Online Resource Material

3 Biotech

Neuroblastoma SH-SY5Y cytotoxicity, anti-amyloidogenic activity and cyclooxygenase inhibition of *Lasianthus trichophlebus* (Rubiaceae)

Mario A. Tan*[1,2,3,4], Mark Wilson D. Lagamayo[1], Grecebio Jonathan D. Alejandro[1,2,3], Seong Soo A. An*[4]

[1]The Graduate School, University of Santo Tomas, Manila, Philippines

[2] College of Science, University of Santo Tomas, Manila, Philippines

[3] Research Center for the Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines

[4] Department of BionanoTechnology, Bionano Research Institute, Gachon University, Seongnam, South Korea

***** Corresponding authors:

Email address: matan@ust.edu.ph (M.A. Tan); seong.an@gmail.com (S.S.A. An)

In vivo Acute Oral Toxicity of the Lasianthus trichophlebus Crude Extract

The experiment protocol (UST-IACUC code number RC2017-890915) was approved by the Institutional Animal Care and Use Committee (IACUC) at the Research Center for Natural and Applied Sciences, University of Santo Tomas, and the Philippine Bureau of Animal Industry and Animal Research Permit.

Six female Sprague-Dawley rats were used to assess acute oral toxicity according to the OECD 425 guidelines. The animals were housed in the UST-RCNAS Animal House and acclimatized to laboratory conditions for seven days before conducting the experiment. They were fed with standard rodent pellets and given access to clean drinking water. The laboratory conditions were maintained at a temperature of 25 ± 3 °C, humidity at 60 ± 4%, and a 12/12 h light/dark cycle.

Acute Oral Toxicity (OECD 425 Guidelines)

All animals were fasted for 24 h to determine their actual weight before receiving the crude extract. One rat served as a normal control group and was treated only with the vehicle in which the respective LTM extract was dissolved. A 500 mg/mL LTM solution was prepared using H_2O as the vehicle. The first animal was given 2000 mg/kg LTM (upper limit dose) via gastric gavage. The animal was observed for 24 hours for signs of toxicity; thereafter, it was observed daily for 14 days. In the event that the first animal died, the main test of the OECD 425 guideline would be used to determine the LD_{50}. If the first animal survived, the other four rats would be administered the same dose of 2000 mg/kg for a total of five animals in the study. The survival of three or more animals would indicate that the LD_{50} was greater than 2000 mg/kg. In principle, the limit test is not premeditated to determine an accurate LD_{50}; rather, it serves as a recommendation to categorize the crude extract according to the expected survival of animals treated with the highest dosage (Roopashree et al., 2009). The animals were observed periodically within the first 24 h, then daily thereafter up to 14 days. The animals were then sacrificed by CO_2 inhalation. Gross necropsy, observation of gross pathological changes, and microscopic examination of all livers, kidneys, and stomachs of the test animals were performed by a licensed veterinarian.

Results of the Acute oral toxicity

Assessment of the acute oral toxicity (Fig S1) of the LTM extract according to the OECD 425 guideline revealed that it was safe and nontoxic up to 2000 mg/kg. No notable variations were observed in the behavioral patterns of female Sprague-Dawley rats during the 14-day observation period. Histopathological analysis by a licensed veterinarian also supported the safety findings, as a cross section of the stomach showed no erosions, ulcerations, or inflammatory infiltrates in the gastric mucosa. Circulatory disturbances were not examined on the other layers of the gastric tissue. Next, the cross sections of both kidneys showed no notable microscopic lesions aside from minimal tubular injury in the proximal tubule region. Ischemic damage, glomerular pathologies, congestion, edema, and acute signs of infiltrated chronic inflammation within the interstitium of the renal parenchyma were not examined. Similarly, the liver cross section presented no signs of hepatocellular degeneration or necrosis, and fibrosis was not observed on any of the portal, periportal, or midzonal areas. In addition, the related inflammatory infiltrates with acute or chronic inflammation were absent in the liver.

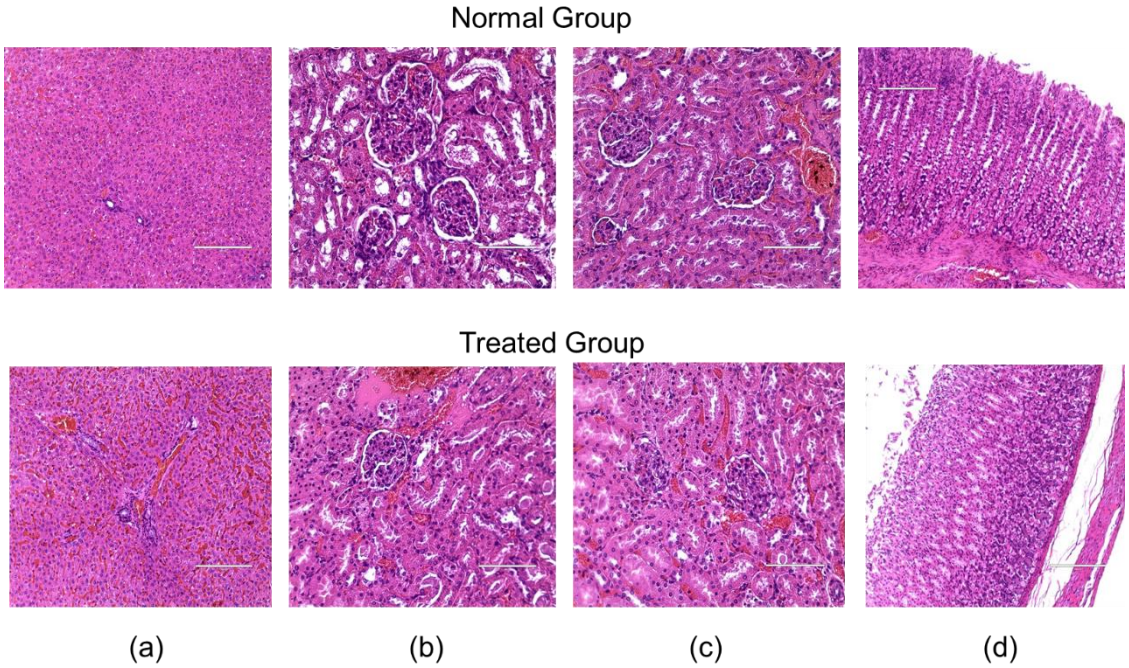

Fig S1. Histopathological Examination of (a) liver, (b) left kidney, (c) right kidney, (d) stomach in normal and *Lasiathus trichophlebus* extract-treated groups.

Reference

Roopashree TS, Dang R, ShobhaRani RH, Narendra C. (2009) Acute oral toxicity studies of antipsoriatic herbal mixture comprising of aqueous extracts of *Calendula officinalis*, *Momordica charantia*, *Cassia tora* and *Azadirachta indica*. Thai J. Pharm. Sci. 33:74–83.