

Toxicologic Analysis of Linlinna-aw (*Peperomia pellucida* Linn.)

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Abstract

This study primarily aimed to perform pharmacological testing on a locally abundant plant that grows widely in the province of Ilocos Sur, commonly known as "linlinna-aw" and known scientifically as Peperomia pellucida Linn.. Specifically, this experimental investigation observed the behavior of the Swiss mice which were administered orally with the test drug, determined the mortality of the Swiss mice after oral administration, and identified the toxicity range (Approximate Lethal Dose or ALD) of "linlinna-aw".

Experimental method was used as the research design employing actual laboratory set-up. The first phase (air-drying and extraction processes) was done at the UNP Chemistry and Natural Science Laboratory and the second phase (pharmacological testing through Approximate Lethal Dose) was done at the Chemistry and Pharmacological Division of the Department of Science and Technology in Bicutan, Taguig, Metro Manila from January 1999 - March 2000.

The ethanol extract of "linlinna-aw" (Peperomia pellucida Linn.) has toxicity effect when administered orally to Swiss mice. The Approximate Lethal Dose (ALD) is 8 g/kg. It is safe to use a dose ranging from 1 g/kg up to 7.5 g/kg. Toxicidrome ranges from decreased motor activity, ptosis, writhing, hyperemia, abnormal gait, ataxia, diarrhea, convulsion, and death of two mice within 30 minutes.

The mortality ratio of Swiss mice administered orally with the ethanol extract is 20:20.

In the autopsy findings, all mice that died immediately and those sacrificed and autopsied after 14 days had grossly normal findings.

Potential Technology. Below 8 g/kg of the sample drug of linlinna-aw can be transformed into tablets and lotion/ointment for the treatment of arthritis and convulsion, and warm poultice for abscesses and boils.

A dose of 8 g/kg or more can be used as pesticides.

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Introduction

Rationale

The Philippines, "The Land of Paradise", is considered a rich country because of its vast natural resources. It is vested with plants which are of ornamental, medicinal, and economic values but only very few are aware of their uses and economic importance. While it's true that plants have been used by man in a variety of ways, some may also be toxic when taken in excessive amount. Plants are used by man for food, clothing, shelter, and medicine. They really play a very important role in the survival of mankind. Most of these Philippine plants are in abundance throughout the year; if these are properly nurtured they serve as raw materials in industry which greatly affect our economic stability. So every Filipino must take part in the proper utilization of these Philippine plants.

"Linlinna-aw" (*Peperomia pellucida* Linn.) is a pantropic species of American origin and is commonly found in damp walls during the rainy season in some areas of the Philippines.

"Linlinna-aw" is a succulent, erect, branched herb, 5-40 cm tall. The stems are round and often 5 mm thick. The leaves are ovate, 1-3 cm long with pointed or blunt tip and heart-shaped base, pale green, pellucid, and shining. The spikes are green, erect, very slender, and 1-6 cm long. The fruit is spherical and less than 1 mm thick.

According to Dr. Eduardo Quisumbing (1981) in his book entitled, "Medicinal Plants of the Philippines", the whole plant (*Peperomia pellucida* Linn.) is used as a warm poultice for abscesses and boils. In the same book, Dalziel mentioned that in Tropical West Africa, the people also use the plant as an ingredient in medicinal infusions for the treatment of convulsions.

