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Acute and Subchronic Toxicity Study of the Ethanol Extracts from *Ficus deltoidea* Leaves in Male Mice

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Abstract

BACKGROUND: *Ficus deltoidea* Jack. leaves have a great potential as traditional medicine, but the safety level of its use is still unknown.

AIM: This study aimed to determine the phytochemical contents of the ethanol extract of *F. deltoidea* leaves and evaluate the level of safety and toxicity through acute and subchronic toxicity tests in mice (Mus musculus).

METHODS: The ethanol extract of *F. deltoidea* leaves was determined for phytochemical contents such as alkaloids, phenolics, flavonoids, coumarin, steroids, saponins, carotenoids, and tannins. In the acute toxicity test, 10 male mice were divided into a control group and the extract treatment group with 2000 mg/kg body weight (BW) dose for 14 days to identify signs of toxicity and mortality. Meanwhile, in the subchronic toxicity test, 25 male mice were divided into control and four treatment groups with various doses (125, 250, 500, and 1000 mg/kg BW), respectively, for 28 days. The toxicological effect was evaluated by observing behavior, signs of toxicity, and changes in BW. At the end of the treatment, hematological and biochemical evaluations were also measured.

RESULTS: *The results showed that* the ethanol extract of *F. deltoidea* Jack leaves qualitatively contains alkaloids, phenolic, flavonoids, coumarin, and steroids, whereas quantitatively total phenolics, flavonoids, and IC50 were 107.6583211 µg GA/mg, 175.9103641 µg CE/mg, and 103.7484 µg/mL. Moreover, 2000 mg/kg BW dose resulted in no symptoms of toxicity and mortality, indicating that the 50% lethal dose (LD50) was above 2000 mg/kg BW. Meanwhile, there were no behavioral changes, significant differences in weight changes, hematological parameters, and serum biochemistry of mice in subchronic toxicity tests.

CONCLUSION: The present study shows that acute and subchronic oral administration of the ethanol extract of *F. deltoidea* leaves for male mice does not induce clinical symptoms of toxicity or mortality. The LD50 of the ethanol extract of *F. deltoidea* leaves for mice >2000 mg/kg is considered as practically non-toxic.

Introduction

In recent years, the use of medicinal plants has risen rapidly and is very popular. The World Health Organization has estimated that 80% of African and Asian residents use traditional medicines to maintain health and treatment of disease. Research on the use of traditional medicines, including herbal medicines, has been rapidly increased, both in developed and developing countries [1], [2], [3]. Indonesia, one of the developing countries, has a high biodiversity of plants that widely used as traditional medicine. One of the medicinal herbs that have been used by Indonesian ancestors is Tabat Barito (*Ficus deltoidea* Jack.) [4], [5].

F. deltoidea is a perennial cultivated plant whose height rarely exceeds 2 m. In some countries, these plants are known by various names such as Mas Cotek in Malaysia, Barito Barat in Indonesia, Agoluran in the Philippines, and Kangkalibang in Africa [6]. In general, this plant lives as an epiphytic plant in the forest and its benefits have been studied in-depth lately. Every part of the plant is known to have benefits as a medicine. The fruit is chewed to relieve headaches and toothache; root powder and plant leaves have been applied outside medicine for wounds [7] and to eliminate rheumatism [8]. Conventionally, these plants are also consumed to help strengthen a woman's womb after giving birth [6], [9] and function as a libido booster for men and women [10].

By detonation of health therapy using plant material, the safety of medicinal plants has become a public health problem [11]. There is an increased awareness in the use of this herbal remedy [12] since most natural products or herbal medicines contain diverse phytoconstituents due to variations in growth patterns, geographical location, harvest time, and storage [13]. The correct identification of

the compound composition in plants is essential to ensure consistent quality, safety, and efficacy [12]. Although *F. deltoidea* has some promising pharmacological potentials, it has not been widely tested for possible side effects. There are not many studies to evaluate the level of safety and toxicity. Further, it is necessary to do further testing in test animals to see whether there are any toxic effects to ensure the safety of their use. Herbal and synthetic raw materials must be ensured of their safety before they can be used as medicine. An important step in ensuring drug safety is conducting an appropriate toxicity test on animal models, where acute and subchronic toxicity tests are one of the main tests that must be carried out [14], [15], [16]. Therefore, the aim of this study was to evaluate the acute toxicity profile and repeated 28-day exposure (subchronic toxicity) from the ethanol extract of *F. deltoidea* leaves.

