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Research Article

ACUTE ORAL TOXICITY STUDY OF THE CRUDE ETHANOLIC LEAF EXTRACT OF *FICUS PSEUDOPALMA* BLANCO (MORACEAE) IN SPRAGUE DAWLEY RATS

Librado A. Santiago[1,2,3*], V. L. M. Valerio[1], and R. T. Yolo[2,4]

1. Research Center for the Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines.
2. Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, Manila, Philippines.
3. Graduate School, University of Santo Tomas, Manila, Philippines.
4. Anatomic Pathology Division, University of Santo Tomas Hospital, Manila, Philippines.

***Corresponding Author: Email** librado_santiago@yahoo.com

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ABSTRACT

Ficus pseudopalma Blanco is an ornamental plant endemic to the Philippines, especially in the island of Luzon. It is commonly used to treat kidney stones and diabetes and used for edible fruits. The leaves are cooked and eaten as vegetable despite the absence of studies on its possible toxic effect. This study was conducted to assess any toxicity of its leaf extract. Acute oral toxicity of the crude ethanolic leaf extract of *F. pseudoplama* was performed according to the guidelines set by OECD 425 on six 8-12 week old female Sprague Dawley rats weighing from 160-210g. One rat was treated with normal saline solution that served as the control. Toxicological and pharmacological observations were completed for 14 days. On day 14, all test animals were sacrificed via cervical dislocation and subjected to gross necropsy; liver samples were subjected to histopathological examination. Gross examination of the rodent's organs was all normal and regarded as unremarkable. Toxicological screening showed that the experimentally treated rats behaved almost normally as the control. Histopathological examination showed no area of hepatic zonal necrosis and tumor formation was identified, no cytological aypia and sinusoid congestion, intact and uninterrupted hepatic lobular architecture, and portal tracts and vessels were unremarkable. These findings strongly suggest that the leaf extract is non toxic and safe for consumption up to 2000 mg/kg BW and may therefore be used for future nutraceuticals and drug development.

Keywords: Botanicals, medicinal properties, food supplements, toxicological evaluation, *Ficus pseudoplama* Blanco.

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INTRODUCTION

Of the more than 800 *Ficus* species in the world, 150 of them are endemic in the Philippines[1]. One of these is *Ficus pseudopalma* Blanco, a species of the fig is known by the common names Philippine fig, dracaena fig, palm-leaf fig, *niog-niogan* (in Tagalog region) and *lubi-lubi* (in Bicol region). It is known elsewhere as an ornamental plant. It belongs to the *Moraceae* family. It is an erect, glabrous, slender, unbranched and palm-like, fast- growing shrub. It is cultivated and used for edible fruits; matured leaves are cooked and sprouts young shoots are eaten as vegetable[2]; leaves are used as food wrap; fodder for livestock; and firewood. In Philippine traditional medicine, the leaf extract is used in the treatment of kidney stones and diabetes[2]. This plant contains triterpenoids namely: squalene, α-amyrin acetate, β- amyrin acetate, lupeol fatty acid ester, lupenone, oleanone, ursenone, and polyprenol[3]. Among these, triterpenoid lupeol and ursenone were found to have antioxidant activities[4].

Furthermore, the fig genus, has been known to be effective as a hypocholesterolemic, gastro protective, anti-

inflammatory and hepatoprotective agent[5]. The genus was also reported to have anti-cancer activity generally connected to the chemopreventive activity of antioxidants[6].

Other than these, there are no other published studies as yet on its toxicity profile. Moreover, the plant is predisposed to very limited biological, biochemical nor pharmacological studies because it remains a lowly ornamental plant. Studies relating to toxicity of some *Ficus* species showed that it is non-toxic and safe. Aqueous extract of the leaves, seeds and bark of *F. platyphylla* Del. Holl. at 3000mg/kg limit dose, proved to be non-toxic in rats[7]. The acute oral toxicity test in rats revealed that ethanolic leaf extract of *F. glomerata* L. at 2000mg/kg dose was safe[8]. Furthermore, the methanolic extract of the bark of *F. racemosa* L. at 2000mg/kg was also found to be non-toxic to rats[9]. The methanolic bark extract of *F. platyphylla* (17-150 mg/kg) decreased exploratory activity in mice, produced peripheral and central analgesic and depressant effects[10]. Wherefore, evaluation of the toxicity of *F. pseudopalma* leaf extract is important.

