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Chronic toxicity evaluation of *Morinda citrifolia* fruit and leaf in mice

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ABSTRACT

Noni (*Morinda citrifolia*) leaf and fruit are used as food and medicine. This report compares the chronic toxicity of Noni fruit and edible leaf water extracts (two doses each) in female mice. The 6 months study showed the fruit extract produced chronic toxicity effects at the high dose of 2 mg/ml drinking water, evidenced through deteriorated liver histology (hepatocyte necrosis), reduced liver length, increased liver injury marker AST (aspartate aminotransferase) and albumin reduction, injury symptoms (hypoactivity, excessive grooming, sunken eyes and hunched posture) and 40% mortality within 3 months. This hepatotoxicity results support the six liver injury reports in humans which were linked to chronic noni fruit juice consumption. Both doses of the leaf extracts demonstrated no observable toxicity. The hepatotoxicity effects of the *M. citrifolia* fruit extract in this study is unknown and may probably be due to the anthraquinones in the seeds and skin, which had potent quinone reductase inducer activity that reportedly was 40 times more effective than l-sulforaphane. This report will add to current data on the chronic toxicity cases of *Morinda citrifolia* fruit. No report on the chronic toxicity of *Morinda citrifolia* fruit in animal model is available for comparison.

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1. Introduction

Morinda citrifolia (family Rubiaceae), commonly known as Noni in America and Mengkudu in Malaysia, as food and medicine has been utilised by the Polynesians for over twenty centuries (Whistler, 1985). The roots, stems, bark, leaves, flowers, and fruits of *M. citrifolia* plant are used in over 40 known recorded herbal remedies in various combinations (Bruggnecate, 1992), for diabetes, high blood pressure, cancer, and many other illnesses (Abbott and Shimazu, 1985), antibacterial (Natheer et al., 2012), antifungal and antihelmintic (Bhawna and Kumar, 2009), antioxidant (Siddiqui et al., 2014), anti-inflammatory (Basar et al., 2010), wound healing, anticancer effects (Wang and Su, 2001), anxiolytic (Kannan et al., 2014), cardiovascular (Gilani et al., 2010), and immune stimulation functions (Palu et al., 2008).

In the last decade, noni fermented and unfermented fruit juice, has become a widely traded dietary supplement globally, with health claims relating to some of its compounds, particularly the flavonoids (Deng et al., 2007; Takashima et al., 2007). Between 2005 and 2011, there have been 7 reports on the hepatotoxicity of noni preparations in humans which has raised health concerns (Yu et al., 2011), including hepatitis in *M. citrifolia* fruit juice products consumers (Stadlbauer et al., 2008). The association between the fruit juice products and hepatitis remains controversial, as no causal link was established between the liver injury cases and the juice consumption (European Food Safety Authority, 2006).

A commercial source of *M. citrifolia* fruit juice from French Polynesia was approved as a novel food by the European Union (Europian Commission, 2003). Subsequent research concluded that the regular intake of noni juice was not likely to cause any toxic effects (European Food Safety Authority, 2006). In vitro hepatotoxicity and oral subchronic toxicity study reported that the noni fruit juice is unlikely to induce adverse liver effects (West et al., 2009a,b). Analyses sponsored by Tahitian Noni, the main global noni juice provider, reported no toxicity from consumption of the product (West et al., 2006, 2009a,b). A short term (28 days) double-blind clinical safety study, concluded that consuming up to 750 mL Tahitian Noni juice daily is safe (West et al., 2009a,b).

The Morinda fruit and leaf were reportedly to contain various compounds (complete list given in Appendices) including acids (Lindsay and Golden, 2012), alcohols, phenols, anthraquinone and anthraquinone glycosides (Takashima et al., 2007), carotenoids, esters (Zhang et al., 2014), flavonoids (Su et al., 2005), iridoids (Akihisa et al., 2010; Takashima et al., 2007), ketones and lactones

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(Farine et al., 1996), lignans (Kamiya et al., 2004; Palu et al., 2008), nucleosides (Su et al., 2005), triterpenoids and sterols (Akihisa et al., 2012; Takashima et al., 2007), and several minor compounds (Pak-Dek et al., 2011; Pawlus et al., 2005; Takashima et al., 2007). Scopoletin, a coumarin derivative, is suggested as a constituent marker for *M. citrifolia* L. quality control (Samoylenko et al., 2006).

Due to the controversial outcomes of *M. citrifolia* toxicity reports, the current investigation aims to demonstrate the long term effects of consuming *M. citrifolia* fruit and leaf aqueous extract in female mice model.

2. Material and methods

Morinda citrifolia leaves (MCL) and fruit (MCF) were obtained from the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). A voucher specimen SK2322/14 was deposited at the Laboratory of Natural Products, IBS, UPM. *M. citrifolia* whole fruits and leaves were oven-dried at 45 °C for 2 days, ground into powder and boiled in 1:10 w/v distilled water for 3 h, filtered and evaporated to dryness at 60 °C to give an aqueous extract of fruits 7.21% and leaves 4.51% w/w yield.

