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PRECLINICAL TEST CONDENSED TANNINS OF *Pluchea indica* DOSAGE BASED ON BEHAVIOURAL PARAMETERS OF MALE *Rattus norvegicus* AS CONTRACEPTIVE CANDIDATE

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ABSTRACT

Herbal medicine's development as an antifertility agent is the condensed tannins of the leaves of beluntas (*Pluchea indica*). This study was to determine the safety of *P. indica* condensed tannin extract based on the behaviour of male *Rattus norvegicus.* This research design was qualitative descriptive with two weeks direct observation of animal models and scoring based on rats' behaviour parameters. Comparative research involving 24 white male rats based on negative control group (aquades), dose groups : I (0.4 ml/KgBW), II (2 ml/KgBW), and III (2 ml/KgBW and 3 ml/KgBW). Parameters observed were body posture, motoric activity, ataxia, righting reflex, gauze test, analgesia, ptosis, and respiratory death or cardiac arrest. The result showed a significant correlation between three of the eight parameters, namely body posture, motor activity, and ptosis based on the dose that had been given. The safe dossage as an antifertility candidate for humans was range I to dose II (0.4ml/KgBW-2 ml/KgBW) because it was included in the practically non-toxic category. Therefore, this research was important to make condensed tannins safe in their application as an antifertility drug for humans in the future.

Keywords**:** Antifertility, Acute Toxicity, Behaviour, Drug Candidate, Lethal Dose

INTRODUCTION

Drugs are the main choice of society in overcoming the symptoms of the disease. However, the use of drugs could be a serious problem for health because its negative effects (Diana *et al*., 2021). The negative impact of synthetic drugs when used in the long term regardless of the dose will cause a dangerous reaction for the body. According to Creagh (2018) drugs can have an addictive stimulatory effect and long-term repeated use could cause damage pathways in the brain. Entering the modern era has not discouraged researchers to seek and study more about traditional medicines which have the advantage of minimizing side effects for the body. Traditional or natural medicines were obtained from plants or natural materials, plant extracts, animals, mineral materials, extract preparations (galenic), or derived from a mixture of these materials with the application of hereditary principles in accordance with the prevailing norms in society (Heliawati, 2017). One of the research developments as an alternative antifertility is condensed tannins extract of *Pluchea indica* leaves or in Indonesia called beluntas. Antifertility can be a drug that has a function to control the rate of population explosion problems (Shah & Jhade, 2018).

Antifertility content in medicinal compounds can control fertility and is commonly referred to as contraception derived from medicinal plants (Daniyal &

Akram, 2015). *P. indica* has been known as a medicinal plant containing lignans, sesquiterpenes, phenylpropanoids, benzoids, monoterpenes, triterpenes, sterols, alkanes, and the leaves contain hydroquinone, tannins, alkaloids, and sterols (Widyawati et al., 2011). These compounds are of course secondary metabolites of *P. indica* that exist in plants with benefits that can be applied especially as alternative medicines. The type of tannins used as antifertility is condensed tannins which has an important role derived from plant secondary metabolites and it's composed of flavan-3-ol units (Ju et al., 2021). The experiment result based on Adnan & Halifah (2000); Sugiantari et al., (2020) mentioned that steroid, alkaloids, flavonoids, and tannins are compounds that have antifertility properties, especially in the male sex. The components of alkaloids, flavonoids, essential oils, and tannins make *P. indica* a source of antifertility compounds Susetyarini et al., (2020). The safety factor is one of the parameters that need to be considered because of the application of traditional medicine using plants that have never been tested before. Preclinical testing is one of the stages that aims to determine the level of safety and accuracy of the efficacy of a compound or substance that has not been proven previously so that it requires a toxicity test and activity test (Mustapa, 2018). Preclinical and safety tests are important stages in drug development to determine the pharmacological effects, pharmacokinetic profiles and toxicity of drug candidates (Hairunnisa, 2019). Development of the application traditional medicine as antifertility which is one of the compounds to inhibit the reproductive system in living organism. This must be done to produce natural contraceptive drugs with clinically tested safety and minimal side effects.

