TOXIC SYMPTOMS IN WISTAR STRAIN RATS ON SINGLE AND REPEATED DOSAGE FROM THE EXTRACT OF RED BETEL LEAF (*Piper crocatum)* AS A TOXICITY TEST OF PERIODONTAL POCKET THERAPY

(GEJALA TOKSIK TIKUS WISTAR PADA PEMBERIAN DOSIS TUNGGAL DAN BERULANG DARI EKSTRAK DAUN SIRIH MERAH (Piper crocatum) SEBAGAI UJI TOKSISITAS TERAPI POKET PERIODONTAL)

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ABSTRACT

Red betel leaf (*Piper crocatum*) is one of the potential medicinal plants known to have properties to cure various diseases. The main components of betel leaf exhibit antiseptic, bactericidal, and antioxidant effects. The betel leaf was concentrated into an essential oil containing the antiseptic chemical. The antibacterial power of betel leaf essential oil is due to the content of phenolic compounds and their derivatives that can denature bacterial cell proteins, so it was considered to have potential as a material that could develop for periodontal therapy. After going through the stages of toxicity testing and repeated dose in terms of mortality, changes in body weight, and relative organ index of Wistar strain rats, the study continued to observe the effects that occurred after

using single and repeated doses of 100, 400, and 1000 mg/kg BW in vivo using red betel leaf extract with the parameters of observing the behavior of rats studied for 14 days. Observation of the behavior of the test animals on toxic symptoms at each visit consisted of motor activity, straub phenomenon, piloerection, ptosis, corneal reflex, pineal reflex, lacrimation, vasodilation, catalepsy, dangling, reestablishment, walking backwards, circular walking, flexion, haffner, writhing, grooming, tremor, body shaking, vocalizations, urination, defecation, heart rate, salivation, mortality, posture, and breath changes before and after dosing for 14 days. From the observations, the analysis of the data obtained is primary data. Primary data in the form of qualitative data was obtained from data on effects that occurred after the rats were given a dose. After processing the data, the data is presented in tabular and narrative form based on the observations by showing the test dose used, the number of animals showing a toxic effect, the time of onset of a toxic effect and the percentage of animals in a group showing a toxic result. Based on the results of the study, red betel leaf extract with doses of 100, 400, and 1,000 mg/kg BW which was observed on the first and 14th days, did not cause toxic symptoms in all groups of rats, both on single administration and repeated administration for 14 days.

Keywords: red betel leaf; toxicity; toxic symptoms

ABSTRAK

Daun sirih merah (Piper crocatum) merupakan salah satu tanaman obat potensial yang diketahui memiliki khasiat untuk menyembuhkan berbagai penyakit. Komponen utama daun sirih menunjukkan efek antiseptik, bakterisida dan antioksidan. Daun sirih dapat dipekatkan menjadi minyak atsiri yang mengandung kandungan kimia antiseptik. Daya antibakteri minyak atsiri daun sirih karena kandungan senyawa fenolik dan turunannya yang dapat mendenaturasi protein sel bakteri sehingga dinilai berpotensi sebagai bahan yang dapat dikembangkan untuk terapi periodontal. Setelah melalui tahapan pengujian toksisitas

dan dosis ulangan dari segi mortalitas, perubahan bobot badan dan indeks organ relatif tikus galur wistar, penelitian dilanjutkan dengan mengamati efek yang terjadi setelah penggunaan dosis tunggal dan ulangan 100, 400, dan 1000 mg/kg BB secara in vivo menggunakan ekstrak daun sirih merah dengan parameter pengamatan perilaku tikus yang diteliti selama 14 hari. Pengamatan perilaku hewan uji pada gejala toksik pada setiap kunjungan terdiri dari aktivitas motorik, fenomena straub, piloreksi, ptosis, refleks kornea, refleks pineal, lakrimasi, vasodilatasi, katalepsi, menjuntai, retablishment, berjalan mundur, berjalan melingkar, fleksi, haffner, menggeliat, grooming, tremor, tubuh gemetar, vokalisasi, buang air kecil, buang air besar, denyut jantung, air liur, kematian, postur dan perubahan napas sebelum dan sesudah pemberian dosis selama 14 hari. Dari hasil observasi, analisis data yang diperoleh adalah data primer. Data primer berupa data kualitatif diperoleh dari data efek yang terjadi setelah tikus diberi dosis. Setelah pengolahan data, data disajikan dalam bentuk tabel dan narasi berdasarkan pengamatan dengan menunjukkan dosis uji yang digunakan, jumlah hewan yang menunjukkan efek toksik, waktu timbulnya efek toksik dan persentase hewan dalam satu kelompok. menunjukkan efek toksik.

