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Research Article

Acute Toxicity of Cashew Nut Shell Extract (*Anacardium occidentale* L.) In Albino Rat (*Rattus norvegicus* Berkenhout 1769)

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Abstract

Cashew plant (*Anacardium occidentale* L.) is a crop producing cashew nut shell that contain phenolic compounds such as lacquer oil (cashew nut shell liquid) which can be used for many studies. This study was conducted to determine the potency of acute toxicity (LD_{50}) of cashew nut shell extract on female albino Wistar rats using Weil method. Twenty rats used in this study. The rats was divided into five groups, each consist of four rats after acclimatization. Each group was given the extract of cashew nut shell orally (force-fed). The amount of cashew nut shell extract that were given to group I, II, III and IV were 2.5, 25, 250 and 2,500 mg kg^{-1} b.wt., respectively, while group V were given 0.5% sodium carboxyl methyl cellulose (CMCNa) solution. Clinical symptoms were observed 24 h after the administration of extract include behavioral changes i.e., licking, scratching, twitching, tremors, wrihing, reactivity to stimuli, cerebral and spinal reflexes, secretions, breath, skin, hair and death. Probit analysis using Weil method was used as an effective dose. The results showed that the potency for acute toxicity (LD_{50}) of cashew nut shell extract was 2,018 mg kg^{-1} which classified as moderately toxic category. The administration of extract also causes behavioral changes in animal including passivity and mucus secretion. All doses of the extract did not affect the development body weight and the weight of organs (spleen, liver, heart, kidneys and lungs) in female rats.

Key words: Cashew nut shell, extract, LD_{50}, albino rats, Weil method

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a tropical plant originating from Brazil, South America. At the beginning of the 16th century, this plant is distributed by Portuguese traders to other areas, such as India, Africa and Southeast Asia, including Indonesia (Madamba *et al*., 1991). Cashew grows in many places in Indonesia, so it is known as several different names like jambu mete (Java), jambu mede, jambu siki (Sunda), jambu dipa (Banjar), buwa yaki (Ternate, Tidore), jambu dare, jambu sereng, buah yakis (Sulawesi), jambu jipang, jambu dwipa, nyambu monyet (Bali) and jambu monyet or gaju (Steenis, 1997). Besides its bark, leaf and fruit, a part of cashew plant that is also useful but is not widely known yet is its nut shell. The cultivation of cashew, which has high economic value, is also producing a lot of cashew nut shell, which is still considered as waste products. However, cashew nut shell contains 30% of Cashew Nut Shell Liquid (CNSL) or lacquer oil (Madamba *et al*., 1991). Lacquer oil is a phenolic compound (Hemshekhar *et al*., 2012), which is primarily consist of 80% anacardic acid, 15% cardol and a small amount of cardol derivate like methyl cardol and cardanol (Sullivan *et al*., 1982; Simpen, 2008). One way to obtain compounds cashew nut shell is the solvent extraction using the chemical. Extraction with ethanol, blackish brown color CNSL (Sornprom, 2007) and the compound obtained is flavonoids, triterpenoids, phenolic and volatile oil (Kannan *et al*., 2009). The CNSL is phenolic compounds consisting of anacardad acid or acid 2-hydroxy-6-alkilbenzoat/acid 6-alkilsalisilat) (6-pentadecenyl salicyclic acid), kardol (5-pentadecenyl recorcinol), cardanol (3-pentadecenyl phenol) and 2 methyl kardol (2-methyl-5-pentadecenyl recorcinol) (Sornprom, 2007; Hemshekhar *et al*., 2012). Anacardic acid is bactericidal, fungicidal and deadly to helminths and protozoa (Kubo *et al*., 1993).