Based on the previous experiments have been tested that tannins in plants could decrease the concentration of male *Rattus norvegicus* spermatozoa in 60 days (Susetyarini, 2013), tannins affected the weight of epididymis of male white rats that could be as antifertility (Delfita, 2014), tannins from papaya seeds were proven as an antifertility (Adani et al., 2017). However, condensed tannins compounds from *P. indica* extract as a candidate antifertility drug that are applied to male *Rattus norvegicus* based on behavioural parameters need to be studied further. The test was based on the behaviour of rats that had been treated with condensed tannins from *P. indica* leaves which were divided into three dosages: I (0.4 ml/KgBW), II (2 ml/KgBW), and III (2 ml/KgBW and 3ml/KgBW). Parameters observed from the acute toxicity test could be seen from changes in body weight, clinical symptoms, haematology, clinical biochemistry, macro pathology, histopathology, target organs, death, and general to specific effects (Siswadi, 2018). Based on the novelty, the purpose of the study was to find the right dose of condensed tannins from *P. indica* leaves from the results of behavioural-based safety tests so that they could be used as candidates for antifertility drugs in the future for human. This research is important to be developed in order to control human reproduction rate medically using natural ingredients found from the *P. indica* leaf plant based on the right dose.

METHOD

The experimental design was The Post Test Only Control Group Design with true experimental as a research model, a research design that uses two groups of subjects in the form of a control group and an experimental group with repeated treatment. The research approach used was a qualitative descriptive, an approach that describes the state of a situation or phenomenon that occurred into sentences, then separated by type to get a conclusion.

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Preparation and Extraction of Condensed Tannins of *P. indica*

The process of obtaining dried simplicial at Materia Medica Laboratory, Malang was by sorting and separating *P. indica* leaves with dirt. The next step was washing with running water to remove soil and dirt. *P. indica* leaves must be chopped before entering the drying process with a small size so that later it will be easy in the milling process. The final step was drying using an oven with temperature 60°C to obtain simplicia can be stored for a long time. This was because the water content has decreased so it did not make the texture of the dry simplicia moist. Process of extraction for condensed tannins started from 5 kg dried chopped *P. indica* leaves at Chemistry Laboratory, State Polytechnic of Malang. Those leaves were extracted by maceration method dissolved in 96% ethanol for 24 hours in a closed condition. Extraction results were evaporated to concentrate the concentration using a rotary evaporator. The generated filtrate was concentrated under the temperature of 40°C, resulting in a condensed extract. The next step was resting and re-processed to get the content of condensed tannins compounds from the simplicia extract of *P. indica* leaves. The final extract stored into dark bottle in the room temperature.

Preparation of Animal Models

Healthy adult 3-months old male Wistar rats (*Rattus norvegicus*) (n=24) weighing about ±120-200 g was obtained from Biomedicine Laboratory, Faculty of Medicine, University of Muhammadiyah Malang. Group housing condition were placed into rats' cage (47cm length x 34cm width x 15cm height) with maximum 3-4 rats for each cage, 27°C at 12 h day/night cycles, and for enrichment environment was using finely shaved wood. The male rats were observed every day and fed with pellets twice a day (morning and evening) also supplied with mineral water which put in the bottle. It was very important to maintain rats in stable condition.

Ethic Code Certification

Description of ethical approval number E.5.a/172/KEPK-UMM/VII/2021, declared to be ethically appropriate in accordance to 7 (seven) WHO 2011 standards, 1) social values, 2) scientific values, 3) equitable assessment benefit, 4) risks, 5) persuasion/ exploitation, 6) confidentially and privacy, and 7) informed consent, referring to the 2016 CIOMS Guidelines. This is indicated by the fulfilment of the indicators of each standard. This declaration of ethics applies during the period July 15, 2021 until July 15, 2022.