Berdasarkan hasil penelitian, ekstrak daun sirih merah dengan dosis 100, 400, dan 1.000 mg/kg BB yang diamati pada hari pertama dan ke-14 tidak menimbulkan gejala toksik pada semua kelompok tikus, baik pada pemberian tunggal maupun pemberian berulang selama 14 hari.

Kata kunci: *daun sirih merah; gejala toksik; toksisitas*

INTRODUCTION

Traditional medicine has a significant role in the health of the Indonesian people, so this traditional medicine has the potential to be developed.[1] Traditional medicine to treat certain diseases is carried out using concoctions of medicinal plants.[2] One medicinal plant still used by the community to cure various diseases is red betel (*Piper crocatum*).[3] Red

betel originated from Peru, then spread to Indonesia and multiple parts of the world. Red betel is a shrub with segmented stems and stemmed leaves and a flat-edged, shiny leaf.[4]

Some conditions must be met when developing a new drug to advance to the following use stage. To ensure safety conditions, it is necessary to test first.[5] Natashya (2021) carried out an antibacterial test, with the results that 25%, 50%, 75%, and 100% red betel leaf extract had an antibacterial effect in inhibiting the growth of *Streptococcus sanguinis.*[6] In addition to antibacterial tests, testing is also needed to see and evaluate the impact of red betel leaf extract with repeated doses. The compound is given every day for approximately 10% of the animal's life.[5] The animals used in this study were rats because the rat response was fast, easy to obtain in large quantities, and gave an idea of what can happen to humans.[7]

Previous research (2020, 2021) was carried out regarding the toxicity test of red betel leaf extract in the *Swiss Webster* mouse viewed from death, weight changes, and the relative organ index of heavy rats with a single and repeated dose in vivo and the results are no toxic symptoms at the whole rat group.[8,9] Based on this research, there is no observation of toxic symptoms with a single and recurring dose that was assessed from the behavior or symptoms of toxicity to rats. The author continues the study by conducting research on the effects that arise on Wistar strain rats after the use of a single and recurring dose, namely with a dose of 100, 400, and 1,000 mg/kg BW in vivo using red betel leaf extract with parameters in the form of observation of mouse behavior that shows toxic symptoms studied for 14 days. This study was conducted to obtain information about the effects of substances that were not detected in previous studies and the possibility of toxic effects after repetitive testing on Wistar strain rats.

The study looked at toxic symptoms with pharmacological screening in rats. Observation of the behavior of the test animals on toxic symptoms at each visit, namely motor activity, straub phenomenon, piloerection, ptosis, corneal reflex, pineal reflex, lacrimation, vasodilation, catalepsy, dangling, reestablishment, walking backwards, circular walking, flexion, haffner, writhing, grooming, tremor, body shaking, vocalizations, urination, defecation, heart rate, salivation, mortality, posture, and breath changes before and after dosing for 14 days. Toxicity tests need to be carried out before using red betel as a treatment to assess the safety that can be used in humans. Based on the background that has been

described, the formulation of the problem can be obtained as follows: Does red betel leaf extract (*Piper crocatum*) cause toxic symptoms in rats?

METHOD

This study's research type is an experimental laboratory with a research design that is Pre-Test-Post Test Control Group Design in vivo. The research was conducted at the Pharmacology Laboratory of the Faculty of Pharmacy, Jenderal Achmad Yani University, Cimahi.

The object used in this study were Swiss Webster rats with the criteria of being healthy and having normal activities. The test animals were obtained from the Bandung Institute of Technology (ITB).

It was extracted using red betel leaf from the Manoko plantation, Lembang, West Java. The red betel leaf is determined first at SITH Bandung Institute of Technology so that the morphological characteristics are satisfactory. 1 kg of red betel leaf was weighed using an analytical balance, then washed with water and dried using a 50C blower oven for 24 hours until dry simplicia was obtained. 200 g of simplicia was mashed with a blender until it became powder and stored in a tight place.[10] 200 gr of red betel leaf powder was weighed. The powder was put into a thimble (filter paper cover) and applied to the soxhlet for soxhletation. 2 liters of 50% ethanol were put into a round bottom flask. Extraction was carried out until the dripping liquid looked clear. After that, freeze-dry was done until the liquid dried up and formed up to 10 grams of powder.[11,12] The determination of the dose was carried out under the guidelines of the Food and Drug Supervisory Agency, which were given in acute toxicity tests for traditional medicinal ingredients. The dose given was two times the maximum dose of 10000 mg/kg BW. The maximum dose was 5000 mg/kg BW, half the dose was 2500 mg/kg BW, a quarter dose was 1250 mg/kg BW, and one-eighth was 625 mg/kg BW. The volume of test preparations administered orally to test animals typically did not exceed or less than 1 ml/20 g body weight. Then for each increase in the dose of red betel leaf extract *(Piper crocatum)* each was dissolved with 20 ml of 0.5% CMC suspension. After calculating the dose, the volume of suspension of red betel leaf extract *(Piper crocatum)* was obtained according to the body weight given to the test animals.

