SHORT COMMUNICATION

HISTOLOGICAL EVALUATION OF THE ACUTE NEPHROTOXICITY OF *THEOBROMA CACAO* LEAF EXTRACT (COALEX-1) IN BALB/C MICE

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ABSTRACT - *Theobroma cacao leaves extract may have potential as a therapeutic agent for the treatment of breast cancer. The antiproliferative activity of cocoa leaf extract is thought to be due to the presence of flavonoids, which are compounds with antioxidant and anti-inflammatory properties. Flavonoids have been shown to have a number of anticancer effects, including inhibiting the growth of cancer cells, inducing apoptosis, and preventing angiogenesis. Hence, more research is needed to confirm these findings. The present study investigated the safety of oral consumption of Theobroma cacao leaves extract (CoaLeX-1) in BALB/c mice for anti-breast cancer study. In the acute toxicity study, 30 mice were randomly assigned into five groups (n = 6), which were the control and four treatment groups of single escalating dose (625, 1250, 2500 and 5000 mg/kg of CoaLeX-1). Animals were observed individually for any clinical signs of toxicity or mortality for 14 days. The histology of the kidneys of the animals was studied at the end of the experiment. During the 14 days observation period, CoaLeX-1 did not induce any toxicity symptom in the mice even at the highest dose (5000 mg/kg). There was no treatment-related mortality of mice at any dose level of CoaLeX-1 tested. The extract did not reveal any marked adverse effects on the kidneys in all treatment groups. Hence, based on the acute toxicity study, treatment with up to 5000 mg/kg of CoaLeX-1 was not toxic to the animals, indicating its safety when a large amount of this plant extract is ingested.*

Keywords: Pre-clinical, cocoa, *Theobroma cacao*, cancer, leaves extract, kidney

INTRODUCTION

Breast cancer is a pressing global health concern, with Malaysia reporting a significant number of new cases, making it the most prevalent cancer and a leading cause of female cancer-related deaths (Htay *et al.,* 2021). However, traditional treatments like surgery, chemotherapy, and radiation therapy face limitations such as drug resistance and toxicity (Chehelgerdi *et al.,* 2023). In response, plant-derived compounds have emerged as promising alternatives in cancer research. Notably, *Theobroma cacao*, or cocoa, has garnered attention for its potential anticancer properties due to its antioxidant content (Baharum *et al.,* 2016). Despite promising findings, research on cocoa's efficacy in treating breast cancer remains limited, leaving a significant gap in understanding its potential effectiveness and safety in breast cancer therapy.

Concurrently, this study aims to conduct an acute nephrotoxicity evaluation of cocoa leaf extract (CoaLeX-1) in BALB/c mice. Through careful assessment of acute nephrotoxicity endpoints, such as mortality, clinical signs, and gross pathology related to kidney health, the study seeks to determine the safety and viability of CoaLeX-1 for potential applications in breast cancer treatment. This acute nephrotoxicity evaluation serves as a crucial preliminary step before further research can be pursued to explore CoaLeX-1's efficacy in breast cancer treatment.

MATERIALS AND METHODS

Plant Material Collection

Fresh cocoa leaves were gathered from cocoa smallholder fields across various locations during the peak fruiting season in mid-2019. A voucher specimen (SK 2434/14) was deposited at the Institute of Bioscience, Universiti Putra Malaysia.

Preparation and Extraction Process

Fifteen kilograms of cocoa leaves were harvested, cleaned, and air-dried in the shade. Once dried, they were ground into a powder and stored in a cool, dark place. This powder was then mixed with distilled water and subjected to extraction using a machine for 2 hours at 100°C. The resulting extract underwent filtration to remove any impurities. After concentration and spray drying, the cocoa leaf extract (CoaLeX-1) was obtained, yielding 0.85%. It was stored at 4°C for further analysis.

Animal Preparation

Female BALB/c mice aged 6 to 8 weeks and weighing between 20 and 30 g were used. They were housed in groups of three per cage under controlled conditions with a 12-hour light/dark cycle, at temperatures of 20-24°C and 40-50% humidity. The mice were obtained from a disease-free animal facility in UiTM Puncak Alam, Selangor and acclimated to the lab for a week, during which they were fed standard chow pellets and had access to water. All procedures involving the mice were approved by the University of Cyberjaya's Animal Care and Use Committee (Approval No.: CACUC/2/2022/3).

Acute Toxicity Study

The toxicity of CoaLeX-1 was tested on 30 mice, divided into five groups (n = 6 mice/group), following to the OECD Test Guideline No. 420 protocol. They were given single escalating doses (625, 1250, 2500, and 5000 mg/kg) of CoaLeX-1 dissolved in distilled water via oral gavage. Meanwhile, control mice received only distilled water. All mice were monitored closely for 14 days, with daily observations of their general condition and weight.

Histopathological Assessment

After euthanasia, the mice's kidneys were examined for signs of toxicity. Kidney samples were weighed, processed, and examined under a microscope for any abnormalities.

