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Cite as: AIP Conference Proceedings 2668, 020005 (2022); <https://doi.org/10.1063/5.0111944>
Published Online: 11 October 2022

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Subchronic Toxicity of *Ficus deltoidea* Jack. Leaves on the Histology of Ventriculus and Intestinum Tenue Male Mice

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Abstract. Leaves of *Ficus deltoidea* Jack. contains flavonoids, steroids, alkaloids, triterpenoids, saponins, tannins, and phenolics which beneficial as traditional medicines. However, level of safety for its use is unknown. Present study purposed to evaluate the subchronic toxicity of ethanolic extract of *Ficus deltoidea* Jack (FDEE) on the histology of ventriculus and intestinum tenue of male mice (*Mus musculus*). In total 25 mice were randomly divided into five treatment groups and treated with leaves of FDEE at doses of 0, 125, 250, 500, and 1000 mg/kg body weight (BW) for 28 days. At the end of the study, the mice were dissected, ventriculus and intestinal tenue organs were taken out and weighed. The organs of the ventriculus and intestinal tenue were prepared for histological preparations. The thickness of the ventriculus wall and the height of the intestinal villi were measured histologically. The results showed that there was no significantly changed on the histological structure of the ventriculus and intestinal tenue of mice treated with 125 and 250 mg/kg BW of FDEE leaves. Meanwhile, at doses of 500 and 1000 mg/kg BW, there was a slightly change on the histological of ventriculus which was indicated by the thickness of the ventriculus wall and the height of intestinal villi. The FDEE leaves above 500 mg/kg BW affected the thickness of the ventriculus wall and the height of the intestinal tenue villi of male mice.

INTRODUCTION

Indonesia has a high diversity of flora, one of which is medicinal plants, namely *Ficus deltoidea* Jack. The *Ficus deltoidea* is a climbing herb, leaves arranged zigzag on slender branches, stalk 0.1-9 cm long, bright yellowish green or yellowish brown with spots, rounded strands of breech eggs to hook, and glossy leaf surface [1, 2]. According to Nugroho *et al.* [3] *Ficus deltoidea* leaves ethanol extract (FDEE) contains alkaloids, phenolics, flavonoids, coumarin, and steroids and total quantitative levels of phenolics and flavonoids are 107.6583211 µg GA/mg and 175.9103641 µg CE/mg. Further, Manurung *et al.* dan Makalalag [4, 5] have also reported that the methanol extract of *Ficus deltoidea* produced by cultivation and wild plants has high flavonoid content and shows high antioxidant activity (IC₅₀ = 72.47 µg/mL), so it has the potential to be used as medicinal plants. Previous research mentioned that the extract of *Ficus deltoidea* which abundances with sterol compounds also shows an anti-cancer or anti-tumor properties [6, 7].

Besides their potential benefits, the FDEE leaves might be having some negative effects because it contains chemical compounds namely ficin, furokumarin, ficucin and psoralen that cause toxic effects, [8-10]. These

chemical compounds are generally found in members of the genus *Ficus*. Diarrhea, vomiting, and irritation of the digestive tract are the common toxic symptom when this plant is ingested, especially if used excessively. Further, the digestive tract such as ventriculus and intestinum tenue can be deteriorated by this plant [11].

Though some studies have been conducted on the effect of the *Ficus deltoidea* extract, study on the sub-chronic toxicity test of the FDEE leaves on the ventriculus and intestinum tenue organs of male mice has not been carried out. Thus, the purpose of current research was to determine whether the FDEE leaves can cause sub-chronic toxicity effects on the ventriculus and intestinal organs of male mice (*Mus musculus*).