The test was carried out on Wistar strain rats. Before the research, the rats were given a period of adaptation (acclimatization), this adaptation period was carried out so that the rats were not stressed and could get to know the new environment. During this adaptation period,

they were fed with standard pellets and given water regularly for five days.

In the single-dose group, the test animals were given a dose once on the first day and observed for 14 days, while in the group with repeated doses, the test animals were given an amount every day for 14 days. A total of 40 rats with a division of 20 males and 20 females were grouped into eight groups consisting of 5 male rats in 1 group and five female rats in 1 group. In the control group, five male rats and five female rats were only given food, drink, and 0.5% of *Carboxymethylcellulose Natrium* (CMC-Na). Then the test group was assigned a test preparation of red betel leaf extract (*Piper crocatum*) with doses of 100, 400, and 1000 mg/kg BW orally using oral probes repeatedly.

This study used 40 rats in each group that was given single or repeated doses, with the distribution of 20 males and 20 females. These 40 rats were divided into eight groups, and each group consisted of five male and five female rats. Rats had to fast for 4 hours before being given a dose so that the red betel leaf extract could be absorbed maximally by the digestive system. One control group was only given food, drink, and 0.5% *Carboxy Methil Cellulose Natrium* (CMC-NA). The other group was given red leaf extract orally with doses of 100, 400, and 1,000 mg/kg BW using a syringe and an oral probe. The volume of dosing to test animals is normally 1ml/100g body weight and a maximum of 5ml. Observations were made on test animals before and after being given the test preparation. The test animals were observed in each treatment group for 14 days. Observations on animals include changes in behavior such as snooping, motor activity, straub phenomenon, piloerection, ptosis, corneal reflex, pineal reflex, lacrimation, vasodilation, catalepsy, dangling, reestablishment, walking backwards, walking in circles, flexion, haffner, writhing, grooming, tremor , body shaking, vocalizations, urination, defecation, heart rate, salivation, mortality, body posture and changes in breathing.[13]

From the observations, the analysis of the data obtained was primary data in the form of qualitative data, namely the effects that arose after the rats were given a dose. After processing the data, the data were presented in tabular and narrative form by indicating the test dose used, the number of animals showing a toxic effect, the time of onset of a toxic effect, and the percentage of animals in a group leading a toxic effect.[14]

This research was conducted after obtaining approval from the Preclinical Ethics Commission of the Faculty of Pharmacy, Jenderal Achmad Yani

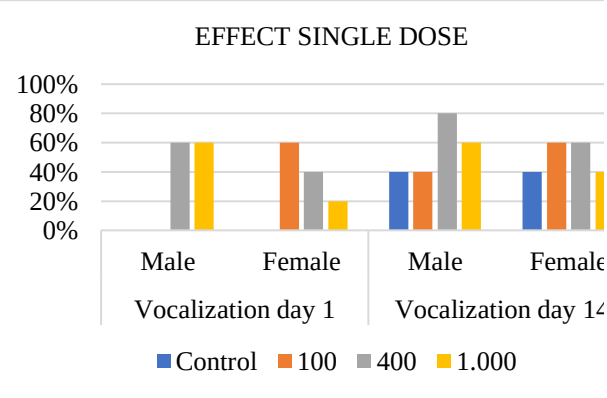
University, with the ethical approval letter no. 7015/KEP-UNJANI/X/2020.