RESULTS

In comparison to the control group, no histological changes, including glomerular dilation and tubular necrosis, were observed in the kidneys of mice across all treatment groups, as shown in Figure 1.1 and 1.2 below.

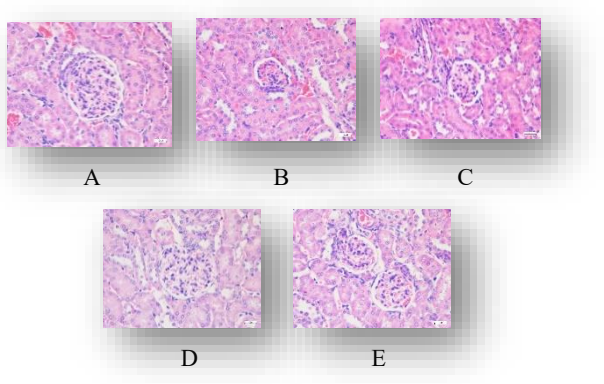

Figure 1.1. The glomeruli of kidney tissue (cortex part) for control and mice treated with different doses of CoaLeX-1 following H&E staining as observed under a light microscope (400× magnification). A: Control; B: Mice fed with 625 mg/kg CoaLeX-1; C: Mice fed with 1250 mg/ kg CoaLeX-1; D: Mice fed with 2500 mg/kg CoaLeX-1 and E: Mice fed with 5000 mg/kg CoaLeX-1.

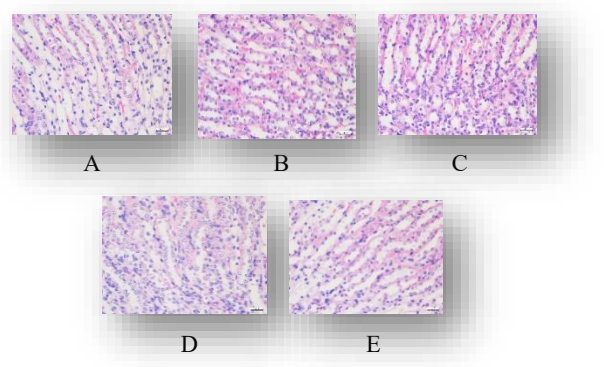

Figure 1.2. The tubular of kidney tissue (medulla part) for control and mice treated with different doses of CoaLeX-1 following H&E staining as observed under a light microscope (400× magnification). A: Control; B: Mice fed with 625 mg/kg CoaLeX-1; C: Mice fed with 1250 mg/ kg CoaLeX-1; D: Mice fed with 2500 mg/kg CoaLeX-1 and E: Mice fed with 5000 mg/kg CoaLeX-1.

DISCUSSIONS

The acute nephrotoxicity assessment of CoaLeX-1 adhered to the Organization for Economic Co-operation and Development (OECD) 420 guideline, with adjustments made in dose selection. Female mice were utilized, as recommended by OECD 420, due to their slightly higher sensitivity. No toxicity effects proportional to the dosage were observed, as the mice exhibited normal health indicators,

including eyes, mucous membranes, fur, and skin condition. Neither treatment nor control groups experienced any mortality, and there was no significant weight fluctuations observed in the treated mice, indicating the absence of wasting syndrome. According to Yazan *et al.* (2015), body weight loss, or wasting syndrome, is a common characteristic observed in toxicity studies.

Furthermore, OECD highlights histopathological examination as a more sensitive indicator of organ toxicity compared to organ weight. The acute toxicity study, as per OECD 420, provides insight into the hazardous properties of substances and allows for classification according to the Globally Harmonized System. With an LD50 cut-off value exceeding 5000 mg/kg for CoaLeX-1, no classification or labelling for hazard is deemed necessary, suggesting its safety or practical non-toxicity. Additionally, all treatment groups exhibited normal kidneys, indicating that CoaLeX-1 treatment did not impact the development of this organ (Begum *et al.,* 2019).

From this study, the histopathological examination of the kidneys further supported these findings, revealing no abnormalities in the organ. Therefore, treatment with CoaLeX-1 doses ranging from 625 to 5000 mg/kg demonstrated no toxicity in the animals, implying its safety even when ingested in significant quantities.

CONCLUSIONS

The experimental data obtained from the present study showed that no mortality and morbidity of mice for all single CoaLeX-1 doses ranging from low to high. The mice still can survive up to 5000 mg/kg of CoaLex-1 when taken orally with single dose. This suggests that further research can be continued to evaluate the potential of CoaLex-1 as a treatment modality in breast cancer management.

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REFERENCES

Abbasalipourkabir, R., Dehghan, A., Salehzadeh, A., Shamsabadi, F., & Abdullah, R. (2010). Induction of mammary gland tumor in female Sprague-Dawley rats with LA7 cells. *Journal of Biotechnology* **9**: 4491–4498.