Materials and Methods

Extract preparation

F. deltoidea fresh leaves were collected, cleaned, dried, and mashed. The mashed leaves were macerated into ethanol 96% solvent for 2 × 24 h, then filtered. The filtrate obtained was collected, and the remaining filtration was soaked again with a new solvent. The process was carried out until the color of the filtrate becomes clear. The ethanol extract was concentrated using a rotary evaporator. Furthermore, the extract was stored for phytochemical screening and animals test. Phytochemical screening test was carried out for alkaloids, phenolics, flavonoids, coumarins, tannins, steroids, saponins, and carotenoids.

Animals test

A group of male mice (8 weeks old, average body weight [BW] 20–35 g) were obtained from Samarinda. The mice were acclimatized in experimental conditions (the light cycle was 12L:12D, temperature 23 ± 3°C, room humidity 50–70%), feed and water were provided *ad libitum* for about 7 days. We followed the ARRIVE (Animal Research: Reporting of *in vivo* Experiments) as a guideline for conducting research with the animal.

Acute toxicity test

Ten male mice were divided into two groups, namely, a control group that was given CMC 0.5% and the extract treatment group dose 2000 mg/kg BW, which was dissolved in CMC 0.5% orally. The mice were observed for possible toxicity every hour for the first 6 h and continued to be observed every day for 14 days. Mice were observed as a sign of toxicity to their physical and behavioral changes and recorded the number of mortality to determine 50% lethal dose (LD_{50}).

Subchronic toxicity test

Twenty-five male mice each were divided into five groups. The extract was suspended in 0.5% CMC with a given volume of 1 mL/100 g BW. The extract mixture was given orally every day for 28 days. The control group was given 0.5% CMC, while the treatment groups were treated with various doses extract of 125, 250, 500, and 1000 mg/kg BW, respectively. Mice were given standard feed and water *ad libitum*. The weight of mice was measured once a week. The signs of toxicity and mortality were observed every day. At the end of the treatment, the experimental animals were fasted overnight, then anesthetized and blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes for hematological analysis, while tubes without EDTA for serum biochemical analysis.

Hematology and serum biochemistry analysis

Hematological analysis of blood such as white blood cell, red blood cell, hemoglobin, hematocrit (Ht), mean corpuscular hemoglobin (MCH) concentration, MCH, mean corpuscular volume (MCV), and platelets was carried out in accordance with the analysis guide for the automatic blood analyzer (Sysmex XS-800i®). Meanwhile, serum biochemical analysis, blood without EDTA, was centrifuged at 3000 rpm for 10 min. Resulted serum was analyzed for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase, bilirubin, total protein, albumin, creatinine, urea, glucose, cholesterol, triglyceride (TG), and lactate dehydrogenase (LDH) using automatic biochemistry analyzer (7020, Hitachi, Japan). All animals were then sacrificed to determine the weight (g) and relative weight (%) of the organs such as the heart, lungs, spleen, stomach, liver, testes, and kidneys.

Data analysis

The results of body, organ and relative organ weight, and hematological and biochemical data were expressed as mean ± standard error. Data were analyzed using one-way ANOVA and followed by least significant difference (SPSS version 22, SPSS, Inc., USA).

Results

Phytochemical screening

The phytochemical analysis of *F. deltoidea* leaves ethanol extract positively contained alkaloids, phenolics, flavonoids, coumarin, and steroids. However,

other secondary metabolites such as saponins, carotenoids, and tannins were not found. Quantitatively, total phenolics and flavonoids of *F. deltoidea* leaves ethanol extract were 107.6583211 µg GA/mg and 175.9103641 µg CE/mg. The extract also has antioxidant activities with the IC_{50} value of 103.7484 µg/mL, which classified as a medium category [17].

Acute toxicity test

In this study, we evaluated the potential for toxicity and an approximate LD_{50} of *F. deltoidea* leaves ethanol extract at a dose of 2000 mg/kg BW after oral treatment of male mice. The results showed that there were no signs of symptoms of toxicity or behavioral changes such as unhealthy or abnormal behavior and mortality within 72 h–14 days after extract administration. The present findings revealed that an increase in BW of the treatment group was shown no significantly different compared to the control group unless the cumulative weight was decreased (Table 1). This research indicated that the administration of *F. deltoidea* leaf ethanol extract at a dose of 2000 mg/kg BW did not affect the BW of mice.