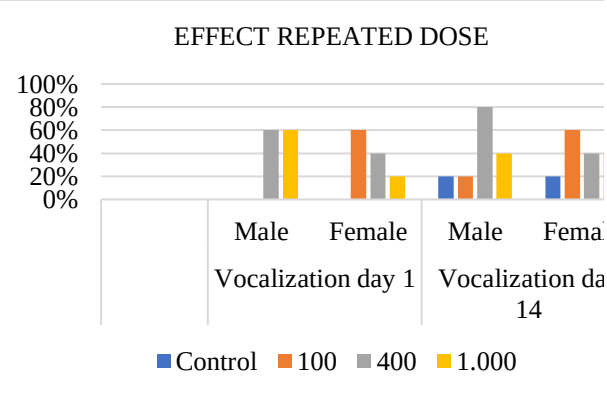
RESULT

Observation of the Gastrointestinal and Urinary Systems

Observations of the gastrointestinal and urinary systems included observing rats' urination, defecation, salivation, and vocalization.

Table 1. Results of Vocalization Observations Day 1 to 14



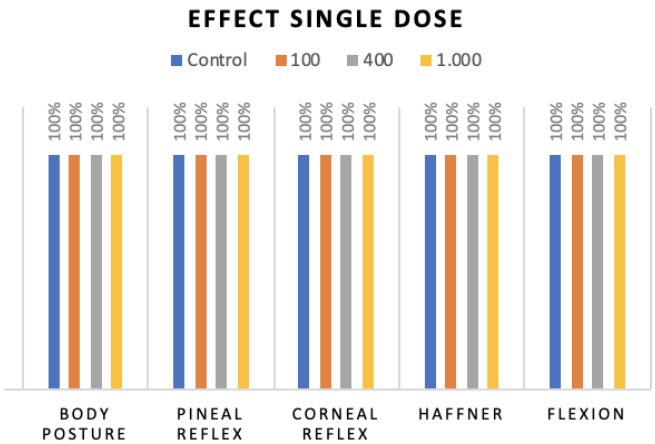


Vocalization is a treatment response such as a louder voice, and vocalization was used as communication before rats mate. Table 1 shows an increase in vocalizations in both the single-dose and repeated-dose groups.

Nervous / Sensory System Observation

Observations of the Nervous / Sensory System include rats' gestures, movements, and responses. Observation of rat body posture was assessed from the fast reaction of rats turning their bodies back to their original position after the researcher stretched the rats' bodies.[15] Observations of responses or reflexes from rats were also carried out, including observing the pineal, corneal, haffner, flexion, dangling, and reestablishment reflexes.

Table 2. Observations of reflexes and posture in male rats on day 1



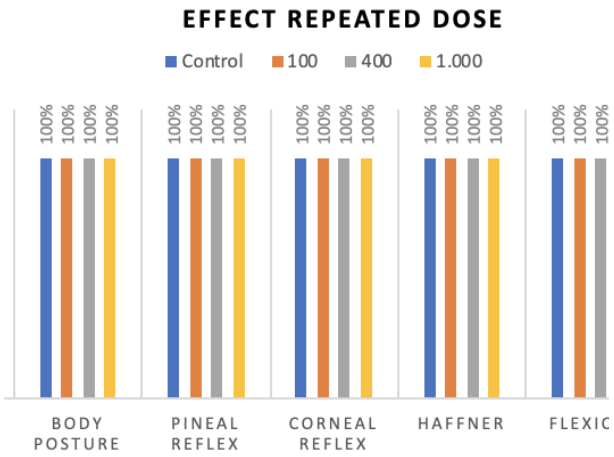
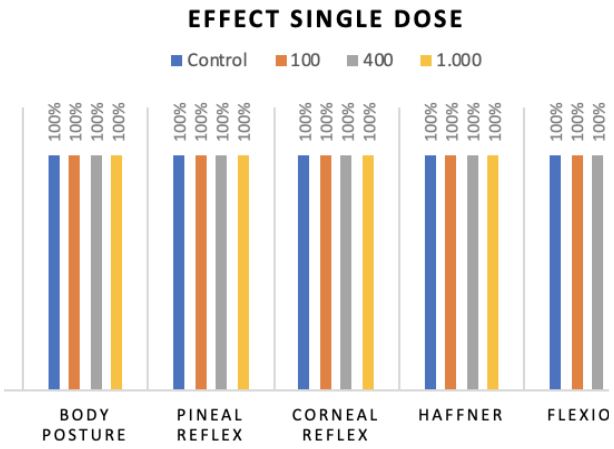


