The effect of *Myrmecodia pendans* extract toward gastric histology in white rats treated with toxic dose of gentamicin: a preliminary report

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**ABSTRACT**

**Introduction:** *Myrmecodia pendans*, or traditionally known as ant nest fruit, is one of popular traditional medicines in Indonesia with a wide range of effects such as antidiabetic, anticancer, and free-radical scavenging agents. Despite of the extensive research, reports regarding its toxicity are scarce. Gentamicin is also one of popular and widely used anti-biotic but it is associated with a wide range of toxicity, especially if administered at high dose. Therefore, this study aimed to evaluate the effect of *Myrmecodia pendans* extract in the mice what received toxic dose of gentamicin.

**Method:** 24 male white rats were used and randomly divided into 4 groups: P0 (negative control), P1 (100 mg/kgBW intraperitoneal gentamicin), P2 (gentamicyn + 250 mg/kgBW myrmercodia extract), P3 (250 mg/kgBW myrmercodia extract) which administered for 7 days and followed by gentamicin. The treatment was given for 10 days and all mice were sacrificed at the end of the study. The stomach was taken from each mouse and examined pathologically using Hematoxylin-Eosin (HE) staining.

**Results:** The necrosis was found in all groups that received gentamicin (P1-P3) with P2 had the most severe necrosis. Hemorrhage was only found in group that received Myrmecodia extract (P2 and P3) and, again, P2 had higher score of hemorrhage compared to P3.

**Conclusion:** The administration of Myrmecodia extract at 250 mg/kgBW together with toxic dose of gentamicin exacerbate the gastric tissue damage in Wistar rats.

**Keywords:** gentamicin, myrmercodia pendans, stomach, toxic

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**INTRODUCTION**

*Myrmercodia pendans*, which also known as ant-nest plant, is originated from Papua but currently, can be found in most regions of South-East Asia.¹ It is an epiphytic plant, commonly attached to other larger trees. Myrmercodia has been used traditionally used as medicine, but researches in the last decade confirmed its pharmacologic potentials.¹⁻³ According to those studies, *Myrmercodia* has anti-cancer, anti-diabetic, anti-bacterial, and antioxidant properties which evaluated through in vitro and in vivo studies. Phytochemical analysis pinpointed to its high concentration of flavonoids as the main reason behind its wide pharmacology effects, but further investigations are needed to evaluate the effect of each flavonoid.⁴⁻⁵ The properties of flavonoids within Myrmercodia also have potential to deter the toxic effect of other drugs but the evidence that support this notion is very scarce.

Gentamicin is one of widely used aminoglycoside antibiotic, originally derived from Streptomyces species.⁶ It is usually the first- or second-line drugs in bacterial infection management both in animals and human.⁷ Despite of its potent effect, gentamicin is associated with several serious side effects such as ototoxicity, nephrotoxicity, and neurotoxicity.⁸⁻¹⁰ These side effects are surprisingly frequent, ranging from 2-25% depending on the dosage, age, and other risk factors.⁶⁻¹⁰ Several observational studies also indicated that gentamicin could cause gastrointestinal discomforts and nausea which might indicate gastric effect of this drug.¹¹ In depth analysis revealed that the main mechanism of gentamicin toxicity is increased production of free radicals that damage neuron, hair cells, and renal tubules, in which administration of anti-oxidant may be beneficial to inhibit this pathological mechanism.⁷⁻⁹

According to those evidence, the initial aim of this study was to assess the beneficial effect of Myrmercodia extract toward the toxic effect of gentamicin. However, it was turned out that Myrmercodia extract produced gastric toxic effect in our research animals, which contradicted the original aim. Nevertheless, the findings of this study are pharmacologically important due to the scarcity
of toxicology study in traditional medicine research. Currently, only one study reported the toxic effect of Myrmecodia extract in renal tissue. Therefore, reports regarding the toxic effect of traditional medicines are needed to be exposed to enhance the awareness of their usage and administration. In this study, we present the toxic effect of Myrmecodia extract toward gastric tissue in mice treated with toxic dose of gentamicin.

