

Short Research Article

PRELIMINARY STUDY ON THE RODENTICIDAL EFFECT OF PONG-PONG (*Cerbera odollam*) SEED POWDER AGAINST SPRAGUE-DAWLEY RATS

ABSTRACT

Pong-pong (*Cerbera odollam*) is a poisonous plant species that has potent cardiotoxic effects. In Malaysia, its fruit, referred to as 'buah tikus' (rat's fruit), has been traditionally used as a rodent repellent. This study evaluated the rodenticidal effects of pong-pong seed powder based on acute oral toxicity and palatability under laboratory conditions against Sprague-Dawley albino rat, *Rattus norvegicus*. Pong-pong fully ripe fruits (red colour) were collected from Sekinchan, Sabak Bernam, Selangor. The fruits were sliced into halves for the seeds, cleaned, dried and ground into powder. The seed powder was administered orally by mixing the prescribed dose into the rat food bait. The acute oral toxicity test was done according to the Organization for Economic Cooperation and Development (OECD) Guideline 420, administering a single high dose of 5 g/kg of body weight. The palatability was determined by using a free choice feeding test between treated bait (mixed with Pong-pong seed powder) with that of the untreated bait. Results indicated a higher susceptibility in female rats, with 83% mortality compared to 33% in males. Based on the palatability results, the acceptance rate of female rats was 25.6% which is lower compared to 44.2% in males. This implies that the females are more sensitive to the seed powder in the baits and significantly reduce their intake of the treated bait when an alternative food is available. Although the preliminary findings demonstrated acute oral toxicity of pong-pong seed powder in rats, further research is needed to confirm its efficacy as a rodenticide.

Keywords: Pong-pong, palatability, rat, seed powder, toxicity

1. INTRODUCTION

Cerbera odollam is a plant species belong to the poisonous Apocynaceae family (Wermuth et al., 2018). The plant grows predominantly in Southern India, Madagascar, Australia, Vietnam, Cambodia, Sri Lanka, Myanmar, and Malaysia (Chopra & Chopra, 2006; Gaillard et al., 2004; Hien et al., 1991; Shankar & Rai, 2009). Its common name includes Grey Milkwood, Othallanga Maram, Mango Laut, Blind rhino, Babuto, Wood octopus and Pong-pong (Chopra et al., 1956; Hien et al., 1991; Ruerkert et al., 2019; Sukmawati, 2016; Tomlinson, 2016). It contains active cardiac glycosides cerberin, cerberoside, odollin that mainly concentrated in the seed (Eddleston & Haggalla, 2008; Gaillard et al., 2004; Guruswami et al., 1970; Laphookhieo et al., 2004). The cardiac glycosides cause electrolyte imbalance by binding and blocking with Na⁺ + K⁺ ATPase pump that leads to heart failure (Hossan et al. 2019). Animal studies have

shown that ingesting very little amounts of the kernel can cause mortality in dogs and cats, with lethal doses of 1.8mg/kg and 3.8mg/kg, respectively (Chopra & Chopra, 2006). In Malaysia, its fruit, referred to as 'buah tikus' (rat's fruit), has been traditionally used to poison rat (Koh et al., 2009). This plant is also planted at the edge of paddy fields since it is thought to deter rodent pests.

Rodentia is the largest order of mammals that make up more than 40 percent of all mammal species, comprises of about 30 families, 481 genera and 2277 species (Wilson & Reeder, 2005). Rats (subfamily Murinae) are omnivores with a predominantly vegetarian diet. They are highly fertile with many species only take two to three months to reach sexual maturity and a gestation period of two to three weeks to give birth to six or seven litters. Additionally, the females are able to get pregnant shortly after giving birth which are known as post-partum oestrus (Quesenberry & Boschert, 2020). Due to all that, rats become a significant pest that are difficult to be control, causing damage to property, crops, contaminate food, destroy livestock feed and spread disease. In Malaysia, there are 19 species of *Rattus* described by Medway (1978), however only four or five are considered as serious agricultural pests and some of the non-pest species have been moved to different genera (Francis, 2019; Payne et al., 1985).