Table 3. Observations of reflexes and posture in male rats on day 14



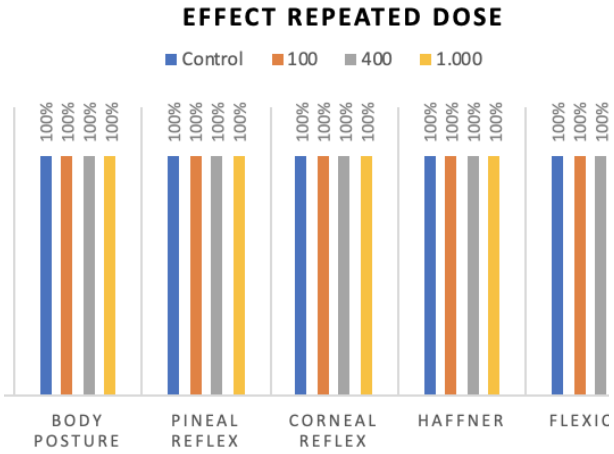
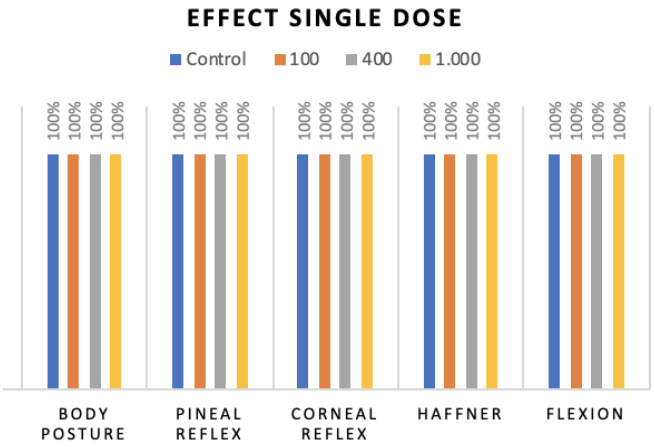


Table 4. Observations of reflexes and posture in female rats on day 1



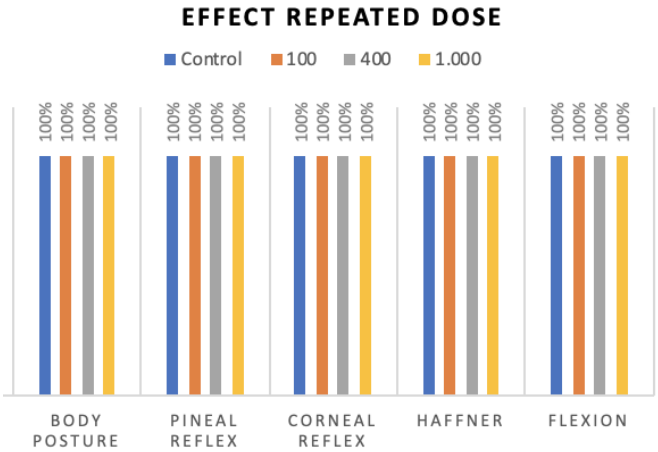
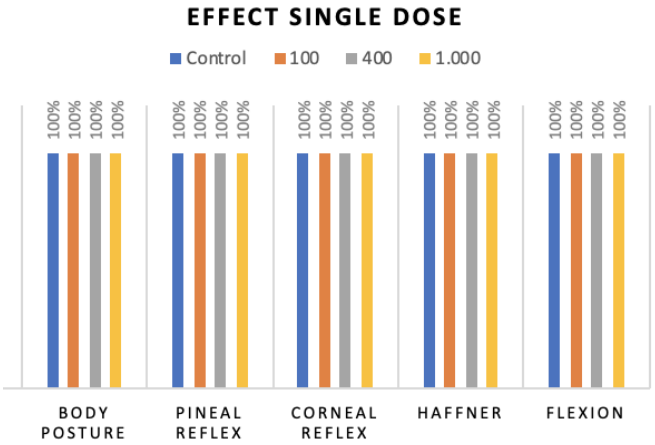
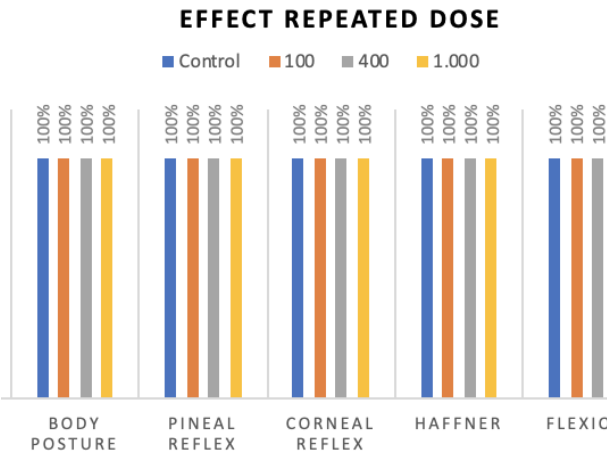