MATERIALS AND METHODS

Extract Preparation

The *Ficus deltoidea* leaves were cleaned, dried, and blended to obtain leaves powder. The leaves powder was extracted using 95% ethanol solvent for two days in an Erlenmeyer flask. The erlenmeyer flask was placed in orbital shaker for 24 hours. After shaking, the Erlenmeyer flask was left for 24 hours followed by filtering and evaporating using a rotary evaporator until a crude extract was obtained. The crude extract was weighed according to the dosage requirement and mixed with 0.5% CMC solution

Animal Test

In total 25 mice were used and randomly divided into five groups of five replications. The treatments of FDEE leaves in 0.5% CMC were carried out orally every day with doses, namely control (0), 125, 250, 500, and 1000 mg/kg BW for 28 days. During the treatment, each mouse was weighed once a week. On the day 28, the mice were dislocated and sacrificed. Ventriculus and intestines of mice were taken out and weighed. After weighing, the ventriculus and intestinal organs of the mice were prepared for histological slides using the paraffin method and the Hematoxylin-Eosin staining

Data Analysis

The histological data of the ventriculus and intestinum tenue of mice were analyzed descriptively. Villi height and ventriculus wall thickness were measured using the software Fiji ImageJ 1.5e National Institutes of Health, USA and analyzed using one way Analysis of Variance (ANOVA), followed by Duncan Multiple Range Test (DMRT) if any significant difference found at $p < 0.05$.

RESULTS AND DISCUSSION

The weight and the height of the ventricular and intestinal organs in the subchronic toxicity were shown in Table 1. It can be seen that the ethanol extract did not affect the weight of the ventricular and intestinal tenue of mice

TABLE 1. The height of villi and thickness of ventriculus wall in male mice (*Mus musculus*) treated with ethanolic extract of *Ficus deltoidea* leaves.

Parameters	subchronic toxicity (mg/kg BW)				
	Control	125	250	500	1000
Ventriculus (g)	0.86±0.15 ^a	0.54±0.09 ^a	0.64±0.12 ^a	0.52±0.10 ^a	0.50±0.08 ^a
Ventriculus (%)	3.63±0.73 ^a	1.61±0.31 ^a	2.81±0.76 ^a	1.84±0.37 ^a	1.56±0.16 ^a
Thickness of the ventriculus wall (µm)	9.42±0.08 ^a	8.75±0.24 ^b	8.33±0.20 ^{bc}	8.21±0.12 ^c	8.17±0.06 ^c
Intestinum tenue (g)	0.18±0.01 ^a	0.16±0.02 ^a	0.16±0.01 ^a	0.25±0.12 ^a	0.20±0.04 ^a
Intestinum tenue (%)	0.75±0.07 ^a	0.46±0.05 ^a	0.67±0.08 ^a	0.88±0.41 ^a	0.62±0.08 ^a
height of the intestinal tenue villi (µm)	3.38±0.06 ^a	3.09±0.10 ^{ab}	3.02±0.12 ^b	2.68±0.14 ^c	2.61±0.05 ^c

Mean ± SE followed by different superscript letters (a, b, c) on the same row indicated significant difference ($p < 0.05$).

Based on the histology observation, the intestinum tenue of mice in the control showed that the villi were still intact, and their structure was regular and normal. There was also no damage on the surface epithelial cells of the villi (Figure 1:C1 and C2). Meanwhile, 125 mg/kg BW (Fig. 1:P1a and P1b) and 250 mg/kg BW (Fig. 1:P2a and P2b) treatment resulted no significantly different on the histological structure and the villi shape compare to control group. This finding confirmed that the FDEE leaves up to a dose of 250 mg/kg BW was not toxic. Further, mice treated with FDEE leaves at 500 mg/kg BW (Fig. 1: P3a and P3b) and 1000 mg/kg BW (Fig. 1: P4a and P4b) exhibited minor damage on the cell layer of the intestinal. The epithelium of the villi and the lamina propria cells were not neatly arranged in the middle of the villi, and the mucous muscles were detached from the intestinal villi. Present results revealed that FDEE leaves extract at dose 500 and 1000 mg/kg BW affected the intestinal villi of mice.

In the control group (Fig0 2: C1 and C2) the ventriculus epithelium layer showed intact and normal. Meanwhile, treatment of 125 mg/kg BW (Fig. 2: P1a and P1b) and 250 mg/kg BW (Fig. 2: P2a and P2b) of FDEE leaves revealed no different with the control group but, the ventriculus lining was still visible. This finding exhibited that mice treated with FDEE leaves up to a dose of 250 mg/kg BW did not cause histological changes. Furthermore, mice treated at doses of 500 mg/kg BW (Fig. 2: P3a and P3b) and 1000 mg/kg BW (Fig. 2: P4a and P4b) with FDEE leaves resulted a mild damage which was seen as desquamation of the epithelium and erosion of the mucosal layer of intestinal organ.