Treatments of Animal Models and Acute Toxicity Studies

Treatment which given to the male rats were by observing their behavior for 14 days and 1 hour in total for each day after being given a dose of plant extract. The treatment has been done by 6 repetitions after given by condensed tannins of *P. indica* through gavage method twice a day and grouped into 4 experimental groups: a) Negative control group aquades treatment; b) Experimental group I. Group treatment dose I: 0.4 ml/KgBW of condensed tannins from *P. indica* leaves; c) Experimental group II. Group treatment dose II: 2 ml/KgBW of condensed tannins from *P. indica* leaves; d) Experimental group III. Group treatment dose III as much as 3 ml/KgBW of condensed tannins from *P. indica* leaves but it went to Lethal Dose so the dosage was reduced into 2 ml/KgBW. Behavioral parameters observed included body posture, motoric activity, ataxia, righting reflex, gauze test, analgesia, ptosis, and

respiratory death or cardiac arrest.

Data Analysis

Data collection was based on the results of observing the behavior of rats during the 60 minutes observation period. Then, the scoring range 0-4 categorization is written in the observation sheets based on the parameter test instrument was adapted from faculty of medicine UMM. Those eight parameters description as follow Table 1.

Table 1. Description about behavioural parameters and scoring animal models (*R. norvegicus*)

Behavioural Parameters	Score Description
a. Body Posture	1 = awake; head and back are in straight position 2 = sleepy; head in straight position, back in tubular position 3 = sleep; head and back are in tubular position
b. Motoric Activity	0 = no movement while touched 1 = decreased motion when touched 2 = spontaneous movement when touched 3 = spontaneous movement
c. Ataxia	0 = can't walk straight 1 = slightly incoordination 2 = incoordination happens 3 = normal
d. Righting Reflex	0 = stay in one position 1 = stay in two positions 2 = stay in supination 3 = normal
e. Gauze test	1 = balanced in posture 2 = fall while gauze is upside down 3 = fall while gauze is in 90° 4 = fall while gauze is in 45°
f. Analgesia	0 = no response while feet are clamped 1 = decreased response while feet are clamped 2 = normal
g. Ptosis	1 = normal (Palpebrae are wide open) 2 = ptosis less than ½ 3 = ptosis ½ 4 = palpebrae are closed
h. Amount of death respiration or *cardiac arrest*	Depends on the amount of death

RESULTS AND DISCUSSION

Tannins can be found in plants or in different parts of herbs commonly called secondary metabolite which are astringent in nature (Sharma et al., 2019). In nature, there are two major sources of tannins i.e., natural and synthetic, which play a significant role in affecting the plants as well as human health in both positive and negative terms. This current research, condensed tannins extract of *P. indica* were tested for antifertility candidate based on the *R. norvegicus* male behavior as a preclinic test for drug safety indicator. The bodyweight of male *R. norvegicus* was weighed on the 14th day before internal organ surgery as supporting data in the preclinical test of condensed tannins on *P. indica* leaves. Bodyweight data is not used as primary data but only as secondary data to determine toxicity effect the *P. indica*

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leaves tannins was on the internal organs of rats during two weeks of observation. Previous study reported that the administration of tannins can reduce the protein digestion process by forming a complex tannins-protein that is difficult to digest and results in weight loss (Manzoor *et al*., 2020). Final rats bodyweight in Table 2.

Table 2. Male *R. norvegicus* Final Body Weight in day 14th

Dose of *P. indica*	Number of experiment (N)	Final Body Weight
Control negative (aquades)	1	156 g
	2	105 g
Dose I (0.4 ml/KgBW)	1	114 g
	2	156 g
Dose II (2 ml/KgBW)	1	117 g
	2	133 g
	3	133 g
Dose III (2 ml/KgBW)	1	129.05 g
	2	161.12 g

Explanation*: Rats final bodyweight were weighed only on day 14th using an analytical scale. Those various data obtained to be supporting data in this research.