To date, there are numbers of plant derived medicines that are already out in the market and are currently used by many in treating different kinds of diseases. Vincristine and Vinblastine are two alkaloids that were isolated from the plant C*atharanthus roseus* which are used as drug constituent and usually cost at around $24,000 and $6,800 per gram, respectively[11]. About 60-80% of the world population still heavily relies on traditional medicines for the treatment of common illness.

Material and Methods

Plant Collection and Preparation

Two kilograms of fresh leaves of *F. pseudopalma Blanco* were collected from Brgy. San Jose, Pili, Camarines Sur, Philippines. Leaf specimens were placed inside newspapers, pressed, and placed inside thick polyethylene bags. The plant was authenticated by the Botany Division of the National Museum Herbarium, Manila. The leaves were air-dried and protected from sunlight for 7-10 days in an air-conditioned room at 18-25°C to avoid decomposition of thermolabile compounds and potential chemical transformation. Dried leaves were ground to fine powder twice using Wiley mill and sieved in 20 mm mesh size. The powder sample was kept in a clean, dried, well-sealed amber glass container to protect it from sunlight.

Plant Extraction

The solvent-to-sample ratio was added in 1:10 ratio as described[12]. The powdered leaves (1.52 kg) were soaked five times with 95% ethyl alcohol in a percolator for two weeks consuming about 15 liters of the solvent or until the last batch of filtrate was colorless. The filtrates after each solvent extraction were concentrated separately using the rotary evaporator (Eyela, USA) at 40°C until syrupy consistency was obtained. The syrupy extract was further evaporated to dryness at 35°C for 5 days. The air-dried crude extract was weighed, obtaining 2.82% yield and kept in amber-colored container under 0°C until use.

The ethanol extract is distinguished by its dark-green to black color and its semi-glossy appearance. Its greasy texture makes it stick to glass and other materials but is easily removed with alcohol. It gives off a distinct oil-like smell. Its pH is almost neutral at 6.82.

Limit Test

The dose (2000 mg/kg) that was used for this study was determined following the Limit Test method as given by the Organization for Economic Cooperation and Development (OECD) Guidelines. This protocol issued by the OECD is a simple test used to approximate the safety of a certain test extract. A total of six healthy randomized and properly identified female 8 weeks old Sprague Dawley rats purchased from the Food and Drug Administration were utilized in the experiment. The experimental protocol that was used to the test animals were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Santo Tomas (UST-IACUC). Prior to dosing the rats were acclimatized for seven days then was fasted overnight (food but not water). Following the period of fasting, the rats were weighed and the test substance was administered. The fasted body weight (BW) of each animal was determined and the dose was calculated according to the body weight[13]. The first two animals were given a dose of 2000 mg/kg BW extract daily for 14 days. Each time the rodents were observed immediately an hour after administration of the extract. Afterwards, the next three animals were also given the same dose of the plant extract,

repeated daily for 14 days. Mortality, signs of toxicity and weight gain were monitored. If three or more animals survive the test, the lethal dose is approximated to be greater than 2000mg/kg BW, and a dose less than that can be used for experimental purpose. The rats were sacrificed via cervical dislocation.

After cervical dislocation, immediate and proper fixation was performed as the livers are resected. The livers were placed in a small container with 10% neutral buffered formalin, with a volume at least 20x the tissue volume, and were weighed using an analytical balance. This is generally used for preservation and storage of surgical, post mortem and research specimens.

The liver of the rats were delivered to Hi-Precision Diagnostics, Quezon City, Philippines to prepare the liver specimen for histopathological examination. In the laboratory, the livers were resected and sectioned using a microtome slicer. This procedure allows proper fixative penetration by the liver samples. The liver samples were excised, weighed, sectioned and sent to the Anatomic Pathology Division of the University of Santo Tomas Hospital for histopathological examination to verify whether organ damage materialized due to the administration of the test extract.

Results and Discussions

There is a growing concern regarding the secure use of botanicals and botanical preparations. Hence, a toxicological evaluation of these extracts is needed. With the aim to assure the safety of the dried extract and due to the scant literature information and established history of food use, the toxicity profile of the crude ethanolic leaf extract of *F. pseudopalma*.

Although used as an ingredient in food preparations and in traditional treatment of specific ailments such as kidney stones and diabetes, there are no published literature information about toxicity of *F. pseudopalma* and established history of it as food in the country or any pre-clinical study, to date.