Animal.research.uiowa.edu. (2020). Anesthesia (Guideline) | Vertebrate Animal Research. Retrieved from https://animal.research.uiowa.edu/iacuc-guidelines-anesthesia

Arbab, I. A., Abdul, A. B., Sukari, M. A., Abdullah, R., Syam, S., Kamalidehghan, B., *et al*. (2013). Dentatin isolated from Clausena excavata induces apoptosis in MCF-7 cells through the intrinsic pathway with involvement of NF-κB signalling and G0/G1 cell cycle arrest: A bioassay-guided approach. *Journal of Ethnopharmacology* **145**: 343–354.

Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P., Thamaraikani, T., Vasanthan, M., Viktor, P., Lakshmaiya, N., Saadh, M. J., Amajd, A., Abo-Zaid, M. A., Castillo-Acobo, R. Y., Ismail, A. H., Amin, A. H., & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. *Molecular cancer* **22(1)**: 169.

Baharum, Z., Akim, M. A., Taufiq-Yap, Y. H., Hamid, R. A., & Rosmin, K. (2016). In vitro antioxidant and antiproliferative activities of methanolic plant part extracts of *Theobroma cacao*. *Molecules* **19**: 18317-18331.

Begum, S., Ahmed, N., Mubarak, M., Mateen, S. M., Khalid, N., & Rizvi, S. A. H. (2019). Modulation of Renal Parenchyma in Response to Allogeneic Adipose-Derived Mesenchymal Stem Cells Transplantation in Acute Kidney Injury. *International journal of stem cells* **12(1)**: 125–138.

Hazirah, A., Zainal, B., & Abdah, M. (2013). Total phenolic content, antioxidant and cytotoxic activity of cocoa (*Theobroma cacao* L.) polyphenols extracts on cancer cell lines. *Malays. J. Nutr.* **19**: 223–232.

Htay, M. N. N., Donnelly, M., Schliemann, D., Loh, S. Y., Dahlui, M., Somasundaram, S., Ibrahim Tamin, N. S. B., & Su, T. T. (2021). Breast Cancer Screening in Malaysia: A Policy Review. *Asian Pacific journal of cancer prevention* **22(6)**: 1685–1693.

Kosem, N., Ichikawa, K., Utsumi, H., & Moongkarndi, P. (2013). In vivo toxicity and antitumor activity of mangosteen extract. *Journal of Natural Medicines* **67**: 255–263.

Lv, Z.-D., Liu, X.-P., Zhao, W.-J., Dong, Q., Li, F.-N., Wang, H.-B., *et al.* (2014). Curcumin induces apoptosis in breast cancer cells and inhibits tumor growth in vitro and in vivo. International *Journal of Clinical and Experimental Pathology* **7**: 2818–2824.

M. A., Chaney, E. J., & Boppart, S. A. (2006). Refractive index of carcinogen-induced rat mammary tumours. *Physics in Medicine and Biology* **51**: 2165–2177.

Mahadi, M. (2007). Cellular apoptosis of 4T1 breast cancer cells induced by V4-UPM Newcastle disease virus. Master's thesis, Universiti Putra Malaysia.

OECD (2002), Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Othman, F., Motalleb, G., Lam Tsuey Peng, S., Rahmat, A., Fakurazi, S., & Pei Pei, C. (2011). Extract of Azadirachta indica (Neem) leaf induces apoptosis in 4T1 breast cancer BALB/c mice. *Cell Journal* **13**: 107–116.

Ozaslan, M., Didem Karagöz, I., Kalender, M. E., Kilic, I. H., Sari, I., & Karagöz, A. (2007). In vivo antitumoral effect of Plantago major L. extract on Balb/C mouse with Ehrlich ascites tumor. *The American Journal of Chinese Medicine* **35(5)**: 841–851.

Pillai, P. G., Suresh, P., Mishra, G., Annapurna, M., & Pradesh, A. (2011). Evaluation of the acute and sub-acute toxicity of the methanolic leaf extract of Plectranthus amboinicus (Lour) Spreng in Balb C mice. *European Journal of Experimental Biology* **1**: 236–245.

Raihan, J., Ahmad, U., Yong, Y. K., *et al*. (2019). Regression of solid breast tumors in mice by Newcastle disease virus is associated with the production of apoptosis related-cytokines. BMC Cancer, 19, 315.

Sodde, V., Dashora, N., Prabhu, K. S., & Lobo, R. (2011). Evaluation of anticancer activity of Macrosolen parasiticus (L.) Danser on Ehrlich's ascites carcinoma treated mice. *International Journal of Cancer Research* **7**: 135–143.

Yazan, L. S., Ong, Y. S., Zaaba, N. E., Ali, R. M., Foo, J. B., & Tor, Y. S. (2015). Anti-breast cancer properties and toxicity of Dillenia suffruticosa root aqueous extract in BALB/c mice. *Asian Pacific Journal of Tropical Biomedicine* **5**: 1018–1026.

Zainal, B., Abdah, M. A., Taufiq-Yap, Y. H., Roslida, A. H., & Rosmin, K. (2014). Anticancer agents from non-edible parts of Theobroma cacao. *Natural Products Chemistry & Research* **2**: 1–8.