The book entitled, "Bioactive Substances from Natural Sources" by E.J. del Rosario, et al. (1996) states that "linlinna-aw" is one of the 11 medicinal plants recommended by the Department of Health (DOH) for the treatment of common health problems of the country. **It was proven that this plant can cure arthritis and rheumatism.**

It is time, therefore, to give medicinal plants just scientific treatment not only as a legitimate area of scientific inquiry but also as an issue for national concern, for the following reasons:

1. Plant preparations are the only medicines many of our folks have easy access to notwithstanding miniature pills and bottled drugs.
2. There is a need to develop local sources of drugs in the name of saving dollars and a self-reliant economy.
3. Medicinal plants have their place in modern medicine. For example, scientists have not been able to prepare alkaloids in serpentina synthetically. The textbook "Plants in

the Philippines" (Merill, 1978) has this to say: "It is observed that many vegetable drugs defy laboratory analysis, and, therefore, their therapeutic constituents cannot be isolated. As a result, there is unavoidable use of the plants in herbal medicine." In other words, plants do not only have some medicinal properties which could not be duplicated by man and, therefore, should be used in their raw or semi-processed form, but they also serve as a cheaper source of medication.

Therefore, we should not take for granted the value of these medicinal plants.

New drugs originate from many different sources. Accidental observations on natural products, unexpected clinical findings on known compounds, basic physiological or biochemical investigations, or even test tube experiments have provided leads to great therapeutic discoveries.

Today, most of new drugs are known by systematic screening methods. The processes are so designed to distinguish useful drug materials from the non-useful ones as rapidly, comprehensively, and unexpensively as possible.

Primary screening provides a general profile of the toxicity, pharmacologic activities, and pharmacokinetics of a new drug. The results obtained from the animal models are used to evaluate the safety of the material, its toxic effect, and its intended therapeutic properties. Thus, it is essential that the pharmacological and toxicological properties of the drug material be established before any clinical trials on man be conducted.

With the reasons stated above, the researchers were greatly interested in looking into the toxicity range of "linlinna-aw" (*Peperomia pellucida* Linn.) using swiss mice as subjects for experimentation. It was greatly hoped that this study would also serve to awaken the interest of the readers to make further studies on the indigenous "linlinna-aw".

Objectives

1. To observe the behavior of the swiss mice administered orally with the sample.
2. To determine the mortality ratio of the swiss mice after oral administration.
3. To determine the toxicity range (ALD) of "linlinna-aw" (*Peperomia pellucida* Linn).

Scope and Delimitation

This study was limited only to the pharmacological testing of "linlinna-aw" (*Peperomia pellucida* Linn.) particularly its toxicity effects, using 90 swiss mice as test animals.

The first phase was conducted at the UNP Chemistry and Natural Science Laboratory and the second phase was done at the Chemistry and Pharmacological Division, Department of Science and Technology (DOST), Bicutan, Taguig, Metro Manila from January 1999-March 2000.

Review of Related Literature

The University of Santo Tomas (UST) is very much interested in the study of medicinal plants. It has come up with the publication of "Acta Manilana", a book on Philippine Medicinal Plants. A supplement of the Acta Manilana is a manual on the procedure of phytochemical, microbiological, and pharmacological screening of medicinal plants (Banez, 1993).

According to Isleta as cited by Banez (1993), Estrada conducted a study on Pharmacological and Toxicologic Analyses of "Lagundi" (*Vitex negundo* Linn.) in 1989. The pharmacologic analysis showed that 100% aqueous extract of "Lagundi" has a lethal dose of 50 of 103 g/kg body weight in the adult albino mouse. Contractions of isolated tissue preparations of the rat duodenum, cat tracheal chain, and rat uterus were depressed. A bioassay method using the rat duodenum for the potency of batches of "lagundi" was established.

The present study is different from the study of Estrada because another plant species was used and swiss mice were subjects for experimentation in toxicity while Estrada's study used the bioassay method using rat and cat as test animals.

The intraperitoneal administration of a number of compounds causes a characteristic response in mice which has been termed writhing, stretching, clamping, squirming, and most recently the abdominal constriction response (Domer, 1971). The number of compounds which cause an almost immediate response is quite varied. However, those which cause the characteristic response in 30 seconds include acetylcholine, adenosine triphosphate, bradykinin, 5-hydroxytryptamine, 2% potassium chloride, 4% sodium chloride, or tryptamine. Following a longer delay, the response is seen following the administration of acetic acid, 1.8% calcium chloride, chlorobutanol, 5-hydroxytryptophan, 2% magnesium sulfate, phenlquinone or tryptophan. As such a wide variety of compounds will evoke this characteristic abdominal constriction response, it is not surprising to find a difference of opinion with regard to the usefulness of such a test for characterizing anti-inflammatory analgesic activity.