Anticoagulant rodenticides have been the primary method used world widely to manage rat populations (Greaves & Ayres, 1967; Pelz et al., 2005) but after some time rat populations became resistance to these chemical treatments (Boyle, 1960; Ishizuka et al., 2008; McNichol, 1985; Pelz et al., 2005; Rost et al., 2004). Rat populations that are resistant to several chemical treatments are common in North America, Europe, Australia and Asia (Pelz et al., 2005). In Malaysia, the development of resistance in rats was reported in the 1980s against warfarin in three different localities, Klang, Teluk Intan and Renggam (Wood & Chung, 1990). A recent study by Noh et al. (2023) reported similar findings on the ineffectiveness of warfarin at reducing rat infestation in oil palm plantations, presumed to be caused by the development of rat resistance. Obviously, the problem is acknowledged as one of the major issues (Ishizuka et al., 2008; Davies and Davies, 2010; Frieri et al., 2017; Gould et al., 2018; Palumbi, 2001; Pelz et al., 2005; Rost et al., 2004). The raises concerns regarding the rat resistance to anticoagulant rodenticides create a significant need for a replacement that maintain the same degree of control over the target pest.

Despite the common belief that pong-pong can be used to control rodent infestations, there is lack of study has been done to verify this. This preliminary study aims to assess the acute oral toxicity and acceptance rate of pong-pong seeds with minimal processing (seed powder) against lab rats.

2. MATERIAL AND METHODS

2.1 Preparation of Pong-pong Seed Powder

Pong-pong fruits were collected from Sekinchan, Sabak Bernam, Selangor. Only fully ripe fruits (red colour) were collected to be used in the preparation of pong-pong seed powder. Pong-pong seed powder preparation method was according to Sungkar et al. (2020). The collected Pong-pong fruits were cut into halves to obtain the seeds. The seeds were washed and air dried to remove excess water before being weighed for its fresh weight. The seeds were then dried in the oven (60 °C) for three days. The dried seeds were crushed and ground into a fine powder. The powder was then sieved using a No. 40 mesh with 0.42 mm sieve opening.

2.2 Laboratory Studies

2.2.1 Handling of animals

Adult male and female Sprague-Dawley albino rats (*Rattus norvegicus*) were used for the experiments. The rats were kept in the Animal Laboratory located at AMTREC Building, MARDI Headquarters, Serdang, Selangor, Malaysia. The rats were kept in a temperature-controlled room ($22 \pm 2^\circ\text{C}$) with relative humidity of $55 \pm 10\%$, and photoperiod 12/12 hours and underwent an acclimatization period of one week prior to the experiments. Rats were caged individually and provided with standard pellet diet daily for a week. Water was supplied ad libitum. The unhealthy and pregnant animals were excluded. Rats were weighed, each was given a reference number.

2.2.2 Acute oral toxicity

The Pong-pong seed powder was analysed for its acute oral toxicity according to the guidelines outlined in the Organization for Economic Cooperation and Development (OECD) Guideline 420 (No, 2002). Groups of 12 rats (six males and six females) were singly caged and used for each treatment including one control group. A single high dose of 5 g/kg of body weight (BW) was used which administered orally by mixing it into the food bait. Each rat received 20 g of the treated bait and the control group was fed with untreated bait. The rats were observed for 14 days for mortality and any toxicity symptoms. Dead rats were autopsied to investigate the symptoms of pong-pong seed powder poisoning.

2.2.2.1 Organ sampling and relative organ weight

At the end of the observation period, the rats were dissected and five main organs were removed; liver, heart, lungs, spleen, and kidneys. All of the organs were rinsed in a salt solution (NaCl, 0.9) and cleaned from fats and blood clots before weighing. The relative organ weight percentage was calculated using the following equations:

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

2.2.2.2 Haematological and biochemical analysis of blood

For haematological and biochemical analysis, the blood was sampled through the vena cava during dissection. Around three to five ml of blood was collected and put into two different blood tubes, a tube with blood anticoagulant (K_3EDTA) and a tube without blood anticoagulant. The blood in the tubes with anticoagulant underwent haematological analysis. The parameters involved were red blood cells, white blood cells, platelets, haemoglobin, and haemocrit. The analysis was carried out using an Exigo (Sweden) blood analysis machine.