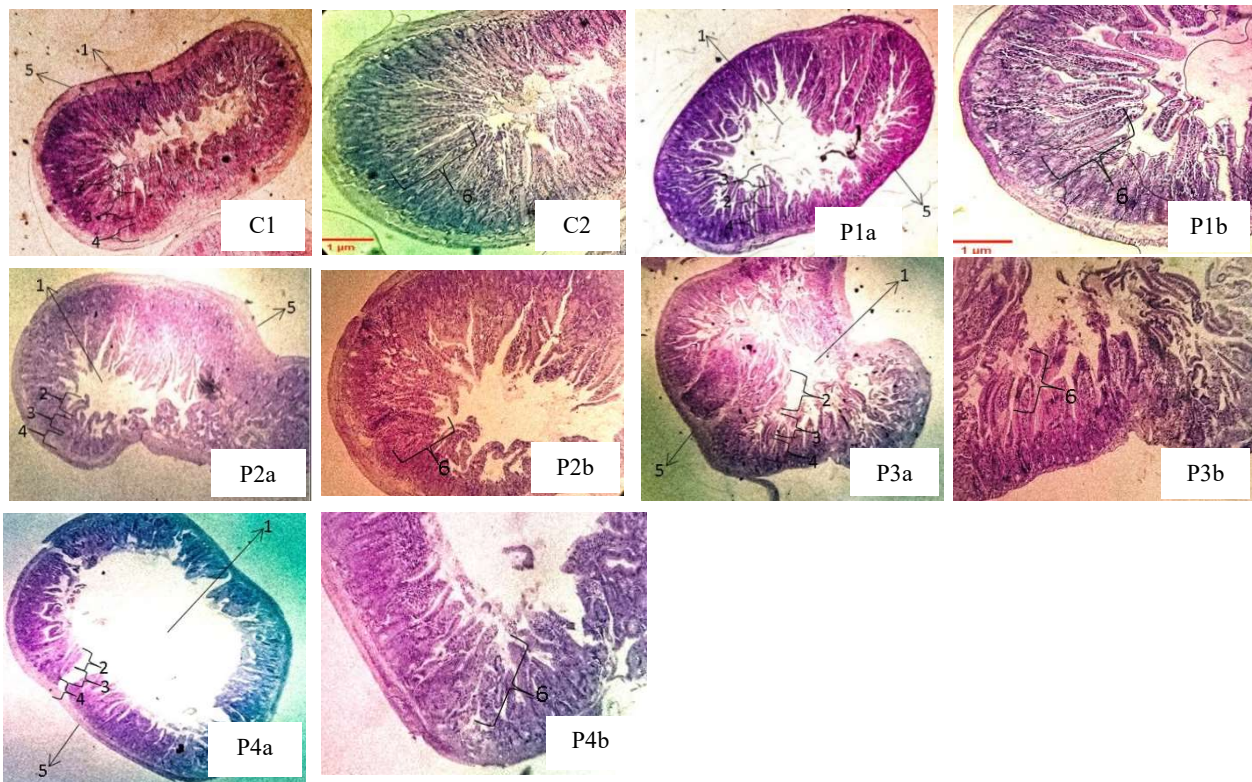


FIGURE 1. Intestinum tenue histology of *Mus musculus* treated with various doses of ethanolic extract leaves of *Ficus deltoidea*. C1 and C2 = Control; Mice treated with various doses of ethanolic extract leaves of *Ficus deltoidea*: P1a and P1b = 125 mg/kg BW; P2a and P2b = 250 mg/kg BW; P3a and P3b = 500 mg/kg BW; P4a and P4b = 1000 mg/kg BW. Paraffin method and Hematoxylin & Eosin staining are used. Each group has magnification 4x10 (C1; P1a; P2a, P3a and P4a); and magnification 10x10 (C2; P1b, P2b, P3b, and P4b). Note: 1. Lumen; 2. Tunica Mucosa; 3. Tunica Submucosa; 4. Tunica Muscularis; 5. Tunica Serosa; 6. The height of Villus.