In this study, the acute toxicity of cashew nut shell extract was determined. The result obtained will be used as an initial information about the dosage needed for further testing. Acute toxicity test is one of some important preclinical trials. This test was conducted on rats as the testing animal using Weil method (Harmita and Radji, 2006). It was designed to determine the toxic effects that will occur within a short time after the exposure or administration of a compound in a particular dose. Quantitative data obtained from this acute toxicity tests is LD_{50} (Donatus, 2001). Median lethal dose (LD_{50}) is the dose given all at once, which causes the death of half the number of test animals. The LD_{50} is one way to measure the short- term toxic potential of a product (Zbinden and Flury-Roversi, 1981). Based on the LD_{50} value, a compound can be classified as extremely toxic material to practically non-toxic material. Qualitative data obtained include the appearance of clinical, morphological and mechanisms of toxic effects (Hodgson and Levi, 2000). Acute toxicity purpose is to detect the toxicity of a substance, determine the target organs and sensitivity, obtaining data danger after acute administration of a compound basis and to obtain preliminary information that can be used to establish dose levels required for subsequent toxicity tests (Lu, 1991). Cashew nut shell extract is used to determine the influence as antifertilitas in female rats.

A research by Harlita (2004) showed that the LD_{50} value on female golden snails (*Pomacea canaliculata*) and its eggs were 62.5 and 50 ppm, respectively. There was no study on the LD_{50} value of cashew nut shell extract against albino mice yet, which can be used as a foundation for the use of a safe dose in the study about the effect of the extract on female albino rats.

MATERIALS AND METHODS

Materials: Cashew nut shell from Wonosari, Gunung Kidul district, Yogyakarta, Indonesia. Twenty female albino rats (*Rattus norvegius* Berkenhout, 1769) Wistar strain, 2 months old with $\pm$130 g b.wt., from the Integrated Research and Testing Laboratory (LPPT) UGM Unit 4. The animals were acclimatized to laboratory conditions for 7 days prior to the experiments. The rats were maintained at a room temperature of 22-24°C with a 12 h light/dark cycle. During acclimatization, the animals were housed in stainless steel cages with a standard pellet diet and tap water *ad libitum*. All procedures in this study were performed according to the Animal Ethics Committee, Universitas Gadjah Mada. Syringe with oral needle No. 14, rats scale, analytical scale, mortar and stamper, stirrer equipped with a heater and a set of glass tools (beaker glass, measuring cup, pipette and flask).

Making of extract sample preparation: Shell of cashew nuts were sliced into small pieces then dried in a dryer cabinet at 50°C for 48 h. Dried cashew nut shells were milled using a blender until becoming powder.

Maceration extraction: Ethanol 96% were added into cashew nut shell powder, stirred for 30 min and incubated for 24 h, then filtered. This process was repeated three times. On each extraction process, the extract solution was separated from the pulp by filtration and after three extraction, the filtrate were mixed together. The filtrate then distilled at 70°C to separate the oil from the solvent using a rotary vacuum

evaporator in water bath. This process produced the viscous extract of cashew nut shell. The viscous extract was poured in a porcelain dish and heated in a water bath while constantly stirred until the extract of cashew nut shell was ready to used.

Acute toxicity (LD_{50}): The value of LD_{50} determined by Weil method (Harmita and Radji, 2006). The study was conducted in two phases using a total of 20 female rats. In the first phase, 20 rats were divided into five groups of 4 rats each. Groups I, II, III and IV animals were given 2.5, 25, 250 and 2,500 mg kg^{-1} b.wt., of the cashew nut shell extract, respectively, to possibly establish the range of doses producing any toxic effect. The extract was given orally. Each rat was given a single dose after at least 7 days of adaptation. In addition, group V was set up as control group and animals in the group were not given the extract, while group 5 were given 0.5% CMCNa solution.

In the second phase, for the same doses (2.5, 25, 250 and 2,500 mg kg^{-1} b.wt., of the extract were administered to 10 rats (two rats per dose) to further determine the correct LD_{50} value. The extract was given via oral route. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 14 days for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity. At the end of 14 days, all surviving rats were sacrificed and then autopsied at the LPPT UGM Unit 4 and then the vital organs including heart, liver, lungs, kidneys.

Data analysis

Observations for toxic symptoms: Toxic symptoms were observed for 24 h and intensive observation was conducted on the first 4 h after administration of the extract. The physical condition of rats and the number of dead rats were observed during this 24 h period. Probit analysis using Weil method (Harmita and Radji, 2006) was used as an effective dose for further treatment. The behavior of rats after administration of a single dose of extract were motor activity, curiosity, effects on the central and autonomic nervous system, as well as defecation, urination and death (Thompson,1985).