Table 1: Body weight analysis of mice treated orally with the ethanol extract of *F. deltoidea* leaves for 14 days

Parameters	Acute toxicity	
	Control	2000 mg/kg BW
Initial weight (g)	23.340 ± 0.145	26.158 ± 1.809
Final weight (g)	27.522 ± 0.689	28.626 ± 2.092
BWG (g)	4.182 ± 0.633	2.468 ± 0.344
BWG (%)	15.015 ± 1.843	8.519 ± 0.670*

Data were represented as Mean ± SEM, *p<0.05 versus control. *F. deltoidea: Ficus deltoidea*, BW: Body weight, BWG: Body weight gain.

Further, the results of the index of heart, lungs, spleen, stomach, liver, testes, and kidneys showed that no enlargement of organs in each mice at a dose 2000 mg/kg BW except at the relative weight of the left testes, while the right testicular weight was decreased (Table 2). However, since no macroscopic or clinical changes were observed, then no biological significance was associated with it.

Subchronic toxicity

The current results stated that daily oral administration of *F. deltoidea* leaves ethanol extract (125, 250, 500, and 1000 mg/kg BW, p.o.) for 28 days did not cause any symptoms of signs of toxicity, changes in behavior, and mortality in mice. The results also found that some mice showed an increase in locomotor activity during the first 30 min after administration and returned to its normal state within 30 min. This condition may cause by the pressure of the gavage treatment [18]. In addition, no obvious general clinical signs were found during the monitoring period.

BW on male mice did not show significant differences between the treatment group and the control group during the treatment period. Meanwhile, the relative weight did not also indicate a significant difference, except at the dose of 125 and 1000 mg/kg BW, which showed a decrease in the percentage of relative weight (Table 3).

Table 2: The weights (g) and relative weights (%) of the organ of male mice treated orally with the ethanol extract of *F. deltoidea* leaves for 14 days

Organs	Unit	Acute toxicity	
		Control	2000 mg/kg BW
Heart	(g)	0.143 ± 0.012	0.139 ± 0.009
	(%)	0.521 ± 0.045	0.502 ± 0.066
Pulmo	(g)	0.174 ± 0.013	0.193 ± 0.023
	(%)	0.637 ± 0.055	0.698 ± 0.110
Liver	(g)	1.464 ± 0.050	1.613 ± 0.168
	(%)	5.341 ± 0.272	5.850 ± 0.890
Spleen	(g)	0.225 ± 0.048	0.211 ± 0.055
	(%)	0.827 ± 0.183	0.772 ± 0.223
Ventriculus	(g)	0.556 ± 0.075	0.812 ± 0.186
	(%)	2.045 ± 0.300	2.915 ± 0.667
Kidney (left)	(g)	0.198 ± 0.008	0.229 ± 0.019
	(%)	0.719 ± 0.023	0.835 ± 0.124
Kidney (right)	(g)	0.200 ± 0.007	0.228 ± 0.019
	(%)	0.727 ± 0.011	0.831 ± 0.120
Testis (left)	(g)	0.123 ± 0.014	0.117 ± 0.024
	(%)	0.155 ± 0.026	0.411 ± 0.079*
Testis (right)	(g)	0.451 ± 0.059	0.117 ± 0.013*
	(%)	0.569 ± 0.100	0.416 ± 0.045

Data were represented as Mean±SEM, *p<0.05 versus control. *F. deltoidea: Ficus deltoidea*, BW: Body weight.

Furthermore, there is no significant difference ($p>0.05$) of all organ weight (g) and relative organ between the treatment group and the control group, including the heart, lungs, liver, spleen, stomach, kidneys, and testes at each dose level (Table 4). However, the relative hepatic weight of mice treated with extracts at a dose of 125 and 500 mg/kg BW found significantly decreased. The heart, lungs, spleen, stomach, kidneys, and testicles did not change after subchronic treatment in male mice. However, all minor fluctuations in the weight value of this relative organ remain within the normal range and are sporadic. No significant changes have been logically observed in the organ between the control group and the treatment group. These results indicated that for 4 weeks, it has no toxic effect on male mice.