Table 3. Data result of control negative group treatment with aquades

Time (minute)	Experiment number (N)	Score Behaviour parameters							
		BP	MA	A	RR	GT	An	P	MY
5	1	3	3	3	3	1	2	3	0
	2	2	2	3	3	1	2	2	0
10	1	3	2	3	3	1	2	3	0
	2	3	2	3	3	1	2	3	0
15	1	1	1	3	3	1	2	2	0
	2	2	3	3	3	1	2	1	0
30	1	1	3	3	3	1	2	1	0
	2	1	2	3	3	1	2	1	0
60	1	1	3	3	3	1	2	1	0
	2	1	3	3	3	1	2	1	0
	Average	1.8	2.4	3	3	1	2	1.8	0

BP=Body Posture; MA= Motoric Activity; A= Ataxia; RR= Righting Reflex; GT= Gauze test; AN= Analgesia; P= Ptosis; MY= Mortality

The observations obtained based on the data (Table 3) of the negative control group with aquades treatment, male *R. norvegicus* with experimental numbers 1 and 2 (Figure 1) obtained almost the same results. At the 5th to 10th minute of observation, the rats tend to be sleepy and asleep. Then, the 15th to 60th minute observations of the rats are in sleepy condition but the body position is awake. In this case, the awake condition means that rats with the head and back upright but not in a state of aggressive activity. The motor activity raised by the two rats tended to move spontaneously on their own or after being given touch stimulation. However, in the 15th minute, there was a decrease in the movement even though it had been touched, it was not caused by signs of poisoning. This could be possible because at the 10th minute the male white rats slept. The muscles of the body when in resting mode

would decrease so that it affected complex movements (Bringmann, 2018). Changes in movement and sensitivity may change from the previous to the next minute that caused by the previous activity such as sleep. Based on Haris *et al*., (2016) stated that group of animals which tend to be active at night, dark time is the right time to move because the regulatory function of animals is active at night due to a collection of nerves in the hypothalamus known as the Suprachiasmatic Nucleus (SCN). In addition, in general, preclinical trials on rodents assess the ability of hypnotics to promote sleep during the active phase (dark period) of nocturnal rats (Gamble *et al*., 2021).

The negative control group was only given aquades, no ataxia behaviour appeared in the form of nervous disorders caused by problems with the brain, balance system, and coordination. The cause of ataxia is usually due to impaired cerebellar function or impaired vestibular or proprioceptive afferent input to the cerebellum (Ashizawa, T., & Xia, 2016). In addition, the symptoms of the righting reflex (still in a certain position) also did not appear. Righting reflex could be seen from the position of the rats can return to its original position or not after treatment (Wijaya *et al*., 2018). The gauze test shows the results do not fall when the gauze is turned over and shaken. The gauze test used a lid from the cage in which the two male rats experimental numbers 1 and 2 were placed on the cage cover and then turned 90° and 45°. This shows that the rats do not experience damage to the coordination system.

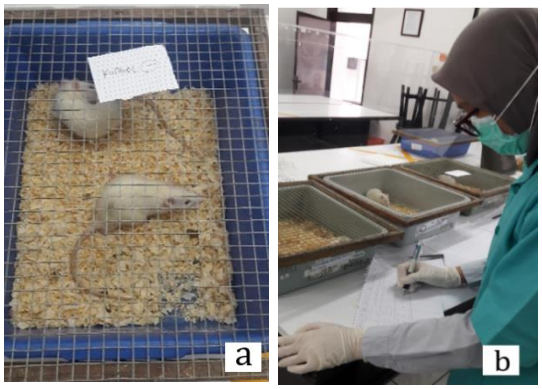

Figure 1. a) Control negative group of male *R. norvegicus* after given aquades for 14 days; b) collected observation result by observer