Parkes and Pickens found that a number of environmental conditions affected the dose-response curves of pain in animals administered with phenylquinone (Banez, 1993). It was found out that less mice were needed to discriminate if the test was carried out at 34°C. Likewise, dieting and fasting the mice before use steepened the dose-response relationship and increased the precision of determining the effectiveness of non-narcotic analgesic activity.

Siegmund injected 0.25 ml of a 0.02% aqueous solution of phenylquinone intraperitoneally in mice. It caused the syndrome which was characterized by intermittent contractions of the abdomen, twisting and turning of the trunk and extension of the hind limbs beginning 3 to 10 minutes after the injection and persisting for more than 1 hour. Only animals that repeatedly showed the syndrome were used. All mice that had not been treated by a potential analgesic compound exhibited the syndrome at least once in a 5-minute period. Therefore, animals were observed for 5 minutes every 15 minutes. As the syndrome occurred, the mice were removed in the cage. Those remaining were considered to show the analgesic effect of drug administration. Drugs were administered orally. Both narcotic and non-narcotic analgesics were active in this procedure, as well as local anaesthetic compounds (Banez, 1993).

Whittle administered test compounds by stomach tube to 20-gm mice. Twenty to 25 minutes later, 0.1 ml of 4% Pontamine sky blue dye was administered intravenously. At 30 minutes, 1 mg of acetic acid was injected intraperitoneally. Twelve mice per dose were used. The animals were placed in a box and the number of squirms within a 20-minute period was recorded. The animals were then killed by cervical dislocation and the viscera were exposed. After allowing 1 minute for the blood to drain away from the abdominal wall and the viscera were irrigated with distilled water while being held over a petridish. The combined visceral washings were filtered through glass wool and made up to a volume of 10 ml. One-tenth milliliter of 0.1N sodium hydroxide was added to each tube to clear any turbidity due to protein. The absorption was read in a spectrophotometer at 590 m μ . Drug activity was expressed as a percent decreased from the control value. Non-narcotic analgesics decreased both the squirming and the leakage of the dye. Narcotic analgesics and central nervous system stimulants decreased only the squinching response. Corticosteroids did nothing in this test

Bradykinin has been found to induce the writhing syndrome. This was injected intraperitoneally 2.5 mg. in mice and caused an abdominal torsion, drawing up of the hind legs to the body, a marked contraction of the abdominal area, and an arching of the back characteristic of the writhing syndrome. Eighty-five percent of the injected animals exhibited this syndrome within 20 minutes. The test compounds were given orally and 10 minutes later the bradykinin was administered. Ten minutes after the bradykinin administration, the number of wriths were counted for a period of 10 minutes.

A number of investigators have used the alteration in capillary permeability following the intravenous administration of a dye as a means of evaluating anti-inflammatory activity in mice.

Banez (1993) conducted pharmacological testing of "sanggumay" orchid (*Dendrobium superbum Reichb.*) and found out that the plant has analgesic effect when administered orally to mice. The ethanol extract has toxicity effect and the approximate lethal dose is 2,500 mg/kg.

Methodology

Experimental method was used as the research design employing actual laboratory set-up. Two phases were included in the conduct of the study.

Phase I. The collection of fresh "linlinna-aw", the air drying process and the extraction process were included in this phase.

Preparation Of the plant extract. Fresh "linlinna-aw" plants were gathered from Ilocos Sur and La Union. They were washed thoroughly and air dried for four days (Figure 1).