Limit Test

The Limit Test for the determination of acute oral toxicity was conducted for 14 days where rats were dozed orally with 2000mg/kg BW of the extract daily. A total of six female Sprague Dawley rats (8-12 week old) weighing from 160-210g were used in the experiment wherein the initial weight of each rat was noted after a seven-day acclimatization period. Each rat was labeled from 1 to 6, where rats 1, 2, 3, 4 and 5 were the test group and rat 6 is the control that received NSS only. During the course of the experiment, two rats, rat 1 and 4, were found dead on the 10th and 12th day of dosing, respectively. Death of these two rats was not expected and may not be related to the administered extract at 2000mg/kg dose. The two rats may already be suffering from illness when purchased from the Bureau of Food and Drugs (BFAD) or the cause of death may be related on the way of administering the extract. Nonetheless, a death of two animals out of five test animals would mean that the toxic dose is definitely lower than 2000mg/kg body weight, according to the OECD Guidelines.

Toxicological Screening

Observations before euthanizing the remaining test animals were done to monitor if there are behavioral changes. Toxicological screening was done as guided by Guevarra (2005)[13].

As listed in Table 1, general observations showed that the extract did not affect the animal's salivation, tail erection, pilomotor reaction, micturation, weight gain and stomach activity. Also, Robichaud test shows that the skin promptly readjusted to the contours of the animal's body. The rats did not move around in circles like the normal control. Intruding activities such as head tapping, grasping and foreleg piercing showed fear and aggression.

The motor activity of each rat did not decrease, however, occasional incoordination and sluggish response with vocalization and attempts to bite or to escape were observed. Upon placing them on their sides, the rats immediately turn to their upright position. Extract treated rats loosed screen grip when tilted to 90° whereas the control rat loosed screen grip when tilted 180°. These observations showed no signs of CNS depression for the treated rats. Also, rats showed visible jerks on loud sounds, they move constantly and rapidly, with some attempt to escape, they also experienced continuous tremor when held which are all the same compared to the normal control.

Ear observation showed that there are no significant changes in the blood vessels and there was no cyanosis present in the

Table 1. Toxicological Screening of Rats Induced with *Ficus pseudopalma* Blanco Leaf Extract

TEST	Rat 2	Rat 3	Rat 5	Rat 6 (control)
A. Central Nervous System (CNS) Depression				
1. Decrease in motor activity	0	0	0	0
2. Ataxia Rating	0	0	0	0
3. Loss of Righting Reflex	0	0	0	0
4. Analgesia	0	0	0	0
5. Anesthesia	0	0	0	0
6. Respiratory Rate and Depth	N/A	N/A	N/A	N/A
7. Corneal and Pinnal Reflex	0	0	0	0
8. Paralysis of forelegs, hind legs, and head	1	1	1	1
9. Loss of screen grip	2	2	2	2
B. Central Nervous System (CNS) Stimulation				
1. Startle reaction	1	1	1	1
2. Increase in motor activity	0	0	0	0
3. Fine body tremors	0	0	0	0
4. Course body tremors	0	0	0	0
5. Fasciculations	0	0	0	0
6. Convulsions	0	0	0	0
7. Respiratory rate and depth	0	0	0	0
C. Eye Observation				
1. Enophthalmus	0	0	0	0
2. Exophthalmus	0	0	0	0
3. Palpebral ptosis	0	0	0	0
4. Pupil size	0	0	0	0
5. Nystagmus	0	0	0	0
6. Lachrymation	0	0	0	0
7. Chromodacryorrhea	0	0	0	0
D. Ear Observation				
1. Blanching	0	0	0	0
2. Hyperaemia	0	0	0	0
3. Cyanosis	0	0	0	0
E. General Observations				
1. Salivation	0	0	0	0
2. Tail erection or Straub response	0	0	0	0
3. Pilomotor erection	N/A	N/A	N/A	N/A
4. Micturation	0	0	0	0
5. Diarrhea	0	0	0	0
6. Colpectasia	0	0	0	0

7. Priaprism	N/A	N/A	N/A	N/A
8. Robichaud test	0	0	0	0
9. Circling motion	0	0	0	0
10. Tail lashing	0	0	0	0
11. Abdominal gripping or writing	0	0	0	0
12. Rectal temperature	N/A	N/A	N/A	N/A
13. Body weight	200g	200g	200g	200g
F. Subjective Test				
1. Head tap test	1	1	1	1
2. Body grasp	0	0	0	0
3. Catalepsy	0	0	0	0
4. Excess curiosity	0	0	0	0