Hematology and blood biochemical analysis

The effects of *F. deltoidea* leaf ethanol extract on some hematological parameters of the treatment and control are presented in Table 5. The results showed that the extract administration during the 28-day trial period did not cause statistically significant changes ($p > 0.05$) on the hematological parameters compared to the control except for the dose of 250 mg/kg BW, the Ht found significantly increased, while the MCV was decreased in comparison with control. Meanwhile, oral administration of the ethanol extract of *F. deltoidea* leaves for 28 days did not cause significant changes ($p > 0.05$) on serum biochemical parameters compared to the control. However, there is a significant increase in AST and ALP in the serum of mice treated with a dose of 1000 mg/kg BW (Table 6).

Table 3: Body weight and body weight gain of mice treated orally for 28 days with ethanol extract of *F. deltoidea* leaves

Parameter	Subchronic toxicity treatment (mg/kg BW)				
	Control	125	250	500	1000
Initial weight (g)	16.048 ± 0.899	29.236 ± 2.109	19.166 ± 2.644	20.714 ± 0.566	26.040 ± 3.228
Final weight (g)	24.766 ± 1.645^{a}	33.936 ± 1.225^{b}	25.004 ± 2.886^{a,b}	28.336 ± 0.405^{a,b}	31.832 ± 3.115^{a,b}
BWG (g)	8.718 ± 1.188^{a}	4.700 ± 1.109^{b}	5.838 ± 0.420^{a,b}	7.622 ± 0.244^{a,b}	5.792 ± 0.595^{a,b}
BWG (%)	34.627 ± 3.395^{a}	14.214 ± 1.109^{b}	24.067 ± 2.165^{a,b,c}	26.945 ± 1.113^{a,c}	19.150 ± 3.209^{b,c}

Data were represented as Mean±SE. The one-way ANOVA followed by LSD test was performed to obtain significantly different among the groups of treatments. The different letter superscripts (a, b, c) in the same row indicate significantly different p<0.05 versus control. BW: Body weight, BWG: Body weight gain, LSD: Least significant difference.

Table 4: Organ weights and relative organ weights of male mice treated orally with ethanol extract of *F. deltoidea* leaves for 28 days

Organ	Unit	Subchronic toxicity treatment (mg/kg BW)				
		Control	125	250	500	1000
Heart	(g)	0.420 ± 0.262	0.189 ± 0.013	0.151 ± 0.012	0.253 ± 0.097	0.159 ± 0.019
	(%)	2.039 ± 1.439	0.563 ± 0.054	0.621 ± 0.042	0.894 ± 0.345	0.500 ± 0.047
Pulmo	(g)	0.218 ± 0.015	0.256 ± 0.009	0.253 ± 0.022	0.298 ± 0.090	0.255 ± 0.025
	(%)	0.899 ± 0.086	0.760 ± 0.050	1.067 ± 0.154	1.054 ± 0.320	0.829 ± 0.098
Liver	(g)	1.486 ± 0.127	1.491 ± 0.036	1.527 ± 0.052	1.287 ± 0.075	1.646 ± 0.199
	(%)	6.110 ± 0.628^{a,b}	4.408 ± 0.141^{b}	6.384 ± 0.634^{a}	4.540 ± 0.255^{b}	5.144 ± 0.256^{a,b}
Spleen	(g)	0.202 ± 0.041	0.222 ± 0.020	0.298 ± 0.041	0.233 ± 0.096	0.301 ± 0.072
	(%)	0.842 ± 0.184	0.654 ± 0.057	1.234 ± 0.194	0.822 ± 0.340	0.901 ± 0.144
Ventriculus	(g)	0.863 ± 0.146	0.536 ± 0.086	0.635 ± 0.123	0.520 ± 0.103	0.505 ± 0.080
	(%)	3.631 ± 0.726	1.614 ± 0.311	2.810 ± 0.756	1.840 ± 0.368	1.558 ± 0.161
Kidney (left)	(g)	0.201 ± 0.018	0.257 ± 0.024	0.214 ± 0.021	0.314 ± 0.091	0.232 ± 0.031
	(%)	0.817 ± 0.056	0.753 ± 0.051	0.875 ± 0.091	1.108 ± 0.323	0.716 ± 0.045
Kidney (right)	(g)	0.196 ± 0.019	0.255 ± 0.019	0.334 ± 0.105	0.325 ± 0.090	0.232 ± 0.027
	(%)	0.791 ± 0.045	0.748 ± 0.039	1.445 ± 0.531	1.149 ± 0.319	0.724 ± 0.046
Testis (left)	(g)	0.106 ± 0.006	0.129 ± 0.007	0.091 ± 0.016	0.230 ± 0.101	0.116 ± 0.015
	(%)	0.436 ± 0.045	0.381 ± 0.011	0.367 ± 0.057	0.813 ± 0.359	0.359 ± 0.027
Testis (right)	(g)	0.106 ± 0.005	0.131 ± 0.006	0.106 ± 0.020	0.213 ± 0.094	0.126 ± 0.019
	(%)	0.435 ± 0.031	0.388 ± 0.022	0.435 ± 0.080	0.753 ± 0.334	0.391 ± 0.046