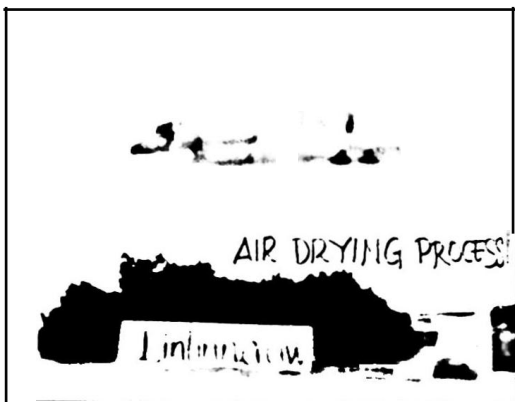


Figure 1. The air drying process of "linlinna-aw"

The plants were finely cut into small pieces and weighed in a balance. The sample weighing 500g was placed in an Erlenmeyer flask. Sufficient amount of ethyl alcohol was added to completely submerge the material. It was stoppered and soaked for 24 hours. Then, it was filtered through a cheese cloth and finally, filtered through a glass funnel. Extraction was done in Soxhlet Apparatus (Figure 2).



Figure 2. The extraction process using ethyl alcohol.

The filtrates were concentrated under vacuo to about 50 ml. The exact volume of the concentrated extracts was measured, placed in two tightly stoppered containers and stored inside a refrigerator. The extracts were ready for pharmacological testing.

Phase II. This included the pharmacological testing using male Swiss mice as subjects of experimentation.

Test drug material, The plant extract (ethanol extract) under study was absolutely free of extracting solvents, thus, this was completely dry or syrupy in consistency.

The drug material was subjected to pharmacological testing together with a standard drug (Normal Saline Solution) as control sample.

Experimental animals. Healthy 90 male Swiss mice, each weighing 20 to 30 g at the start of the experiment, were kept in individual observation cages. All animals were made to fast from food and water 16 hours before the test. Two hours after administration of the drug, the animals were given free access to food and water.

Procedure. Eight increasing doses of the test substance were given orally to the animals in eight groups of 10 animals. Another group of 10 animals was given the control sample equivalent to the volume used for the highest dose of the test substance. The number of deaths and other adverse/abnormal signs and manifestations were closely observed and noted for the first two hours after administration of the test sample. This was continued in the next 24-48 hours, daily up to 14 days.

Figure 3 shows the schematic flow of the research method from collection of "Linlinna-aw" plants to the pharmacological test.

