calculated using the formula:

$$\text{Range } LD_{50} = \text{Log m} \pm 2\text{d } .\delta\text{f}$$

Log m: log $LD_{50}$ value. d: log multiples of dose. f: factor in Weil's table.

*2.3. Observation of Anatomical Pathology (PA)*

Anatomical pathology observations were carried out by necropsy on rats that died from treatment and rats that were killed at the end of treatment using chloroform [1]. Necropsy is helpful to determine changes in the condition of the organs in the rat after treatment. Information obtained from necropsy

results is also helpful in comparing effects on organs at all treatment levels. Necropsies were performed by dissecting rats and looking at changes in internal organs such as the liver, stomach, intestines, lungs, and brain.

The first stage of a necropsy is to cut the skin and muscles from the ventral to the lower part of the neck. Next, an incision is made on the scalp to facilitate the extraction of the brain. At the time of surgery, internal organs such as the liver, stomach, intestines, lungs, and brain are removed and separated using tweezers and surgical scalpels. The last stage is observing the internal organs of the rat. Changes in internal organs were observed, recorded, and documented as qualitative data.

*2.4. Data processing*

The experimental design used was a completely randomized design with five replications for each treatment. The conversion consumption of rat to the feed was processed using Microsoft Excel 2013. The conversion data were analyzed for variance using the Statistical Analysis System (SAS) for Windows version 9.1.3 with a further test at a significance level of =5%. Toxicity analysis was carried out by finding the $LD_{50}$ value and range using the Thompson and Weil formula. At the same time, the qualitative data from the anatomical pathology test results were presented as a description.

## 3. Results and Discussion

Mortality is the main variable observed in the toxicity test. Test animal mortality data is needed in determining the $LD_{50}$ value. Based on the data in Table 1, it can be seen that there are differences in rat mortality at each treatment level. Rat mortality increased with increasing doses. In control and at a dose of 25 ppm of body weight, mortality did not occur. Higher doses, i.e. 50, 100, and 200 ppm of body weight resulted in mortality of 20%, 40%, and 100%. This value indicates that the leaf extract of *T. vogelii* at a certain dose can cause mortality in rats.

**Table 1.** Mortality of rats after application

| Dosage (ppm of body weight) | Number (head)[a] | Dead (head)[b] | Mortality (%) |
|---|---|---|---|
| 0 (Control) | 5 | 0 | 0 |
| 25 | 5 | 0 | 0 |
| 50 | 5 | 1 | 20 |
| 100 | 5 | 2 | 40 |
| 200 | 5 | 5 | 100 |

[a] number of repetition; [b] number of dead rats

The leaves of *T. vogelii* contain the active ingredients tephrosine, deguelin, and rotenone which belong to the rotenoid compounds. Rotenoid compounds can inhibit the transfer of electrons in the mitochondria so that the formation of ATP in cellular respiration is reduced, so cells lack energy [20]. Cellular respiration is a catabolic (energy-producing) pathway that breaks down complex molecules into simpler compounds. The primary process of catabolism is cellular respiration which breaks down sugars and other organic materials into carbon dioxide and water. After the reshuffle, the energy stored in organic molecules can be used to carry out cellular work. Energy is the basis for all metabolic processes [21]. Administration of leaf extract of *T. vogelii*, whose active ingredient group is rotenoid, is thought to inhibit or reduce ATP formation so that metabolic processes in rats are disrupted and can cause mortality at high doses.

The rat mortality data was processed to find the $LD_{50}$ value, the lethal dose of 50% of the test animals calculated in ppm of body weight. The uses of the $LD_{50}$ value include classifying the degree of toxicity

of chemicals, evaluating the impact of accidental poisoning, as a basis for planning sub-acute and chronic research in animals, as well as providing information on the reactivity of an animal population [22].

Based on the results of Thompson and Weil's calculations, it is known that the $LD_{50}$ value of the leaf extract of *T. vogelii* is 92.90 ppm of body weight, meaning that half the population of the test animals will experience mortality at that dose. The range of values for the $LD_{50}$ leaf extract of *T. vogelii* was 92.19 to 93.61 ppm of body weight. Based on the classification of chemical toxicity according to WHO, the leaf extract of *T. vogelii* is classified as highly toxic, because the $LD_{50}$ of the extract in its liquid form is between 20 – 200 ppm of body weight. The lower the $LD_{50}$ value, the more toxic the pesticide is. Pesticides will be dangerous if they exceed a specific dose [16].

The toxic effect of one compound is not only indicated by mortality in test animals. However, it can also be described by other variables such as its effect on feed consumption and body weight, which is a graded response [22]. In this study, daily consumption data was taken from rats for five days before and 14 days after application. The feed chosen was grain, with the consideration that the grain contained the elements needed by rats. The basic needs of rats are carbohydrates 50%, protein 25%, fat 5%, crude fibre 5%, ash 5%, and vitamins [3]. The needs of these rats can be met by grain which contains 75% carbohydrates and 8% protein, as well as other constituents such as fat, fibre, and ash [23].

The effect of *T. vogelii* leaf extract on feed consumption and body weight are shown at Tables 2 and 3. Based on Table 2, the feed consumption decreased at a specific dose of treatment. The control and quantity of 25 ppm of body weight, and feed consumption before and after treatment was not significantly different. A significant decrease in feed consumption occurred in rats given extract doses of 50 and 100 ppm of body weight. Consumption data before and after treatment at a dose of 200 ppm of body weight could not be compared because 100% of the test animals died after application. The toxic effect of *T. vogelii* leaf extract on reducing feed consumption rate only occurred at doses of 50 and 100 ppm of body weight, whereas in the control and treatment doses of 25 ppm body weight, there was no toxic effect. This condition occurred because the rats experienced a recovery effect due to the treatment at relatively low doses [22].