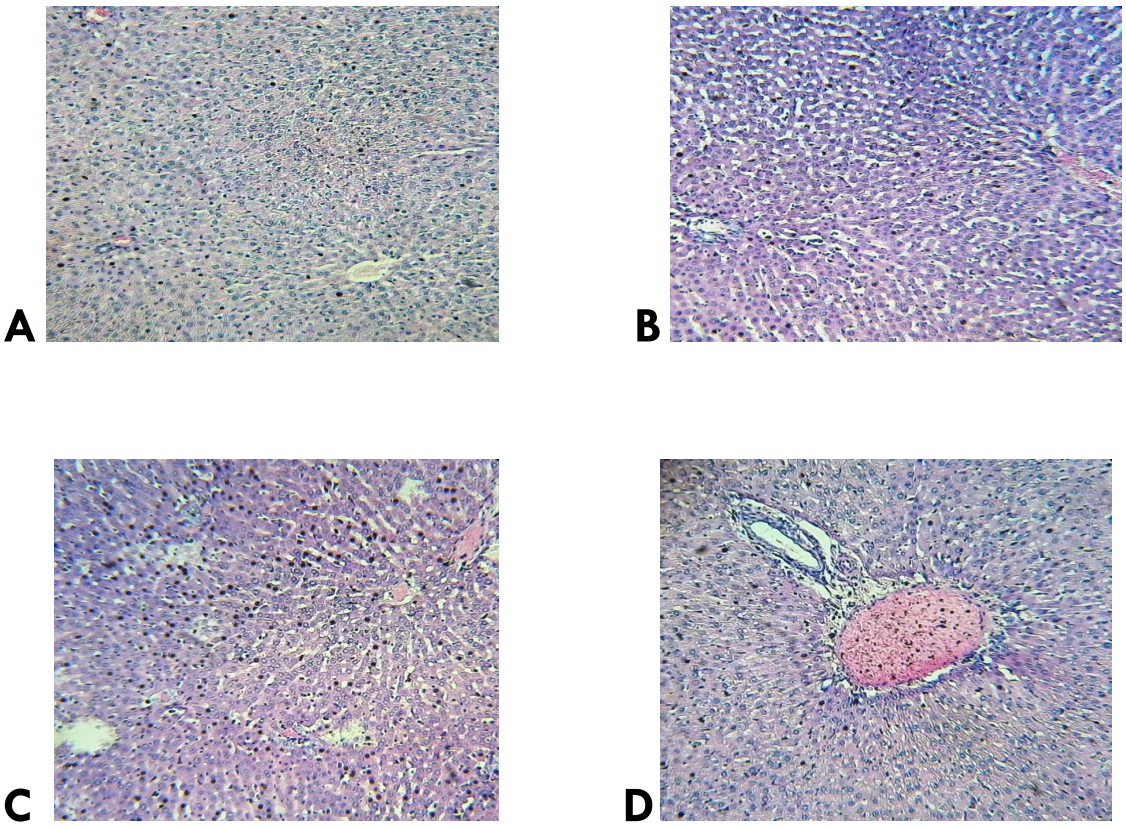

Figure1. Cross section from the livers of each rat treated with the crude ethanolic leaf extract of *F. pseudopalma* at 2000mg/kg BW dose. Histopathologic analysis of livers of the experimentally treated rats (Figure 1A, 1B and 1C) using H&E staining showed that the rat's hepatic cells are normal and showed no toxic signs as compared to the liver of the control rat treated with NSS (Figure 1D). Viewed at 200x magnification.

treated animals. Eye observations did not note any sign of enophthalmos and exophthalmos. The eyelids are non-drooping and the pupil showed normal contractile response. Therefore, the extract did not show any side effect on the visual of the test animal.

Gross Necropsy and Histopathological Analysis

On the 14th day, the weight of each rat was recorded before they were sacrificed by cervical dislocation. Gross examination of the rats showed that all organs were normal and did not present any toxic signs. Examination of the rat liver showed that there was no discoloration and it has a smooth and firm surface.