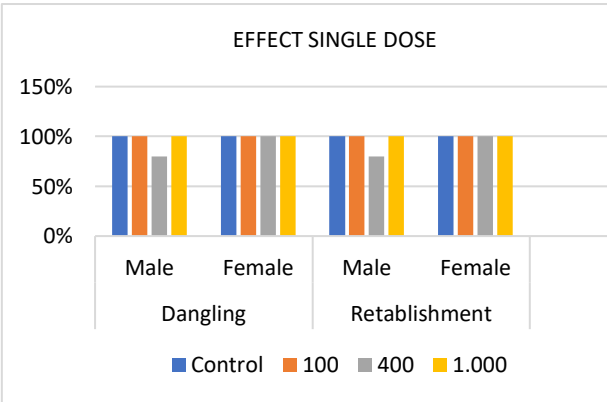
Table 5. Observations of reflexes and posture in female rats on day 14





From the observations in Tables 2 to 5, it was found that the red betel leaf extract in the control dose groups, 100, 400, and 1,000 mg/kg BW had 100% results, which means that the test material did not interfere with the eye, ear, tail or foot reflexes of the male and female rats. It showed that the red betel leaf extract does not inhibit the activity of the rat's sensory nerves.

Table 6. Observations of dangling and reestablishment of rats on day 1



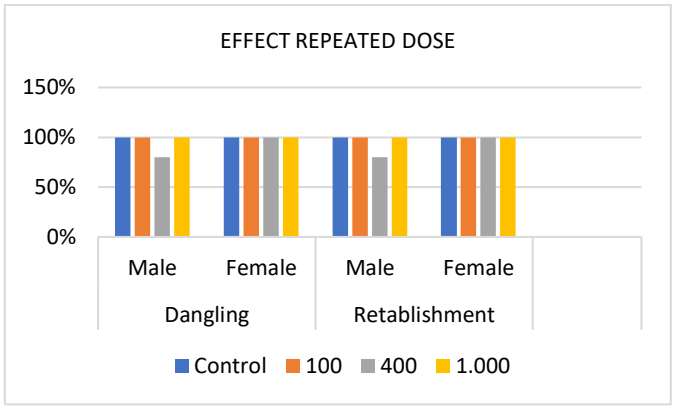
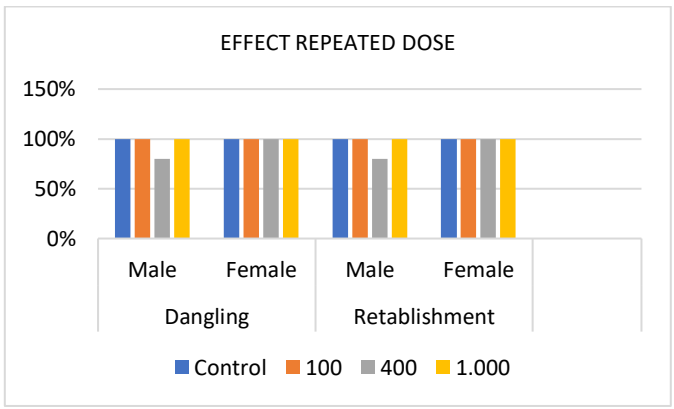
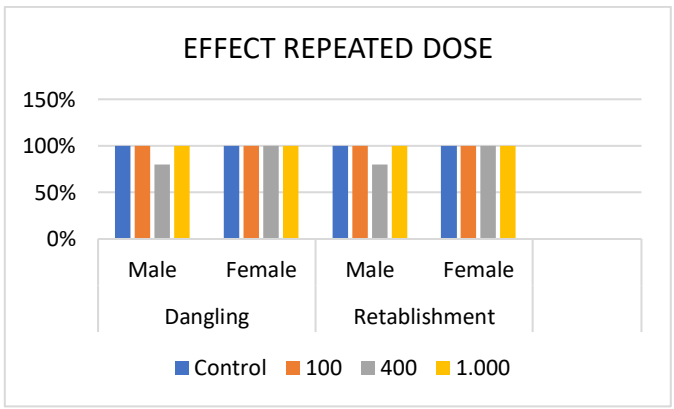