Statistical analysis: The statistical analyses were carried out using statistical package for statistisal science (SPSS computer package). Percentage organ body weight ratio and rats' body weight were expressed as Mean±SD. Values in all groups were compared using the analysis of variance (ANOVA). For all analyses the level of statistical significance was fixed at $p<0.05$ (Murray, 1992).

RESULTS

Potency of acute toxicity (LD_{50}): The following data were obtained from the toxicity test of cashew nut shell extract on female albino rats in Table 1.

Observation for toxic symptoms: Observation for toxic symptoms was carried out intensively in the first 4 h and the observation was continued until 24 h. Toxic symptoms observed include behavioral changes (motor activity, restlessness), licking, scratching, twitching, tremors, wrihing, reactivity to stimuli, cerebral and spinal reflexes, secretions, breath, skin, hair and death. The results of qualitative examination of rats can be seen in Table 2.

Body weight and internal organ weight: On the development of body weight rats for female 14 days after administration single oral dose for each treatments dosage extract shown in Fig. 1. Vital organ body weights both in female rat were recorded as shown in Table 3.

DISCUSSION

Potency of acute toxicity (LD_{50}): Table 1 shows that the extract of cashew nut shell did not kill the rats except at high dose (2,500 mg kg^{-1}), while CMCNa 0.5% as control also did not give toxic effects. Based on the data in Table 1, potency for acute toxicity of cashew nut shell extract can be calculated with probit analysis using Weil methods that expressed as LD_{50} which is 2,018 mg kg^{-1} (Appendix 1). This amount of dose is classified as moderately toxic in toxicity category. According to Lu (1991), LD_{50} of 0.5-5 g kg^{-1} is classified as moderately toxic category. It also supported by stated that the dose of 500-5000 mg kg^{-1} were moderately toxic (Table 4). The LD_{50} of 5,000-15,000 mg kg^{-1} is classified practically non toxic (Table 4). While, according to Hodgson and Levi (2000),

Table 1: Number of female albino rats were died during 24 h after oral administration of a single dose of cashew nut shell extract

Groups	Treatments	N	No. of dead rats	Response (%)	LD_{50}
I	2.5 (mg kg^{-1} b.wt.)	4	0	0	
II	25 (mg kg^{-1} b.wt.)	4	0	0	
III	250 (mg kg^{-1} b.wt.)	4	0	0	2,018 (mg kg^{-1} b.wt.)
IV	2,500 (mg kg^{-1} b.wt.)	4	4	100	
V	Control (CMCNa 0.5% solution)	4	0	0	

LD_{50}: Lethal dose 50, $CMCN_a$: Sodium carboxy methyl cellulos

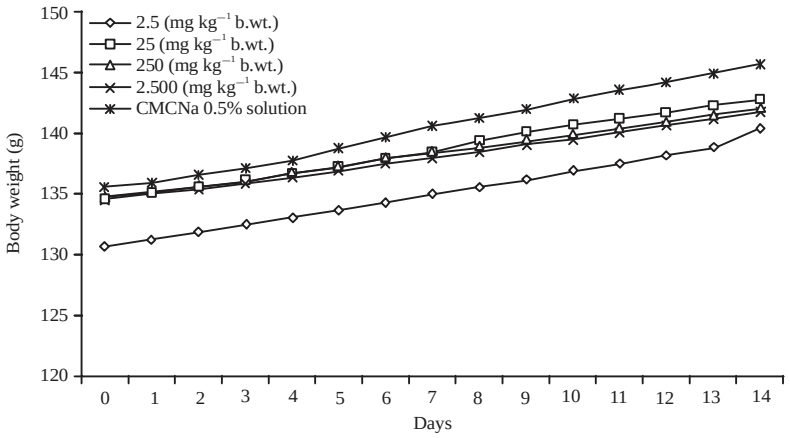

Fig. 1: Changes in average body weight (g) of female rat for 14 days after administration of a single dose of cashew nut shell extract