The analgesia test by pinching one of the legs of the rats using tweezers showed normal results and the response was spontaneously lifting its leg and making a sound of pain. This is because male white rats do not receive drugs or compounds that cause pain reduction without losing consciousness (Wulan *et al*., 2017). Ptosis is related to body posture because ptosis can be seen from the eyelids of rats closing or opening. In the control group the number of deaths is 0 male white rats were given sufficient feed in the form of bran which had been mixed with air and aquades as drinking water 2 times a day. Final body weight of rats experimental number 1 that showed in the Table 1 was 156 g and rats experimental number 2 was 105 g. The treatment of condensed tannins from the leaves of *P. indica* was carried out in the first dose group of 0.4 ml/KgBW (Figure 2), the second dose group of 2 ml/KgBW (Figure 3), and the third dose group of 2 ml/KgBW and 3 ml/KgBW (Figure 4). The duration of time used for the trial was 14 days. Based on the results of Table 4 after

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being given the first dose, the behaviour of the two rats with experimental numbers 1 and 2 for 60 minutes that could be seen are body posture, motor activity, and ptosis. The body posture of the experimental number 1 and 2 rats at the 5th minute is sleeping. Then, at 15 to 60 minutes both male white rats tend to be awake.

Table 4. Data result of Dose I Group with Dose of Condensed Tannins *P. indica* 0.4 ml/KgBW

Time (minute)	Experiment number (N)	Score behaviour parameters							
		BP	MA	A	RR	GT	AN	P	MY
5	1	3	2	3	3	1	2	2	0
	2	3	2	3	3	1	2	2	0
10	1	1	2	3	3	1	2	4	0
	2	1	2	3	3	1	2	4	0
15	1	2	3	3	3	1	2	4	0
	2	1	3	3	3	1	2	1	0
30	1	1	3	3	3	1	2	4	0
	2	1	3	3	3	1	2	1	0
60	1	2	3	3	3	1	2	4	0
	2	1	3	3	3	1	2	1	0
	Average	1.6	2.6	3	3	1	2	2.7	0

Explanation* : those data were collected on the day 14th by scoring based on the behaviour of rats for each minutes. For certain parameters such as ataxia, righting reflex, gauze test, analgesia, and mortality in the dosage 2 and 3 didn't show any change (same result). In this case, those parameters in the Table 5 and 6 were not included. BP=Body Posture; MA= Motoric Activity; A= Ataxia; RR= Righting Reflex; GT= Gauze test; AN= Analgesia; P= Ptosis; MY= Mortality

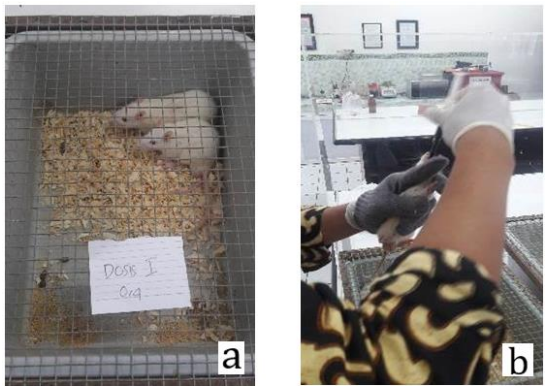

Figure 2. a) Male *R. norvegicus* dose I (0.4ml/KgBW) group; b) Treatment with condensed tannins through gavage method

The motor activity that appears is the reduction of movement after being given a stimulus in the form of touch at the 15th minute to the 60th minute still giving a response in the form of movement when touched. A decrease in motor activity can be an indication of the body's reaction to incoming toxins, causing rats to tend to be passive rather than active (Natawigena *et al*., 2018). Tannins also can have positive

and negative impact on animal performance depends on the concentration, the higher concentration, negative impact could possibly appears (Hassan *et al.,* 2020).

Ptosis can be an indicator of toxic symptoms which can be observed directly from the eyelids closed or not (Sujana *et al*., 2020). However, ptosis that occurred in the dose group I of both experimental number 1 and experimental number 2 rats dominantly showed lid closure (eyelids) of less than a half. In addition, lid closure was related to the condition of the rats' posture when the rats started drowsy and when the rats were in the awake position the lids were fully open. The results of other behavioural test parameters did not show significant changes and showed the same results as the control group, namely there was no ataxia, the straightening reflex could still return to its original position, the test did not fall when the gauze was turned over and shaken, and the analgesia response remained normal. The number of experimental animal deaths in the dose group I did not appear (0 death) either due to respiratory death or cardiac arrest. The final body weight (Table 1) in the dose group I after the treatment was given, was 114 g for the rats experimental number 1 and 156 g for the rat experimental number 2.