The blood in the tube without anticoagulant was centrifuged at 6,000 rpm for 10 minutes to obtain the blood serum. The serum was analysed using a DIRUI CS300 (China) clinical biochemical analysis machine which evaluated the toxicity effect on liver and kidney function. The levels of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), protein, albumin, globulin, and bilirubin in the blood were the parameters of liver function, while urea and creatinine levels were the parameters to assess renal function.

2.2.3 Bait acceptance

Free choice feeding test were used to determine the acceptance rate of the treated bait (mixed with Pong-pong seed powder) with that of the untreated bait. Groups of 12 albino rats (six males and six females) were singly caged and used for each treatment including one control group. The dose was 5 g/kg BW which administered orally by mixing it into the food bait. The palatability test method was conducted according to Palmateer (1974). Each rat was given a free choice between 20 g of treated and 20 g of untreated bait. In control group, both diet containers contained untreated bait.

The diet containers used in the experiment were identical in terms of type and size. Their position was exchanged daily to avoid location preference. Each container was given equal and consistent diet throughout the test. The weight of consumed food was recorded daily for five successive days. After five days, the treated baits were removed, and survived animals were fed and monitored for nine additional days on normal diet. Bait acceptance and mortality was recorded by using Buckle and Smith (2015) equation:

$$\text{Acceptance \%} = \frac{\text{Consumed amount of treated bait}}{\text{Consumed amount of treated bait} + \text{challenge diet}} \times 100$$

2.3 Statistical Analysis

The statistical analysis of toxicity tests was conducted using Minitab 19 for Windows. For comparing the experimental group with the control group, data was analyzed by using one-way ANOVA with LSD post hoc test. Statistical significance was defined as $p < .05$.

3. RESULTS AND DISCUSSION

3.1 Acute Oral Toxicity

3.1.1 Mortality and time to death

The study revealed that pong-pong seed exhibited acute oral toxicity against Sprague-Dawley albino rats (*Rattus norvegicus*) even with minimal seed processing (in powder form). The mortality rate of the female was higher than the male rats. Out of the six treated female rats that were observed, five mortalities (83%) were recorded while only one survived with clinical signs of toxicity such as lethargy and abnormal posture. For male, only two mortalities (33%) were recorded while the rest four were alive until the end of the observation period (Table 1). However, clinical signs of toxicity such as decreased mobility or physical activity were either almost non-existent or very slight in the male survivors.

Other than that, the time to death in female was also shorter than in male. The range was four to 10 days in female with the mean of 6.6 ± 2.41 days. In male, the range was between 11 to 13 days with the mean of 12.0 ± 1.4 days (Table 1). There were no mortalities recorded in control group of both sexes.

Table 1. Mortality and time to death following five days of administration.

Treatments	% Mortality	Time to death (days)	
		Range	Mean \pm SD
Female	83	4 – 10	6.6 \pm 2.4
Male	33	11 – 13	12.0 \pm 1.4

Control female	0	N/R*	N/R
Control male	0	N/R	N/R

* Not relevant

Based on the results of mortality and time-to-death, female rat was found to be more susceptible while male rat was more tolerant to the toxicity of pong-pong seed powder. It's typical that female rats exhibited a slightly higher sensitivity to toxic effects than male rats (Ballesteros-Ramírez et al., 2024). Since female rats are the key to population growth, it is advantageous that females are more susceptible to the toxic seed powder. This may directly limit the breeding capacity of the rat population. According to Jacob (2006), a reduction in the pest population's reproductive rate could reduce the population growth and lessen harm to crops.