In order to observe the effects of the plant extract on the liver cells of the rats, the fresh rat livers were collected and weighed. These liver samples were prepared for Hematoxylin and Eosin (H&E) staining. In histology, both eosin and hematoxylin are used as a routine stain and is better known as the hematoxylin and eosin (H&E) stain. This is the primary stain used to give a visible look at the nucleus of cells, which can give a general conclusion if there is abnormal growth or division in the nucleus of the cells.

In the study, histopathological observation was performed at the UST Hospital. The pathologist disclosed in his independent report unremarkable liver tissues. The hepatic lobular architecture of each liver was intact and uninterrupted. The portal tracts and portal vessels were unremarkable. The hepatocytes are arranged in a radical formation converging from the centrilobular veins and showed no cytologic aypia. No abnormal accumulation of bile pigment was observed and the hepatic sinusoids were not congested. Lastly, no area of hepatic zonal necrosis and tumor formation was identified. The pathology report was officially documented and formally recognized as a UST research effort and was entered in the UST Anatomic Pathology log dated November 26, 2012.

CONCLUSION

The study showed that the ethanolic leaf extract of *F. pseudopalma* Blanco possess no toxicity and safe for consumption. This attribute makes *F. pseudopalma* as a good agent for nutraceuticals and drug development.

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REFERENCES

[1] Pancho JV. (1983). Vascular flora of Mount Makiling and Vicinity (Luzon, Philippines) Part 1 Kalikasan. Philipp. J. Biol. Suppl. 1: 67-111.

[2] Stuart G. (2008) An illustrated compilation of Philippine Medicinal Plants. Available online at: http://www.stuartxchange.com/Niyog.html.

[3] Ragasa CY, Tsai PW, Shen CC. (2009). Terpenoids and Sterols from the Endemic and Endangered Philippine Trees, *Ficus pseudopalma* and *Ficus ulmifolia*. Philipp J Sci. 138 (2): 205-209.

[4] Topçu G, Ertas A, Kolak U, Ozturk M, Ulubelen A. (2007). Antioxidant activity tests on novel triterpenoids from *Salvia macrochlamys*. ARKIVOC. 7: 195-208.

[5] Sirisha N, Sreenivasulu M, Sangeeta K, Madhusudhana C. (2010). Antioxidant Properties of Ficus Species – A Review. Int J PharmaTech Res. 2 (4): 2174-2182.

[6] Joseph B, Raj SJ. (2010). Phytopharmacological Properties of *Ficcus racemosa* Linn - An Overview. Int J Pharm Sci Rev Res. 3(2): 134-138.

[7] Ugwah-Oguejiofor CJ, Bello SO, Okolo RU, Etuk EU, Ugwah MO, Igbokwe VU. (2011). *Ficus platyphylla* promotes fertility in female *Rattus norvegicus* Wistar strain: a preliminary study. Reprod. Biol. Endocrin. 9: 145.

[8] Prasanna KV, Sunil V, Venugopal JP. (2011). Evaluation of acute and chronic toxicity studies of ethanolic extract of *Ficus glomerata* L. Int. J. Pharm. Dev. Tech. 1 (2): 66-70.

[9] Kumar CP, Chandra DS, Kumar DS. (2012). Anti-inflammatory activity of methanolic extract of bark of *Ficus racemosa* L. and root of *Cissampelos pareira* L. var *Hirsuta* (DC) forman. Int. J. Res. Phar. Chem. 2 (4): 1128-1133.

[10] Wakeel OK, Aziba PI, Ashorobi RB, Umukoro S, Aderibigbe AO, Awe EO. (2004). Neuropharmacological activities of *Ficus platyphylla* stem bark in mice. African J. Biomed Res. 7: 75-78.

[11] Rao CK. Database of Medicinal Plants. Available online at: www.medicinalplants-kr.org

[12] Green, R.J. (2004). Antioxidant Activity of peanut plant tissues Available online at: http://repository.lib.ncsu.edu/ir/bitstream/1840.16/3371/1/etd.pdf.

[13]Guevarra BQ: "A Guidebook to Plant Screening: Phytochemical and Biological", Research Center for the Natural and Applied Sciences, University of Santo Tomas, Manila, 2005.

[14] Anjana S., John E. T., "Analysis of cytotoxic potential of the aqueous leaf extracts of pogostemon Auricularius (l.) Hassk. Using allium cepa root tip assay" Int. J. Res. Dev. Pharm. L. Sci., 2013, 2 (5), pp. 562-566.

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