Data were expressed as Mean±SE. The one-way ANOVA followed by LSD test. The different letter indexes (a, b, c) in the same row indicate significantly different p<0.05 versus control. *F. deltoidea: Ficus deltoidea*, BW: Body weight, LSD: Least significant difference.

Table 5: Hematological parameters of male mice treated orally with the ethanol extract of *Ficus deltoidea* leaves for 28 days

Parameters	Treatment (mg/kg BW)				
	Control	125	250	500	1000
WBC (10^3/µL)	1.65 ± 0.31	1.82 ± 0.14	1.83 ± 0.45	2.25 ± 0.59	2.03 ± 0.28
RBC (10^6/µL)	0.96 ± 0.31	0.81 ± 0.25	1.55 ± 0.26	1.08 ± 0.15	0.88 ± 0.15
Hb (g/dL)	7.60 ± 0.51	7.88 ± 0.14	7.38 ± 0.47	8.20 ± 0.38	7.88 ± 0.25
Ht (%)	3.28 ± 0.41^{a}	4.42 ± 1.05^{a,b}	7.50 ± 0.96^{b}	5.74 ± 0.69^{a,b}	5.48 ± 0.95^{a,b}
MCV (fL)	60.32 ± 1.46^{a}	49.76 ± 1.25^{b}	42.52 ± 2.53^{c}	60.40 ± 0.94^{a}	61.44 ± 0.97^{a}
MCH (pg)	141.80 ± 2.19	158.14 ± 9.66	127.70 ± 6.79	138.54 ± 14.03	158.96 ± 11.82
MCHC (g/dL)	240.86 ± 1.83	239.06 ± 47.20	184.32 ± 24.35	153.66 ± 16.99	181.80 ± 20.11
PLT (10^3/µL)	2499.00 ± 14.71	3795.60 ± 421.92	2507.00 ± 683.33	3782.40 ± 307.42	2891.40 ± 213.88

Data were expressed as Mean ± SE. The one-way ANOVA followed by LSD test. The different letter indexes (a, b, c) in the same row indicate significantly different p <0.05 versus control. MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, Ht: Hematocrit, PLT: Platelet, MCV: Mean corpuscular volume, LSD: Least significant difference, *F. deltoidea: Ficus deltoidea*, BW: Body weight.

Table 6: Biochemical serum parameters of male mice treated orally with the ethanol extract of *F. deltoidea* leaves for 28 days