Table 2: Results of qualitative examination for the toxic symptoms on female albino rats for 24 h after oral administration of a single dose of cashew nut shell extract

Groups	Treatments	No.	Toxic symptoms
I	2.5 (mg kg^{-1} b.wt.)	4	-
II	25 (mg kg^{-1} b.wt.)	4	-
III	250 (mg kg^{-1} b.wt.)	4	-
IV	2,500 (mg kg^{-1} b.wt.)	4	+
V	Control (CMCNa 0.5% solution)	4	-

-: No toxic symptoms found, +: Toxic symptoms found, $CMCN_a$: Sodium carboxy methyl cellulos

Table 3: Relative organ weights of rats treated with cashew nut shell extract (*Anacardium occidentale* L.) after acute toxicity study

		Organs weights (g)				
Groups	Treatment	Spleen	Liver	Heart	Lungs	Kidneys
I	2.5 (mg kg^{-1} b.wt.)	0.60±0.01	4.60±0.08	0.76±0.01	1.57±0.07	0.70±0.02
II	25 (mg kg^{-1} b.wt.)	0.55±0.02	4.30±0.07	0.70±0.02	1.54±0.01	0.65±0.01
III	250 (mg kg^{-1} b.wt.)	0.50±0.01	4.20±0.05	0.70±0.01	1.43±0.03	0.65±0.02
IV	2,500 (mg kg^{-1} b.wt.)	0.42±0.01	3.89±0.09	0.67±0.02	1.40±0.04	0.64±0.04
V	Control (CMCNa 0.5% solution)	0.65±0.01	4.60±0.08	0.76±0.01	1.58±0.08	0.72±0.02

Values are expressed as Means±SD (n = 2 for each group), relative organ weight as calculated as (organ weight/body weight)×100%, $CMCN_a$: Sodium carboxy methyl cellulos

Table 4: Hodge and sterner toxicity scale (1980)

Toxicity rating	Commonly used term	LD_{50} (rat oral)
1	Extremely toxic	Less than 1 mg kg^{-1}
2	Highly toxic	1-50 mg kg^{-1}
3	Moderately toxic	50-500 mg kg^{-1}
4	Slightly toxic	500-5,000 mg kg^{-1}
5	Practically non-toxic	5,000-15,000 mg kg^{-1}

Source: (Yance and Tabachnik, 2007), LD_{50}: Lethal dose 50

LD_{50} is a single dose that is able to cause the death of 50% of test animals. After obtaining this value, the safe dose range can be determined for further treatment.

A research by Harlita (2004) showed that the LD_{50} value on female golden snails (*Pomacea canaliculata*) and its eggs were 62.5 and 50 ppm, respectively. These findings are consistent with research conducted Leite *et al.* (2015) which examines toxicity natural Cashew Nut Shell Liquid (iCNSL) and Technical Cashew Nut Shell Liquid (tCNSL) on *Artemia salina*, obtained for 50% lethal concentration (LC_{50}) value was 36.96 and 91.67 g mL^{-1}, respectively. According Arcanjo *et al.* (2012) plant extracts with LC_{50} values under 1,000 g mL^{-1} are considered to be active and to have toxic activity. Therefore, although iCNSL and tCNSL had different LC_{50} values, both are considered to have shown toxic effects in the acute toxicity test with *A. salina*.

Observation for toxic symptoms: Based on the qualitative observation for toxic symptoms on female albino rats after cashew nut shell extract administration (Table 2) groups I-III and group V did not show any toxic symptoms, no adverse effect on the behavioural responses of the tested rats up to 14 days of observation. Physical observations indicated no

signs of changes in the skin, fur, eyes mucous membrane, rats. This is consistent with research Mir *et al*. (2013) methanolic extract Tridex procumbens a dose of 300 mg kg $^{-1}$ b.wt., in Spague Dawley's rat, showed wellness parameters namely skin, fur, eyes, mucous membrane, behavioral pattern, salivation, were found to be normal. Tremors, lethargy, diarrhea and coma did not occur in any of the animal, however at 2000 mg kg $^{-1}$ b.wt., dose lethargy, convulsions, tremors, diarrhea, morbidity and mortality was found. Rats in group IV showed an increase in activity, anxiety, increased breathing, secretion of mucus and nasal fluids. They stretched out and rested in the corner of the cage, began to close their eyes looked calm and finally died.