METHODS

Study Design and Animal Selection
An experimental animal study was conducted at pharmacology and pharmacy lab and pathology lab, Faculty of Veterinary Medicine, Univeritas Udayana. This study used 24 male white rats, 2-3 months old, and weighed 200-250 grams which were obtained in Denpasar, Bali. The animals were acclimated to the lab environment for 7 days prior to experimentation. Then, the mice were divided into 4 groups as follow:

- **P0** = Negative control
- **P1** = Positive control; The animals were treated with gentamicin at a dose of 100 mg / kg BW for 10 days
- **P2** = Treatment I; The animals were treated with 250 mg / kg BW Myrmecodia extract + 250 mg / kg BW gentamicin for 10 days.
- **P3** = Treatment II; The animals were treated with 250 mg / kg BW Myrmecodia extract for 7 days, followed by 250 mg / kg BW Myrmecodia extract + 100 mg / kg BW gentamicin for 10 days.

**Myrmecodia pendans** Extraction
Ant nests are washed and drained, then dried and aired in indirect sunlight. Then the dried ant nest, chopped into small pieces, put in a blender until smooth. The powdered blender is put into a clean jar. The powder was then macerated with 96% ethanol solution for 48 hours and the filtrate was taken by filtering method with Whatman filter paper no. 42 so that the ethanol extract was obtained. The filter results are then evaporated in a vacuum rotary evaporator at a temperature of 45°C, until all the solvent evaporates and is stored in the refrigerator at 10°C.

**Sample preparation, Staining, and Sample Examination**
The stomach from each mouse was cut to a size of 1x1x1 cm and fixed in 10% Neutral Formalin Buffer (BNF). The organ samples were further cut to fit the size of compartment in tissue cassettes and tissue were fixed for 18-24 h. Then, the samples were dehydrated by immersing them sequentially in 70% alcohol, 80% alcohol, 90% alcohol, 96% alcohol, absolute alcohol, toluene, and paraffin. The organ samples were embedded with liquid paraffin and then cooled. The cold blocks were cut using a microtome with a thickness of ± 4-5 microns. Finally, the specimens were stained with Hematoxylin-Eosin and observed under a microscope at 100x magnification at five fields of view.

Necrosis, hemorrhage, apoptosis, and inflammation were assessed in every specimen. Each variable was graded as follow:

- Score 0 = no change
- Score 1 = focal (mild)
- Score 2 = is multifocal (moderate)
- Score 3 = diffuse (severe)

**Statistical Analysis**
The data was tabulated and descriptively presented to obtain the general view of data distribution in each variable. The analysis was conducted using non-parametric Kruskal-Wallis to analyze the difference in all groups. Subsequently, post-hoc analysis was conducted using Mann-Whitney test to analyze the difference between two groups. P-value <0.05 was considered significant.

**RESULTS**
According to histological observation, no lesion was observed in P0 specimens while the other three groups had at least one type of lesion. In P1, focal necrosis was observed in the mucosal tissue while P2 and P3 both had inflammation and necrosis. The necrosis observed in both groups was at higher degree compared to P1. Interestingly, P2 experienced higher severity of inflammation and necrosis compared to P3 which might be correlated to the administration technique of the Myrmecodia extract (Table 1). The mean score of each lesion in every group is presented in Figure 1 for easier comparison. The representative microscopic images of necrosis and inflammation are presented in Figure 2 and 3.

To obtain an objective comparison, the lesion’s scores were analyzed using Kruskal-Wallis non-parametric comparison test. The result showed that inflammation and necrosis differ significantly among those groups. However, Kruskal-Wallis cannot pinpoint the specific differences between two groups. Thus, the analysis was continued using post-hoc test (Mann-Whitney).

Post-hoc analysis showed that the level of inflammation was significantly higher in both P2 and P3 groups. The highest level of inflammation was observed in P2 and it was significantly higher compared to the other groups. Similar finding was
also observed in necrosis level. The necrosis score was significantly higher in P2 group compared to all others. In fact, the mean score was roughly double than mean necrosis score in P3 (P2 vs. P3: 1.47±1.3 vs. 2.8±0.77).