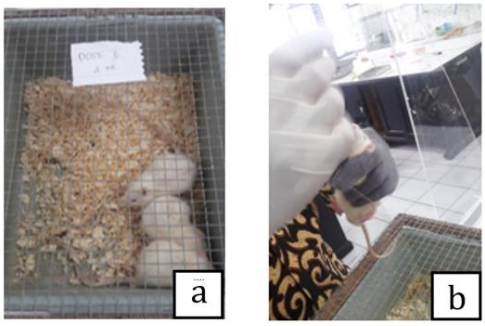

Figure 3. a) Male *R. norvegicus* dose II (2 ml/KgBW) group; b) Treatment with condensed tannins through gavage method

The results of the second dose group based on Table 5 after being given 2 ml/KgBW of condensed tannins from the leaves of*P. indica* for 14 days (Figure 3) and after being observed for 1 hour (60 minutes) the behavior shown by three male white rats for parameters the behavior of a bent body posture with the head upright and the back flat despite the previous body position. The body parameters appear based on observations caused by the nature of the rats themselves which are nocturnal animals or the body's response to the impact of condensed tannins content so that it can be an indicator of toxicity symptoms. Body posture related to the reaction with symptoms of condensed tannins toxicity can be directly related to the motor activity of the white rats themselves, then in the 10th minute the experimental number 3 rats experienced a decrease in movement that did not cause any movement after being touched. However, for male white rats experimental numbers 1 and 2 until the 30th minute, they were still in a state of spot movement when touched. Then, at the 60th minute, the male white rat experimental number 3 showed the same response as the 10th minute called non-spontaneous movements even though they were given touch stimulation. According to Endeswari (2020) stated that the decrease in movement along with an increase in the test dose can cause some animals to experience a decrease in motor activity.

The other rats not to experience this, it could be because the body's defense system captures different responses. Factors that support this to happen is the

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maximum consumption of food and drink. Based on Luthfiyah (2020), stated that while food intake is not consumed sufficiently, it causes a lack of nutrition so that metabolic performance decreases and causes the immune system to decrease and be susceptible to disease. For the ptosis parameter, the rats only closed less than a half and the results of other parameter tests such as ataxia, righting reflex, gauze test, analgesia showed the same results as the control group and dose group I. Death of experimental animals as one of the toxicity responses did not occur in the three male white rats. Both respiratory death and cardiac arrest. The final body weight (Table 1) of the three rats was 117 g for experimental 1,133 g for rats experimental number 2 and 3.

Table 5. The result of dose II group with amount of condensed tannins *P. indica* 2 ml/KgBW

Time (minute)	Experiment number (N)	Score behaviour parameters		
		Body Posture	Motoric Activity	Ptosis
5	1	3	2	2
	2	2	1	4
	3	1	2	1
10	1	2	2	4
	2	2	1	4
	3	2	0	4
15	1	3	2	2
	2	2	2	2
	3	2	2	1
30	1	3	2	2
	2	2	2	4
	3	1	2	1
60	1	2	2	4
	2	2	1	4
	3	2	0	4
Average		2.1	1.5	2.9

The experimental data obtained previously from the dose group III with the previous dose of 4 ml/KgBW experienced a Lethal Dose (LD) due to the concentration of condensed tannins in the leaves of *P. indica* which was very concentrated, causing the death of model organism where the initial symptom during the test was loss of weight, appetite, and drinking. As a result of this, on the 13th day the model organism died and had to be re-experimented for 14 days with the new rats, while the other one was taken from one of the groups from dose II. Thus, male white rats from the second dose group had to undergo follow-up observations for 14 days so that a total of 4 weeks or 28 days of observation with a fixed dose of 2 ml/KgBW. The data result of dose III treatment can be seen in Table 6.