3.1.2 Toxicity effects on different organs

3.1.2.1 Organ relative weight

The relative organ weight was used to identify abnormalities in the physical structure of the organ; the values may indicate swelling or shrinkage of the organ. The data show that the relative weight of the five major organs did not significantly differ between the treatment and control group of rats (Table 2).

Table 2. Relative organ weight (% of body weight) of rats. Data are presented as mean ± standard deviation (SD).

Treatment	Heart	Liver	Lung	Spleen	Kidney
Female	0.49±0.00 ^a	3.58±0.00 ^a	0.62±0.00 ^a	0.12±0.00 ^a	0.92±0.00 ^a
Male	0.41±0.03 ^a	3.37±0.07 ^a	0.59±0.03 ^a	0.15±0.03 ^a	0.84±0.01 ^a
Control female	0.39±0.03 ^a	3.57±0.15 ^a	0.70±0.05 ^a	0.18±0.01 ^a	0.89±0.03 ^a
Control male	0.37±0.02 ^a	3.69±0.15 ^a	0.49±0.03 ^a	0.17±0.03 ^a	0.77±0.02 ^a

Means that do not share a letter within a column are significantly different at $p < .05$

3.1.2.2 Haematological and biochemical analysis of blood

Haematological analysis revealed that most parameters, including red blood cells, white blood cells, haemoglobin and haematocrit, showed no significant differences between the treated group and control group. This is consistent with reports from Bernshteyn et al. (2020) in a human poisoning case where the levels of red blood cells, white blood cells, haemoglobin and haematocrit were all found to be within normal ranges.

However, platelet levels in both treated male and female rats were higher than the control group albeit only treated female showed a significant difference (Table 3). This elevation indicates an abnormality in the heart function of the treatment group, particularly in the female rats that are more susceptible to the toxicity of pong-pong seeds. According to Gadi et al. (2009), elevated blood platelet activity can be linked to cardiovascular diseases. The value of blood platelet shows several dysfunctions related to cardiovascular pathologies such as arterial hypertension (Mekhfi et al. 2004). Previous studies reported that Pong-pong seed poisoning causes elevated blood pressure in dogs and cats followed by a sudden fall and death (Guruswami et al., 1970; Misek et al., 2018).

Table 3. Blood haematology of rats. Data are presented as mean ± SD.

Treatment	Red blood cell (10 ¹² /l)	White blood cell (10 ⁹ /l)	Platelet (10 ⁹ /l)	Hemoglobin (g/dl)	Hematocrit (%)
Female	10.22±0.00 ^a	7.00±0.00 ^a	1290±0.00 ^a	18.50±0.00 ^a	52.80±0.00 ^a
Male	9.79±0.22 ^a	7.65±1.64 ^a	1001.75±41.39 ^b	18.28±0.64 ^a	50.50±1.58 ^a
Control female	10.07±0.27 ^a	9.14±1.63 ^a	871.40±22.80 ^b	19.30±0.33 ^a	52.94±0.85 ^a
Control male	10.76±0.39 ^a	9.58±0.93 ^a	876.60±37.16 ^b	20.54±0.70 ^a	55.86±1.93 ^a

Means that do not share a letter within a column are significantly different at $p < .05$

The liver function analysis (Table 4) shows no significant difference between the treated group and control group of rats in most parameters including alanine transferase (ALT), aminoaspartate transferase (AST), total protein, albumin, globulin and albumin to globulin ratio. Only the alanine phosphatase (ALP) value in the treated female was significantly higher compared to the control group. An increase in this parameter can be linked to a decompensated heart failure (Shamban et al., 2014). This validates the mortality results where higher mortality recorded in the treated female presumed due to heart failure. In human poisoning case study, cerberin which is a cardiac glycoside found in pong-pong seeds, disrupts cardiac electrical activity that leads to heart failure (Misek et al., 2018).

Table 4. Liver function analysis of rats. Data are presented as mean ± SD.