DISCUSSION

Myrmecodia pendans is widely known as one of traditional medicines in some areas of South-East Asia, including Indonesia.\(^1\) Several studies had shown its therapeutic potentials, which include anti-diabetic, anti-cancer, anti-bacterial and antioxidant.\(^1\)\(^-\)\(^5\) Additionally, local people in several regions in Indonesia has used this plant regularly as traditional remedy.\(^1\) However, the lack of reports regarding the potential side effect and toxic dose of traditional medicine is concerning and the occurrence of the side effects could be largely under-reported. Therefore, toxicology studies are urgently needed since traditional medicines are used extensively among people, especially those who live in urban or under-developed areas.

Despite of the deviation from the original aim, the findings of this study is important. Currently, there are only four studies that evaluated the toxicology of Myrmecodia extract and all of them conducted and published in Indonesia.\(^12\)\(^-\)\(^15\) However, none of them evaluated the toxic effect of Myrmecodia to stomach tissue. Even among clinicians and veterinarians, the importance of stomach is often ignored despite it is the first digestive organ that received the administered extract. The extract or the solvent potentially damage the gastric tissue if administered at high dose.\(^16\) Accordingly, the findings of this study are different than previous ones. The toxic dose of Myrmecodia extract from previous reports ranged from 300 mg/KgBw to as much as 3.162 g/KgBw.\(^12\)\(^-\)\(^15\) At the highest toxic dose, all of tested mice suffered from acute toxicity which manifested as hyperactivity and convulsion, and dead within an hour after administration.\(^15\) On the other hand, administration at 300 mg/KgBw resulted in marked renal toxicity as reported by Manullang et al.\(^14\) The other dosages evaluated in that study was 100 mg/KgBw and 200 mg/KgBw. Meanwhile, we used 250 mg/KgBw dose which never evaluated before and the potential toxicity at this dosage might be missed in previous studies. However, it should be noted that the degree of damage in P3 was significantly lower than P2, which might indicate that long term exposure to the extract could still have some degree of protective effect (the mice received Myrmecodia extract only for the first 7 days).

Nevertheless, the mechanism of toxicity by Myrmecodia extract is still unexplained as no...
pathomechanism reports can be found regarding the interaction between Myrmecodia chemical content with animal and human physiology. Potential interaction between Myrmecodia extract with gentamicin is also cannot be excluded in this study because as seen in P1, gentamicin alone can induce mucosal necrosis. Detailed assessment about the pharmacodynamic of this extract is needed and very crucial in understanding its chemical and molecular interaction as well as in determining the therapeutic dose of Myrmecodia.

Because of preliminary nature of this study, there are several shortcomings that should be addressed. First, the positive control group that only received Myrmecodia extract did not included in this study because as seen in P1, gentamicin alone can induce mucosal necrosis. Detailed assessment about the pharmacodynamic of this extract is needed and very crucial in understanding its chemical and molecular interaction as well as in determining the therapeutic dose of Myrmecodia.

Because of preliminary nature of this study, there are several shortcomings that should be addressed. First, the positive control group that only received Myrmecodia extract did not included in this study, so the sole effect of Myrmecodia could not be assessed. Second, the potential effect of ethanol which used as solvent in the extract also could not be excluded. Ethanol administration could also induce mucosal necrosis as reported by Yang et al. Therefore, further studies are needed to assess the therapeutic dose, pharmacodynamic, and pharmacokinetic of Myrmecodia extract.

CONCLUSION

Toxic dose of gentamicin was potent enough to induce tissue necrosis in gastric tissue of Wistar mice. The administration of 250 mg / kb BW Myrmecodia extract in addition to gentamicin, induce a higher degree of necrosis and hemorrhage. Further studies with inclusion of extract-only control group are needed to assess the therapeutic dose, pharmacodynamic, and pharmacokinetic of Myrmecodia extract.

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CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this article.

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ETHICS APPROVAL

This study had been approved by Animal Ethics Committees of Faculty of Veterinary Medicine, Universitas Udayana with ethical clearance number 1780a/UN14.2.9/PD/2018

AUTHOR CONTRIBUTION

All authors contributed equally in the research process, writing, and revising this article.

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