The prominent behavior of male white rats experimental numbers 1 and 2 from 3rd dosage group are the body posture that tends to sleep with the position of the head and back flat, and motor activity begins to decrease when given a touching stimulation. Both indicators are included in the response of the rats body to the

toxicity of condensed tannins compounds in *P. indica* leaves. However, the results of other parameters, such as ataxia caused by lack of muscle control or coordination of spontaneous movements, such as walking or picking up objects (Hadjivassiliou, 2017), righting reflex, gauze test, and analgesia showed the same results as the control group, dose I, and dose II, that is, no ataxia occured, the righting reflex can still return to its original position, the gauze test does not fall when the gauze is turned and shaken, and the analgesia response remains normal. Symptoms of ptosis after administration of dose III (2 ml/KgBW for experimental number 1 rats and 3 ml/KgBW for experimental number 2 rats) dominantly closed the eyelids.

Table 6. Data result of dose III group with amount of condensed tannins *P. indica* 2 ml/KgBW and 3 ml/KgBW

Time (minute)	Experiment number (N)	Score Behaviour Parameters		
		Body Posture	Motoric Activity	Ptosis
5	**1** 2 ml/KgBW (4 weeks)	3	1	3
	2 3 ml/KgBW (2 weeks)	1	1	1
10	**1** 2 ml/KgBW (4 weeks)	2	1	2
	2 3 ml/KgBW (2 weeks)	2	1	3
15	**1** 2 ml/KgBW (4 weeks)	3	2	3
	2 3 ml/KgBW (2 weeks)	3	2	3
30	**1** 2 ml/KgBW (4 weeks)	2	1	3
	2 3 ml/KgBW (2 weeks)	3	1	3
60	**1** 2 ml/KgBW (4 weeks)	3	1	2
	2 3 ml/KgBW (2 weeks)	3	1	3
	Average	2.5	1.2	2.6

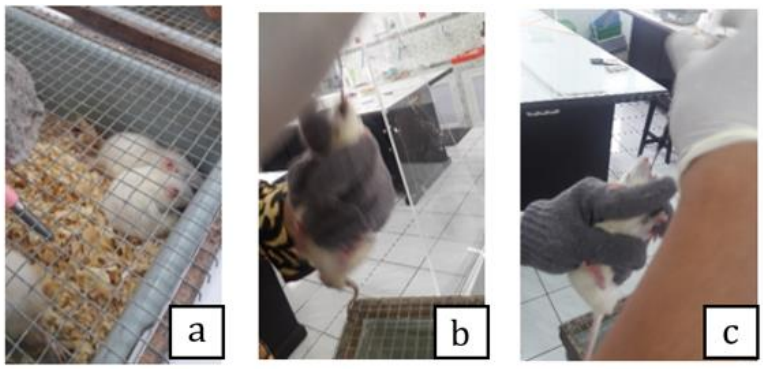

Figure 4. a) Male *R. norvegicus* dose III group; b) Treatment with 2 ml/KgBW condensed tannins through gavage method; c) Treatment with 3 ml/KgBW condensed tannins through gavage method

The third dose of 3 ml/KgBW did not cause the death of experimental animals and no symptoms of respiratory death or cardiac arrest were found. The final body weight (Table 1) of the male white rats in the group III dose experimental number 1 (2 ml/KgBW) was 129.05 g, while the experimental number 2 rats (3 ml/KgBW) was 161.12 g. Lethal dose (LD) can be used as an indicator of safety in determining a