Treatment	Alanine transferase (ALT) (U/l)	Aminoaspartate transferase (AST)(U/l)	Alanine phosphatase (ALP) (U/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Albumin: Globulin ratio
Female	50.00±0.00 ^{ab}	129.00±0.00 ^a	222.00±0.00 ^a	70.70±0.00 ^a	40.74±0.00 ^a	30.00±0.00 ^a	1.36±0.00 ^a
Male	66.00±5.00 ^a	106.25±3.53 ^a	282.00±23.78 ^a	70.40±3.47 ^a	39.92±1.37 ^a	30.48±2.13 ^a	1.32±0.05 ^a
Control female	49.20±2.90 ^b	105.20±11.10 ^a	152.40±19.32 ^b	76.30±1.30 ^a	43.40±0.98 ^a	32.90±0.87 ^a	1.32±0.05 ^a
Control male	61.60±5.25 ^a	120.60±2.22 ^a	235.20±16.82 ^a	70.02±2.83 ^a	41.15±1.02 ^a	28.94±1.82 ^a	1.43±0.05 ^a

Means that do not share a letter within a column are significantly different at $p < .05$

Results on kidney function analysis (Table 5) shows no significant difference in urea and creatinine values in the treated group compared to the control group of rats. This suggests that no significant renal damage occurred in the treated rats group.

Table 5. Kidney function analysis of rats. Data are presented as mean ± SD.

Treatment	Urea (mmol/l)	Creatinine (µmol/l)
Acute Female	4.76±0.00 ^a	17.00±0.00 ^a
Acute Male	4.90±0.14 ^a	18.00±1.31 ^a
Control Female	9.53±0.86 ^a	20.60±0.23 ^a
Control Male	4.48±0.35 ^a	18.40±0.77 ^a

Means that do not share a letter within a column are significantly different at $p < .05$

3.2 Bait Acceptance

The palatability test revealed that both the treated male and female rats consumed significantly more untreated bait than the treated bait. The acceptance rate in treated female rat is only 25.6% which is lower compared to 44.2% in male (Table 6). This indicates that female rat was

more sensitive to the presence of Pong-pong seed powder in the baits. Despite finishing the treated bait in the acute oral toxicity test, mostly for survival, females in the free choice feeding test drastically lower their consumption of the treated bait when an alternative food was available. In contrast, male rats were found to be less sensitive since they consumed significantly more of the treated bait compared to the females. All rats in the palatability study stay alive until the end of the observation period (14 days) and did not show any clinical symptoms throughout the study period.

Table 6. Mean \pm SD intake of food and palatability following five days of administration.

Treatments	Average bait consumption/ day (g)		% Acceptance
	Treated bait	Untreated bait	
Treated female	2.82 \pm 2.71 _d	8.99 \pm 5.3 _c	25.6
Treated male	7.02 \pm 4.45 _c	9.66 \pm 6.13 _c	44.2
Control female*	13.30 \pm 5.73 _b		100
Control male*	20.00 \pm 5.27 _a		100

Means that do not share a letter within a column are significantly different.

**Both diet containers in control group contained untreated bait.*

In general, the palatability study implies that both sexes were able to detect the presence of pong-pong seed powder in the bait. Therefore, in order to enhance the potential of the seed powder, the diet needs to be mixed with an attractant and formulated as bait. According to Buckle and Smith (2015), taste influences dietary preferences and recognition which affects the effectiveness of poison baits. Hence, attractants including strong odour, flavouring or food in bait helps to reduce shyness in rodenticides (Prakash, 2018).

4. CONCLUSION

Results of the preliminary study showed that pong-pong seed powder has acute oral toxicity effects on Sprague-Dawley albino rat, *Rattus norvegicus*. However, the acceptance rate was low particularly in female rat and in future the diet needs to be mixed with an attractant and formulated as bait. Findings from this study were insufficient to confirm the efficacy of pong-pong seed powder as a highly potential rodenticide. More research is needed to fully investigate and validate the rodenticidal effects of Pong-pong specifically its effectiveness in the field.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the MARDI animal ethics committee.

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