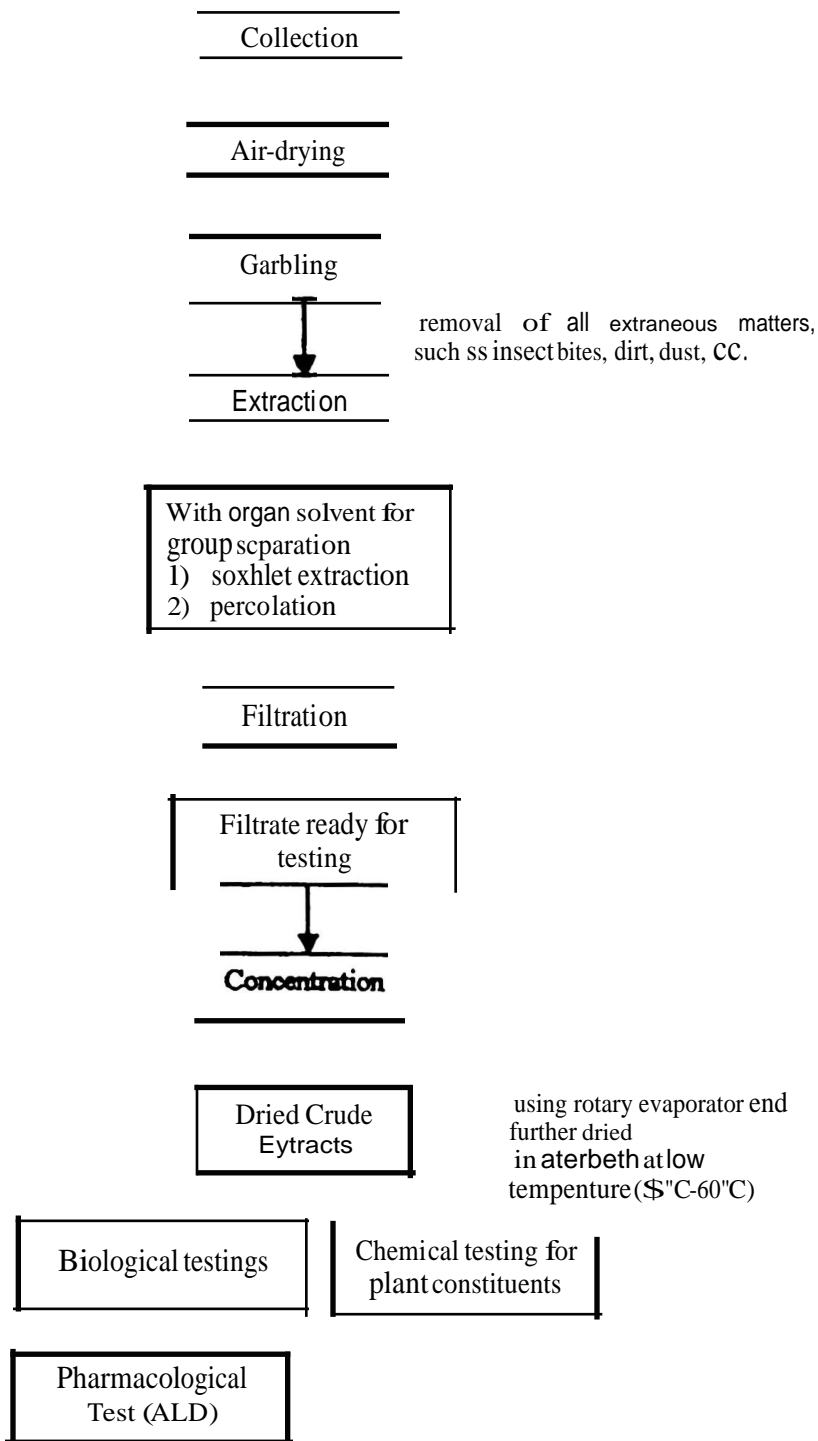


Figure 3. Schematic flowchart of the research method.

Discussion of Results

Behavior of Test Animals

Behavioral observation of the results of Approximate Lethal Dose (ALD) using Swiss mice as test animals is exhibited in Table 1. Doses of 2-7.5 g/kg ethanol extract were given orally to six groups of 10 animals. Five minutes after dosing, the mice manifested decreased motor activity and ptosis lasting for two hours. This indicates that using the drug with this amount is still safe.

On the other hand, two groups were given 8 g/kg and 12 g/kg ethanol extract, respectively. Five minutes after dosing, the mice manifested decreased motor activity, ptosis, writhing, hyperemia, abnormal gait, ataxia, diarrhea, convulsion, and death of 10 mice within 30 minutes.

It was observed that upon giving the drug orally to the test animals, the Swiss mice showed abnormal depression, drooping of the upper eyelid due to paralysis or atrophy of the levator palpebrae. Active hyperemia occurred in physiologic activity of glands and other organs and in inflammation. Due to selective toxic effect, a severe acute infection on the spinocerebellar tracts occurred. Abnormal gait was also observed; the feet were raised high, thrown forward, and brought down suddenly, the whole soles striking the ground at once; it was also characterized by lesions of the posterior column of the spinal. Finally, convulsion occurred leading to the death of the mice.

In the control group, normal saline solution (NSS) was used. The same volume as that in the highest dose (12 g/kg) was administered orally to the mice and normal condition was observed.