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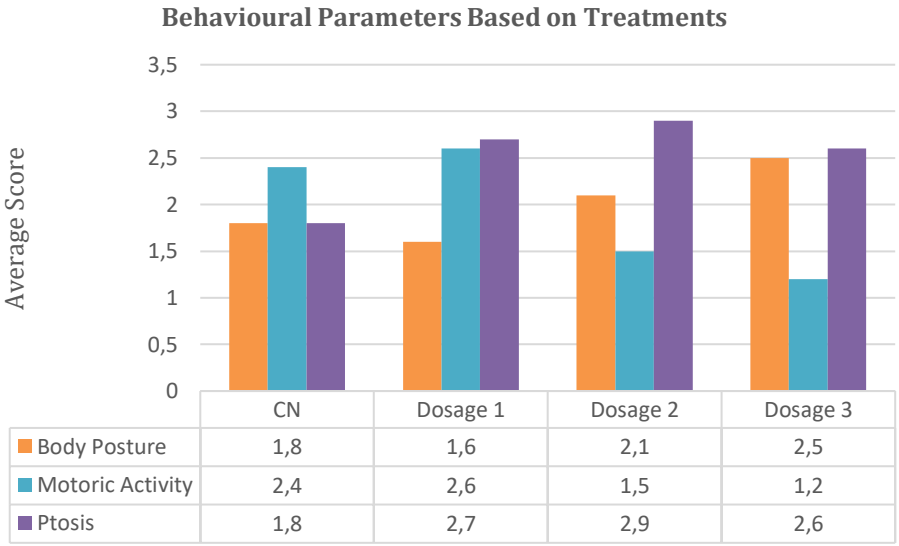
single dose of a drug or compound before being applied to the target object (Ayun *et al*., 2021). The dose level was reduced from 4 ml/KgBW to 3 ml/KgBW because the high dose caused male white rats to experience acute toxic symptoms until they died. The treatment for dose III condensed tannins *Pluchea indica* to the rats can be seen in Figure 4. Observational analysis as determining the right dose based on toxicity test parameters are body posture, motor activity, ataxia, righting reflex, gauze test, analgesia, ptosis, and death showed significant results on the parameters of body posture, motor activity, and ptosis. From the dosage has a correlation with the mortality of animal models (*R. norvegicus*) it can be seen that from the negative control treatment with aquades, dose I (0.4 ml/KgBW), dose II (2 ml/KgBW), and dose III (2 ml/KgBW and 3 ml/KgBW) did not cause mortality of experimental animals, either in terms of respiratory death or cardiac arrest, so the graph that appears is a straight line pointing to the 0 mortality rate.



	CN	Dosage 1	Dosage 2	Dosage 3
Body Posture	1,8	1,6	2,1	2,5
Motoric Activity	2,4	2,6	1,5	1,2
Ptosis	1,8	2,7	2,9	2,6

Figure 5. Graph shows average score of behavioural parameters based on treatments which already given to animal models (*R. norvegicus*)

The lethal dose was caused by the condensed tannins compound that was given too concentrated, causing intolerance and toxic effects on experimental animals. The results of research by Robinson (2013) stated that the acidic nature of tannins can cause toxic effects on rats and certain types of rats when given intravenously or after entering the subcutaneous tissue. The dose that can be used for the application of antifertility drugs to the right male white rats can be taken from the highest dose. This is supported by the results of research from Sulastra *et al*., (2020) states that the acute toxicity test has been carried out and does not cause the death of the experimental animal after being given a single dose of the test compound, to determine the LD_{50} value of the extract using the highest dose that has been given to previously tested animals. Figure 5 shows the average score from three behavioural parameters that have significant result from each treatment that already given to the rats. Those average score could be as a prediction that the side effect of condensed tannins *P. indica.*

CONCLUSIONS

The preclinical test results of condensed tannins of *Pluchea indica* leaves based on behavior of *Rattus norvegicus* have significant results, which can be seen from three of eight parameters, namely body posture, motor activity, and ptosis. Those behavior shows specific result based from different dosage comparing with negative control group. A safe dose to be applied as an antifertility drug candidate is range dose I to II (0.4−2 ml/KgBW) which is practically non-toxic. Moreover, condensed tannins extract of *P. indica* also might have side effect so in this experiment need to be further studied.

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