Table 7. Observations of dangling and reestablishment of rats on day 14





Tables 6 and 7 show the ability of male and female rats to perform dangling and reestablishment, and good presentation results were obtained. There were no significant changes after being given red betel leaf extract with varying doses to the ability of rats to dangle and to return the

body position to the top after hanging upside down (reestablishment). One male rat in the 400mg/kg BW dose group was able to perform dangling and reestablishment, but did not last for 5 seconds, so the percentage in that group was recorded as 80%. The rats did not experience disturbances in the central nervous system and muscles because, overall, the rats were able to perform dangling and retablishment.[12]

Cardiovascular Observation

Cardiovascular observations were carried out by observing whether there was an increase or decrease in the heart rate of the rats and the vasodilation seen on the surface of the ears of the rats after being given various doses of red betel leaf extract. The results obtained on days one and 14 showed no increased or decreased rat heart rate, vasodilation, or bleeding experienced.[16]

Muscle Observation / Neuromuscular

Neuromuscular observations were carried out to observe whether there was an increase or decrease in muscle function of rats from the administration of red betel leaf extract in the dose variation group. In neuromuscular observation, the observation was made on the motor activity of rats. From the observations, it was found that the motor activity of rats was inversely proportional to the increase in the dose of red betel leaf extract given.[12]

In addition to observations of motor activity, observations of catalepsy, writhing, tremors, straub phenomena and mortality in rats were also carried out.[18] During the 14-day observation, none of these things happened to all rats in the control group or the group with varying doses, so it was found that the extract did not decrease muscle function or did not block the neuromuscular transmission pathway.

Rat Behavior Observation

Observations of rat behavior were carried out visually by observing the position of the head, pointing up and down, commonly referred to as head-dipping, grooming response, walking backward, and walking in circles. Head-dipping observations were made to find out the curiosity of the rats.

Table 8. Observations of head-dipping of Rats on Day 1

Observed effect Single dose		**Control**	**100**	**400**	**1.000**
Head-dipping male	Up	21	32	27	33
	Down	14	38	13	21
Head-dipping female	Up	33	43	34	34
	Down	28	20	13	45

Observed effect Repeated dose	**Control**	**100**	**400**	**1.000**

Head-dipping male	Up	22	23	25	20
	Down	23	25	14	21
Head-dipping female	Up	28	30	31	21
	Down	18	22	19	20

Table 9. Observations of head-dipping of Rats on Day 14

Observed effect Single dose		**Control**	**100**	**400**	**1.000**
Head-dipping male	Up	23	29	24	29
	Down	26	20	15	21
Head-dipping female	Up	23	43	35	31
	Down	28	14	22	28

Observed effect		**Control**	**100**	**400**	**1.000**
Head-dipping male	Up	21	38	46	58
	Down	33	24	23	31
Head-dipping female	Up	22	53	50	37
	Down	32	31	22	22

Observations of head-dipping were done by observing the rats for 1 minute and then counting the number of rats raising their heads and lowering their heads. The greater the number of head-dipping was, the more curious the rats were. [16,17] In Tables 5.8 and 5.9, the results showed that in the variation group, the number of head-dipping tended to increase, while in the control group, it was observed to decrease, and the whole group of rats did not walk backward/circles.

Table 10. Grooming response Observations Day 1-14

Observed effect Single dose		**Control**	**100**	**400**	**1.000**
Grooming day 1	Male	0	20 %	40 %	40 %
	Female	40%	60 %	60 %	60 %
Grooming day 14	Male	0	0	20 %	20 %
	Female	0	0	40 %	60 %

Observed effect Repeated dose		**Control**	**100**	**400**	**1.000**
Grooming day 1	Male	0	40 %	40 %	40 %
	Female	20%	60 %	60 %	60 %
Grooming day 14	Male	0	20 %	0	20 %
	Female	40%	20 %	40 %	40 %

Grooming activity is a normal reaction in rats, namely the movement of the rat's hand lifting towards the chest. Still, grooming can also indicate a response from the rat's body after being exposed to a foreign object so that it can adjust. The data in Table 10 show grooming activity in male and female rats at a single dose, indicating that the higher the dose, the higher the grooming activity, especially in females. It proved that female rats are more sensitive than male rats. The high grooming response in rats that were given a dose could cause by the taste of red betel leaf extract, which is very bitter and uncomfortable in the mouths of rats.[45] In Table 10, repeated doses show that the grooming response has decreased

because the rats can adapt to a foreign environment. At the beginning of being given red betel leaf extract, several groups of rats showed a high percentage, presumably due to the bitter taste of red betel leaf extract, so the rats felt discomfort in their mouths.[12]