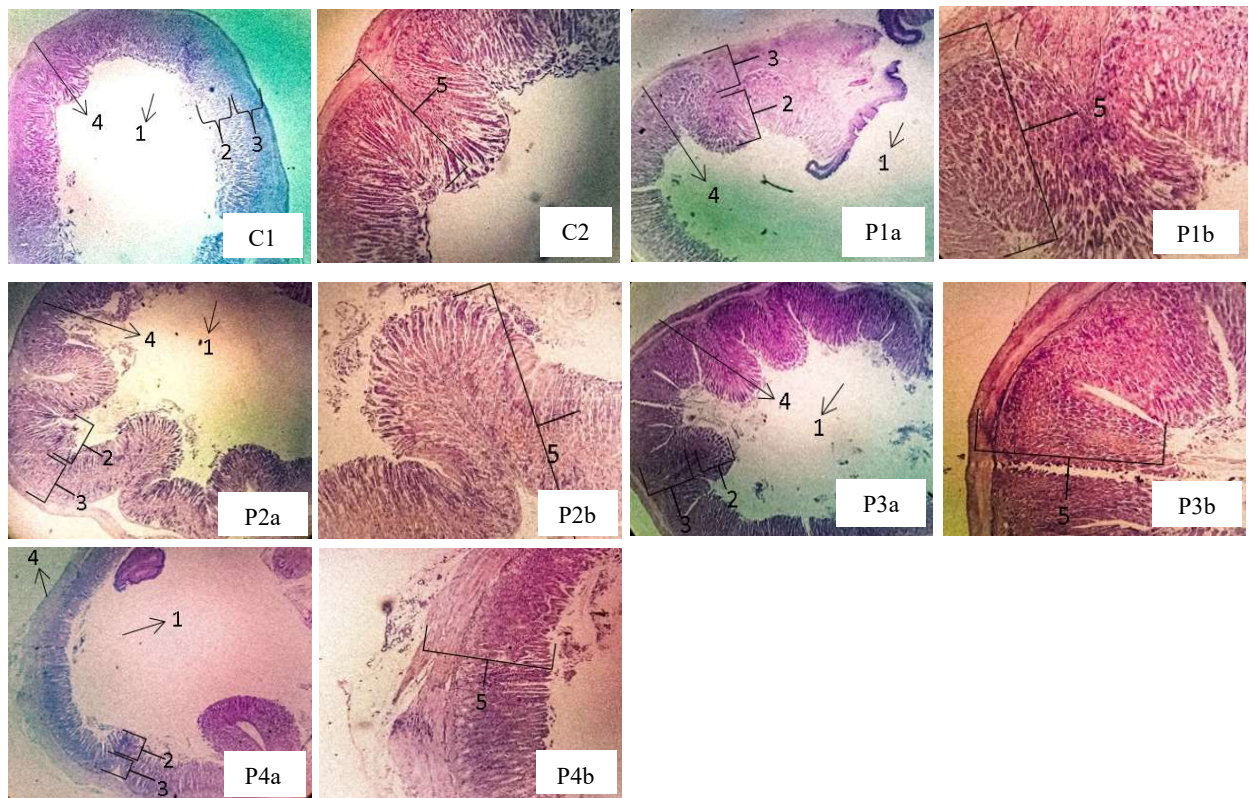


FIGURE 2. Ventriculus histology of *Mus musculus* treated with various doses of ethanolic extract leaves of *Ficus deltoidea*. C1 and C2 = Control; Mice treated with various doses of ethanolic extract leaves of *Ficus deltoidea*: P1a and P1b = 125 mg/kg BW; P2a and P2b = 250 mg/kg BW; P3a and P3b = 500 mg/kg BW; P4a and P4b = 1000 mg/kg BW. Paraffin method and Hematoxylin & Eosin staining are used. Each group has magnification 4x10 (C1; P1a; P2a, P3a and P4a); and magnification 10x10 (C2; P1b, P2b, P3b, and P4b). Note 1. Lumen; 2. Tunica Mucosa; 3. Tunica Muscularis; 4. Tunica Serosa; 5. The thickness of ventriculus wall