Body weight and internal organ weight: Figure 1 shows that an increase in body weight group of rats treatment with the control group did not differ significantly from day to day for 14 days observation. From the statistical data processing using one-way ANOVA results that there was no significant difference between the increase in body weight in the treatment groups the control group ($p>0.05$).

Thus it can be stated with a single oral dose the fourth extract to doses of 2,500 mg kg $^{-1}$ b.wt., did not effect on growth and development body weight of female for 14 days of observation after administration of the test substance. However, weight gains were observed in all animals administered with cashew nut shell extract. It can be stated that the cashew nut shell extract did not interfere with the normal metabolism of animals. A decrease in body weight in a day does not reach 5% without showing the influence behavior in test animals, is a common result treatment. Oral administration of methanolic extract Tridex procumbens a dose of 2,000 mg kg $^{-1}$ b.wt., in Sprague Dawley's rat, showed no significant changes were observed in body weight (Mir *et al*., 2013). The body weight changes serve as a sensitive indication of general health status of animals (El Hilaly *et al*., 2004).

Table 3 shows that neither body weight nor vital organs weight of the treated rat was significantly changed relative to the control group. Gross examination of the vital organs revealed no pathological abnormality relative to the control group based on the macroscopic observation (data not shown). Size, tumors, colours and textures are parameters that observed in macroscopic observation. This is consistent with study of Ping *et al*. (2013), the oral administration methanolic extract of Euphorbia hirta at a dose of 5,000 mg kg^{-1} had no adverse effect the relative organ weights (heart, liver, spleen, kidneys and lungs) in their study.

Organ weight also is an important index of physiological and pathological status in animals. The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant (Michael *et al*., 2007; Roopashree *et al*., 2009). The administration of cashew nut shell extract did not show any adverse affect on organs weight of all important organs. Hence, it can be suggested that, cashew nut shell extract is virtually nontoxic.

CONCLUSION

Based on the analysis of quantitative and qualitative data it can be concluded that potency for acute toxicity (LD_{50}) of cashew nut shell extract on female albino rats is 2,018 mg kg^{-1}, a moderately toxic category. The extract of cashew nut shell also caused some changes in the behavior of animals including of passivity and fluid secretion. All doses of the extract did not affect the development body weight and the weight of organs (spleen, liver, heart, kidney and lung) in female rats.

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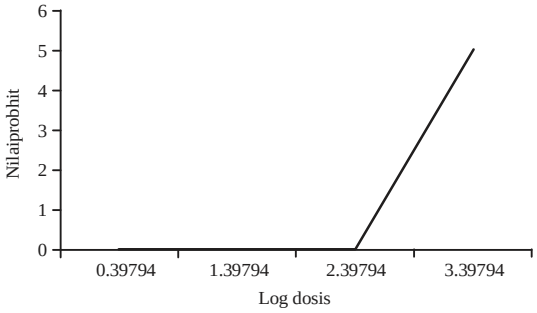
Appendix 1: Determination of dosage log

Concentration	Log of concentration(x)	Prohibit (y)
2.5	0.39794	0
25	1.39794	0
250	2.39794	0
2,500	3.39794	5



Appendix: Continue

Considering y = ax+b,

For point (2.505;1)
(2.705;1)
(3.105;4)

so:

4 = 3.105a + b
1 = 2.505a + b _
3 = 0.6a
a = 5

for a=5, so

1 =12.525 + b
b = -11.525

for a=5 and b=-11.525, so

y = 5x – 11.525

4 = 3.105a + b
2 = 2.705a + b _
2 = 0.4a
a = 5

for a=5, so

4 = 15.525 + b
b = -11.525

for a=5 and b=-11.525, so

y = 5x – 11.525

LD_{50} CALCULATION

y = 5x – 11.525
5 = 5x – 11.525
x = 3.305

If x is log of concentration, so the concentration is 2,018

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