Eye Observation

Observations on the eyes were carried out by observing the ptosis and lacrimation reactions. Ptosis occurs when the rat's eyelids wilt or close by about 50%, and lacrimation is the release of tears from the rat. From the observations on the first day and the 14th day, all groups of rats did not get ptosis and lacrimation, so this shows that the extract did not affect the optic nerve.[13]

Skin Observation

Dermal observations were carried out by observing the appearance of rat fur and the occurrence of swelling. Observations on the fur of the mice were whether the hairs were standing or normal after being given red betel leaf extract with various doses. On the results of observations of the skin, it was found that there was no piloerection (rat hair stood up).[17]

DISCUSSION

Undesirable effects of herbal ingredients generally occur due to non-standardized ingredients, incorrect dosages, effects of combinations of constituent compounds, hygroscopic and volumetric properties, or possible contamination by other microbes. Undesirable effects can also arise due to the addition of compounds in herbs. Unwanted side effects and misuse can cause harm.[17]

Oral administration of ethanolic extracts of herbal ingredients causes the active substances contained in the extracts of these herbal ingredients to be absorbed into the digestive tract. The active substance then undergoes distribution and metabolism processes. Toxic metabolic products work as enzyme inhibitors for the next metabolic stage; the reaction between active substances and receptors in effector organs causes poisoning symptoms. Each sample used will give a different response at a specific dose. The difference in the sensitivity level of each sample caused the difference in response.[18]

This research looked at the toxic symptoms with pharmacological screening in rats. Observation of the behavior of the test animals was carried out on toxic symptoms at each visit, namely observations of motor activity, straub phenomenon, piloerection, ptosis, corneal reflex, pineal reflex, lacrimation,

vasodilation, catalepsy, dangling, reestablishment, walking backwards, circular walking, flexion, haffner , writhing, grooming, tremor, body shaking, vocalizations, urination, defecation, heart rate, salivation, mortality, posture, and breath changes before and after dosing for 14 days.[19,20]

Observations of the gastrointestinal and urinary systems included observing rats' urination, defecation, salivation, and vocalization. The study's results showed that the higher the extract dose, the darker the yellow color of the urine and the darker greenish green the feces were, while in the control group, the urine was clear yellowish, and the feces was black. This shows that the higher the red betel leaf extract dose, the higher the antidiarrheal activity. The main significant ingredients include flavonoids, tannins, essential oils, and alkaloids. Flavonoids, especially quercetin, inhibit intestinal contractions; tannins could reduce intestinal peristalsis, increasing urine production, while alkaloids and essential oils can kill microorganisms in the intestine.[17,19]

In this study, the administration of single or repeated doses for 14 days showed an increase. It happened because it is one of the mouse responses to new things.[15]

Head-dipping is a motor activity that shows the curiosity of a rat characterized by a movement or a rat's defensive reaction. This reaction usually occurs when the mouse was placed in a new environment. The number of head dipping indicates the rat's inquisitive nature. Straub phenomenon is the erection of the rat's tail that occurred after the spinal cord and anal sphincter was stimulated after dosing. Pyloerection shows a condition in which the rat's fur is erect and coarse.

Ptosis is when the upper eyelid droops due to a disturbance in the eyelid muscles. Reflex tests carried out were corneal and pineal reflex, characterized by eye and ear movements when stimulated. There is also a flexion reflex examination to examine the neuromuscular system in the legs and a Hafner reflex examination to determine the reflex in the tail by pinching the base of the rat's tail using tweezers.[20] In this study, it was found that the red betel leaf extract in the control dose group, 100, 400, and 1,000 mg/kg BW had 100% results, which means it did not interfere with the eye, ear, tail, or foot reflexes of male and female rats. It showed that the red betel leaf extract does not inhibit the activity of the rat's sensory nerves.

Lacrimation is an event when a rat or test animal sheds tears. It is called chromodacyorrhea if the water that comes from the eyes is red or there are red spots. Vasodilation is the widening of blood

vessels that occurs due to a decrease in oxygen supply and an increase in body temperature, which can be seen in the red blood vessels in the ears of rats. Grooming response is a common behavior that rats do to foreign objects to adapt. Dangling is a way to test the rat's physical ability to recover its body position after being hung on a horizontal wire for 5 seconds, while reestablishment is the rat's ability to return its body position after being hung upside down.[20]

CONCLUSION

The conclusion is that the ethanol extract of red betel leaf (Piper Crocatum) with doses of 100, 400, and 1,000 mg/kg BW given to rats in vivo is safe and does not cause symptoms or harmful effects.

CONFLICT OF INTEREST

We declare no conflict of interest in the scientific articles we wrote.

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