Present study also found that there were no toxic symptoms or death in subchronic toxicity for 28 days in all treatment groups of mice. Toxic symptoms such as physical condition of hair and skin, eyes, behavior patterns of mice, and lethargy were not found in either control or treatments group. These results are in accordance with past research of Farsi *et al* [12] revealed that *F. deltoidea* methanolic leaves extract up to a dose of 5000 mg/kg BW for 14 days showed no significant changes in the behavior of mice such as apathy and hyperactivity. Ilyanie *et al.* [13] also found that no signs of toxicity and mortality in diabetic rats which treated with methanolic extract of *F. deltoidea* up to a dose of 6400 mg/kg BW for 14 days. No signs of toxicity and mortality was also found in diabetic rats treated with ethanol extract of *F. deltoidea* up to a dose of 2000 mg/kg BW for 14 days [3].

Current results also revealed that the FDEE leaves did not affect on the weight of the ventricular and intestinal tenue of mice. This finding is similar to previous study stated that treatment of methanol extract of *F. deltoidea* Jack. leaves at a dose of 750, 1250, 2500 mg/kg BW in subchronic toxicity was not affect on the organ weight (heart, kidney, liver, lymph, lung) compared to the control group. In addition, Saragih [14] stated that the higher the villi ratio, the higher the digestion rate and nutrient absorption while, Laudadio *et al.* [15] also mentioned that the higher the villi, the greater the nutrient absorption area. The decrease in the surface area of the villi limits the absorption of food juices [16]. The damaged of villi also interferes with the process of absorption of food in the intestinum tenue [17]. In addition, Farsi *et al.* [12] stated that giving methanol extract of *F. deltoidea* leaves caused mild inflammation on the lung histology. The changes in the structure of the ventriculus wall thickness caused by a high dose of ethanolic extract of *F. deltoidea* leaves affected the thickness of the ventriculus wall. This is due to the fact that *F. deltoidea* leaves contain chemical compounds such as flavonoids, alkaloids, saponins, tannins that might

be having a toxic effect when used with high doses. Wagner [18] revealed that alkaloids and flavonoids are toxic because it can act as respiratory toxins and even alkaloids can cause ventriculus poisoning, while, tannins might block the digestion process and cause water absorption disorders in organisms. Moreover, saponins can result in decreasing the activity of digestive enzymes and food absorption, inhibiting development and interfere to growth.

The presence desquamation of epithelial cells on several walls of the ventriculus mucosa, possibly the side effect of *F. deltoidea* leaves ethanol extract treatment. According to Jahra *et al.* [19], desquamation of epithelial cells is characterized by exfoliation or peeling of surface epithelial cells. This is a response to the ventriculus mucosa due to a decrease in mucus secretion (cytoprotective) and the process of desquamation of epithelial cells that can cause erosion of mucosal cells. Cell desquamation occurs due to the decreasing in ventriculus cytoprotective levels which can be caused by oxidative stress, triggered by Reactive Oxygen Species (ROS) substances such as alcohol, acidic foods or drinks, stress factors, and drugs. *Ficus deltoidea* Jack. itself is an herbal plant that can be used as a raw material for medicinal herbs. Herbal plants contain chemicals that can cause side effects such as toxicity at high doses, namely mucosa irritation [20, 21]

CONCLUSION

Ethanol extract of *F. deltoidea* Jack. leaves above 500 mg/kg BW affected the thickness of the ventriculus wall and the height of the intestinal tenue villi of male mice. Present finding also concludes that the treatment of ethanolic extract on mice shows no toxic symptoms or death in subchronic toxicity for 28 days in all treatment groups of mice.

ACKNOWLEDGMENTS

All researchers acknowledge to Direktorat Jenderal Kementerian pendidikan tinggi, Riset dan Teknologi Pendidikan Tinggi (Ristekdikti), Republic Indonesia for the fund, contract number 200/UN.17.41/KL/2019. The researcher also wants to thankful to Fakultas MIPA, Mulawarman University, Samarinda, East Borneo, Indonesia, for any kind of support.

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