The 6–7 weeks female Imprinting Control Region (ICR) mice were from the Faculty of Veterinary Medicine, UPM, and acclimatized for 7 days by housing and maintaining on a 12-h light/dark cycle at 25 ± 2 °C. They were given standard pellet food (Gold Coins from A Sapphire Enterprise, Serdang, Malaysia) and drinking water ad libitum. This study was approved by the Institutional Animal Care and Use Committee, UPM, with approval number UPM/IACUC/AUP-R022/2013. The chronic toxicity was evaluated according to the OECD (Organisation for Economic Co-operation and Development) guideline 452 for female mice. Due to time and funding budget limitation only females were used. Mice were then grouped (n = 5) into: A: Control (distilled drinking water DW); B: 1 mg MCL/ml DW; C: 2 mg MCL/ml DW; D: 1 mg MCF/ml DW; and E: 2 mg MCF/ml DW. Animals were weighed weekly and observed for any toxicity symptoms. At the end of the 6 months, all animals were anaesthetized using ketamine (50 mg/kg) and xylazine (5 mg/kg) and exsanguinated by cardiac puncture for blood and organ examination. The liver and kidneys were harvested, cleaned with saline, weighed and preserved for histopathology analysis.

Blood were collected into serum separation tube, allowed to clot at room temperature for 30 min before centrifugation (5000 g for 15 min) for liver and kidney biomarker analysis: albumin, creatinine, and urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Harvested tissues were fixed in buffered formaldehyde solution (10%), dehydrated by serial ethanol solution, diaphonized with ethanol-benzene and enclosed with paraffin. After processing, the tissues were sectioned to a 4 μM thickness using a rotary microtome and oven-dried overnight at 37 °C. The sections were stained with hematoxylin and eosin (H&E) and examined under compound light microscope (40× magnifications).

All data are mean ± S.D, and significant differences between groups were determined using one-way analysis of variance (ANOVA), and further evaluated by Duncan's post hoc test and considered significant at $p < 0.05$. All statistical analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

3. Theory

Long term consumption of *M. citrifolia* fruit (MCF) or leaf (MCL) aqueous extract at high or low dose may or may not damage mammalian tissues/organs and the possible cause for the injury.

4. Results

The yield of the water extracts are 7.2 ± 0.5% for MCF and 4.5 ± 0.5% for MCL. Daily administration of MCL (1 and 2 mg/ml) for 6 months did not show any signs of toxicity or mortality. However, the mice consuming MCF especially at 2 mg/ml water showed significant retarded body weight increase after the first month, as compared to the control mice (Fig. 1a). The liver weights and lengths, showed shrinkage in the 2 mg/ml MCF rats which was significant for the length but not for the liver weight when compared to control group (Table 1).

The mice on 2 mg MCF/ml showed 2 mortalities on day 68 and 93, respectively, with obvious toxicity symptoms (Table 1), while those on 1 mg MCF/ml water showed some liver histological changes. Table 1 also shows the drinking water consumption, the calculated extract dosage and the equivalent human dose for the extract.

The chronic consumption of *M. citrifolia* aqueous extract (MCL and MCF at both concentrations) showed no significant changes in the liver damage biomarker enzyme ALT, and serum creatinine levels, as compared to control group (Fig. 2). The normal ranges of these biomarkers for mice are marked within the rectangle. The tissue injury biomarkers that were outside these ranges were only for AST (2 mg/ml MCL & MCF) and albumin (2 mg/ml MCF) which are indicative of liver and kidney injury respectively. The blood AST increase indicated AST leakage from the injured hepatocyte membrane. The decreased blood albumin levels indicated albumin loss via damaged renal tubules or glomeruli. However, there were significant reductions in ALT levels, and urea levels (for the 1 mg MCL/ml, 2 mg MCL/ml, & 1 mg MCF/ml treated mice) compared to control mice, but they were still within the normal ranges. Any values outside the normal range indicate either severe tissue damage, membrane leakage, necrosis or hypertrophy.

Administration with both concentrations of MCL did not cause observable adverse effect on the hepatocytes histo-architecture (Fig. 3). However, mice treated with the fruit extracts MCF (1 and 2 mg/ml) showed dose-dependent hepatocellular necrosis. The kidneys treated with both concentration of MCL and MCF showed normal tubule structures (Fig. 4).

5. Discussion

Morinda citrifolia, or noni, is considered the second most important medicinal plant in the Hawaiian Islands. The mice consuming *M. citrifolia* leaves aqueous extract (MCL; 1 and 2 mg/ml) did not show any observable toxicity effects and were consistent with previous *M. citrifolia* leaves toxicity evaluations (Lagarto et al., 2013a,b; Serafini et al., 2011; West et al., 2007), although the AST values for the 2 mg/ml dose increased significantly above the normal range after 6 months continuous daily consumption.

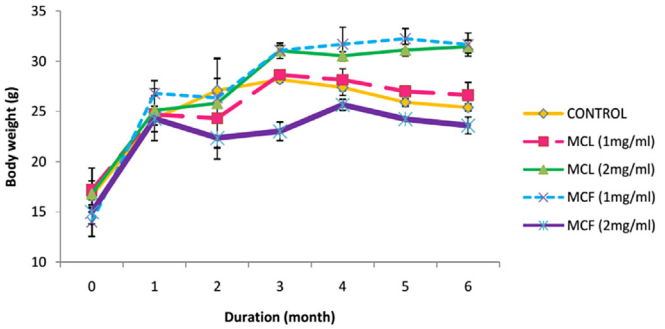

Fig. 1. Body weights of control and *M. citrifolia* aqueous extract treated mice after 6 months. Values are expressed as mean ± SD (n = 5). Means with different superscript letters within the same graph are significantly different ($p < 0.05$).

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