**Table 2**. The consumption rate of treated rat on un-hulled rice before and after extract treatment

| Dosage (ppm of body weight) | Feed consumption (g/rat/day) | |
|---|---|---|
| | Before | After |
| 0 (Control) | 6.30 ± 0.29 a | 6.38 ± 0.29 a |
| 25 | 6.58 ± 1.00 a | 6.29 ± 1.10 a |
| 50 | 6.14 ± 0.35 a | 4.99 ± 0.42 b |
| 100 | 6.17 ± 0.90 a | 3.89 ± 1.68 b |
| 200 | 6.79 ± 0.96 a | - |
| Average Consumption | 6.40 ± 0.28 | 5.39 ± 1.18 |

[a] Numbers in the same row followed by the same letter showed values that are not significantly different at the 5% test level (Duncan's multiple range test)

Table 3 shows a decrease in the weight of the rats at all doses on the first day after treatment, except for the control. The highest weight loss on the first day after treatment occurred at a dose of 200 ppm of body weight. This happened because the rats had diarrhea after the treatment. This condition describes a disturbance in the organs in the rat digestive system. However, the weight of the rats increased again starting on the second day after treatment. The increase in body weight occurred due to the treatment of a short dose or a relatively low dose level so that the toxicant effect on rats could be recovered [22].

**Table 3.** Average body weight of rats before and after extract treatment

| Time | Body weight of rats (g) at dosage (ppm of body weight) | | | | |
|---|---|---|---|---|---|
| | Control | 25 | 50 | 100 | 200 |
| Before application | 168.90 | 174.43 | 169.02 | 188.50 | 161.43 |
| After application (day-) | | | | | |
| 1 | 169.11 | 172.17 | 164.58 | 180.88 | 152.21 |
| 2 | 170.69 | 176.27 | 160.29[a] | 200.41[b] | - |
| 3 | 171.22 | 174.75 | 156.50 | 200.30 | - |
| 4 | 173.57 | 179.42 | 160.82 | 203.00 | - |
| 5 | 174.19 | 182.82 | 160.96 | 205.26 | - |
| 6 | 174.85 | 181.34 | 163.06 | 208.21 | - |
| 7 | 177.43 | 185.44 | 166.78 | 211.21 | - |
| 8 | 177.97 | 184.38 | 164.01 | 209.79 | - |
| 9 | 177.58 | 186.17 | 164.70 | 209.42 | - |
| 10 | 180.13 | 190.53 | 166.66 | 214.11 | - |
| 11 | 180.94 | 191.46 | 166.70 | 212.90 | - |
| 12 | 182.06 | 191.63 | 168.45 | 215.55 | - |
| 13 | 182.67 | 192.79 | 166.62 | 217.53 | - |
| 14 | 183.27 | 192.41 | 170.64 | 218.33 | - |

[a] four repetitions; [b] three repetitions until day-14 after application

Observations of anatomical pathology show that, in general, detrimental effect of leaf extract on internal organs of treated rat occurs only in the liver and lungs (Table 4). At all doses, the liver was blocked, both in dead rats and rats that were killed at the end of the treatment. This happens because the liver is the largest organ involved in the metabolism of toxicants and has a higher capacity to bind chemicals than other organs [22]. The susceptibility of the liver to toxicants is related to its functions related to blood circulation and the substances contained in it. In control, the liver was blocked because the rats were euthanized using chloroform which can induce acute liver damage [15]. Changes in the condition of the lungs also occurred at all doses. The highest doses not only cause changes in the liver and lungs but also in the intestines and stomach. Inflammation of the intestines and stomach is an indicator of disruption of the gastrointestinal system [22].

**Table 4**. Observation of anatomical pathology on treated rat

| Dosage (ppm of body weight) | The detrimental effect of leaf extract on the internal organs of treated rat | |
|---|---|---|
| 0 (Control) | Liver | : Congestion |
| | Lungs | : Congestion |
| | Intestine | : No changes |
| | Stomach | : No changes |

| | | |
|---|---|---|
| 25 | Liver | : Congestion |
| | Lungs | : Congestion |
| | Intestine | : No changes |
| | Stomach | : No changes |
| 50 | Liver | : Congestion |
| | Lungs | : Congestion |
| | Intestine | : No changes |
| | Stomach | : No changes |
| 100 | Liver | : Congestion |
| | Lungs | : Congestion |
| | Intestine | : No changes |
| | Stomach | : No changes |
| 200 | Liver | : Congestion |
| | Lungs | : Congestion |
| | Intestine | : Inflammation (enteritis) |
| | Stomach | : Inflammation (gastritis) |

## 4. Conclusions and Suggestions

*4.1. Conclusion*

The $LD_{50}$ value of the leaf extract of *T. vogelii* was 92.90 ppm of body weight, with a value range of 92.19 to 93.61 ppm of body weight. Increasing the dose was directly proportional to the increase in rat mortality. Doses of 50, 100, and 200 ppm of body weight caused death by 20%, 40%, and 100%, respectively. Treatment of the extract caused a decrease in the weight of the rats on the first day after treatment, then increased again due to recovery. Changes in internal organs include damming in the liver and lungs, and inflammation of the intestines and stomach.

*4.2. Suggestion*

Further research is needed on the sub-chronic and chronic toxicity test of the leaf extract of *T. vogelii*.

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