Table 1. Behavioral observation/toxidrome after oral administration of sample to male Swiss mice.

DOSE g/kg	N	OBSERVATION
0	10	Normal Condition
2	10	
4	10	
6	10	Five minutes after dosing, the mice manifested decreased motor activity.
6.5	10	
7	10	
7.5	10	
8	10	
12	10	Five minutes after dosing, the mice manifested decreased motor activity, ptosis, writhing, hyperemia, abnormal gait, ataxia, diarrhea, convulsion, and death of 10 mice within 30 minutes.

- - Control; normal saline solution was used at the same volume as that in the highest dose.

Mortality Ratio

Table 2 presents the summary of mortality ratio of mice administered orally with the ethanol extract.

Table 2. Summary of mortality ratio of mice administered orally with the sample.

GROUP NO.	DOSE g/kg	N	Day 1	MORTALITY RATIO			
				Day2	Day 3	Day7	Day 14
I	O	10	0/10	0/10	0/10	0/10	0/10
II	2	10	0/10	0/10	0/10	0/10	0/10
III	4	10	0/10	0/10	0/10	0/10	0/10
IV	6	10	0/10	0/10	0/10	0/10	0/10
V	6.5	10	0/10	0/10	0/10	0/10	0/10
VI	7	10	0/10	0/10	0/10	0/10	0/10
VII	7.5	10	0/10	0/10	0/10	0/10	0/10
VIII	8	10	10/10	10/10	10/10	10/10	10/10
IX	12	10	10/10	10/10	10/10	10/10	10/10

O– control, the same volume as in highest dose

Group 1 was given nonnal saline solution (NSS) at the same volume as that in highest dose (12 g/kg) and no mice died until the 14 day. In group II-VII which were orally administered with 2-7.5 g/kg ethanol extract no mice died until Day 14. In group VIII, 10 mice were given 8 g/kg ethanol extract and it was observed that in Day 1 all of them died and so also did the mice in Group IX which were given 12 g/kg test drug. The mortality ratio in groups VIII and IX is 20:20.

Toxicity Range

The data indicate that dosage of ethanol extract of "linlinna-aw" ranging from 1-7.5 g/kg is safe to use. The approximate lethal dose (ALD) of the sample ethanol extract of "linlinna-aw" is 8 g/kg. This implies that anything taken in excess is toxic and ethanol extract at a dose of 8 g/kg or more can be used as pesticides.

In the autopsy findings, all mice that died immediately and those sacrificed and autopsied after 14 days had grossly normal findings.

Conclusions

Based on the findings, the following conclusions were drawn:

The ethanol extract of "linlinna-aw" (*Peperomia pellucida* Linn.) has toxicity effect

when administered orally to Swiss mice. The Approximate Lethal Dose (ALD) is 8 g/kg. It is safe to use a dose ranging from 1-7.5 g/kg. Toxidrome ranges from decreased motor activity, ptosis, writhing, hyperemia, abnormal gait, ataxia, diarrhea, convulsion, and death of two mice within 30 minutes. A dose of 8 g/kg or more can be used as pesticides.

The mortality ratio of Swiss mice administered orally with the ethanol extract is 20:20. In the autopsy findings, all mice that died immediately and those sacrificed and autopsied after 14 days had grossly nonnal findings.

Recommendations

Based on the conclusions, the researchers present the following recommendations.

I. A follow-up study should be conducted to quantify, isolate, and identify the types of chemical constituents present in "linlinna-aw".

2. Further studies on the plant's therapeutic properties like anti-inflammatory, antipruritic, analgesic, etc. are recommended to be undertaken by interested researchers.

3. The toxicity level of "linlinna-aw" (*Peperomia pellucida* Linn.) is recommended to be listed in the compilation and documentation of medicinal plants in the Philippines through the Philippine National Science Society, Department of Science and Technology, and the University of the Philippines.

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