Parameters	Treatment (mg/kg BW)				
	Control	125	250	500	1000
Glu (mg/dL)	145.00 ± 8.51^{a,b}	117.20 ± 12.56^{a,b}	130.40 ± 13.54^{a,b}	108.80 ± 5.08^{b}	171.20 ± 23.53^{a}
AST (U/L)	244.60 ± 13.12^{a}	265.60 ± 52.77^{a,b}	227.20 ± 18.62^{a}	567.00 ± 92.36^{b}	1155.80 ± 125.22^{c}
ALT (U/L)	87.20 ± 13.36	116.80 ± 11.01	116.00 ± 24.58	193.00 ± 49.73	186.80 ± 48.71
ALP (U/L)	75.60 ± 4.01^{a}	95.00 ± 10.38^{a}	77.80 ± 11.33^{a}	96.60 ± 7.00^{a}	147.20 ± 9.87^{b}
BiL-T (µM)	0.10 ± 0.01^{a}	0.07 ± 0.01^{a,b}	0.05 ± 0.01^{b}	0.07 ± 0.01^{a,b}	0.08 ± 0.01^{a,b}
BiL-D (µM)	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.01
TP (g/L)	5.18 ± 0.30^{a}	6.08 ± 0.19^{b}	5.92 ± 0.13^{a,b}	5.46 ± 0.21^{a,b}	5.56 ± 0.13^{a,b}
ALB (g/L)	2.94 ± 0.09	3.30 ± 0.26	3.34 ± 0.12	3.34 ± 0.17	3.16 ± 0.09
TC (mg/dL)	90.20 ± 6.41	109.60 ± 6.32	95.80 ± 1.85	91.20 ± 7.97	86.60 ± 6.93
TG (mg/dL)	96.00 ± 11.66^{a,b}	117.40 ± 9.99^{a}	114.60 ± 11.61^{a,b}	95.60 ± 0.93^{a,b}	78.40 ± 5.59^{b}
UA (mg/dL)	3.22 ± 0.29^{a,b}	2.20 ± 0.25^{b}	3.32 ± 0.23^{a,b}	3.22 ± 0.45^{a,b}	3.60 ± 0.30^{a}
BUN (mg/dL)	42.50 ± 2.15^{a}	65.96 ± 3.96^{b}	44.04 ± 3.17^{a,b}	47.46 ± 8.55^{a,b}	62.94 ± 6.27^{a,b}
Cr (mg/dL)	0.04 ± 0.01^{a}	0.011 ± 0.01^{b}	0.07 ± 0.01^{a,b}	0.09 ± 0.01^{b}	0.06 ± 0.01^{a,b}
LDH (U/L)	1323.20 ± 101.04^{a,b}	1479.80 ± 215.59^{a,b}	1481.80 ± 127.68^{a,b}	1584.80 ± 120.21^{b}	817.80 ± 214.97^{a}

Data were expressed as Mean±SE. The one-way ANOVA followed by LSD test. The different letter superscripts (a, b, c) in the same row indicate significantly different p<0.05 versus control. Glu: Glukosa, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, Bil-T: Bilirubin total, Bil-D: Bilirubin direct, TP: Total protein, ALB: Albumin, TC: Total cholesterol, TG: Triglyceride, UA: Uric acid, BUN: Blood urea nitrogen, Cr: Creatinine, LDH: Lactate dehydrogenase, *F. deltoidea: Ficus deltoidea*, BW: Body weight, LSD: Least significant difference.

Discussion

The plant has long been used for human needs for food making and medicinal herbs. It is usually accepted as very safe compounds to consume and contains some important secondary metabolites [19], [20], [21], [22]. The secondary metabolite or bioactive compounds (phytochemicals) of phenolic, such as existing flavonoids in plants, have been reported to be responsible for various biological processes in the body [23], [24], [25], [26]. Although bioactive compound from plant including flavonoid is widely used, the use of plants itself may be able to cause unexpected effects of toxicity. The toxicity might be derived from several plant compound that causes hematotoxic [27] and hepatotoxic [28], which can provoke insertional and carcinogenesis [29], [30]. Recently, there is still a lack of scientific data evidence on the toxicity and safety of many plants. Therefore,

the scientific evaluation of the effectiveness, as well as the safety of herbal medicine, is urgent to be done. Meanwhile, the first priority in herbal research with toxicological studies to determine the toxicity of the product and choose a safe dose in humans using various experimental models needs to be conducted [31].

The acute toxicity test in animals can be used to obtain the hazard classification requirement of LD_{50} value. The acute toxicity is usually to be the first step to be done and can provide preliminary information about the toxic action of a substance, determining the dose in animals [32]. It is the basis that the acute toxicity assessment of the ethanol extract of *F. deltoidea* leaves was carried out in mice. In general, abnormal changes in general behavior and BW have been used as critical parameters for objective evaluation of the test substance effect on experimental animals because these changes are often the first sign of toxicity [33], [34].

The present findings stated that mice treated with the ethanol extract of *F. deltoidea* leaves in the acute and subchronic toxicity did not either cause mortality or even did not appear to have abnormal changes in general behavior, a significant variation of the weight associated with the treatment of extracts, or food/water intake as a sign of critical poisoning in test animals. Therefore, it can be assumed that the LD_{50} of the extract is >2000 mg/kg, which suggests that extracts are generally considered as nontoxic in acute ingestion, based on the method of classification of acute toxicity [35]. Moreover, the present results regarding LD_{50} evaluation revealed that a single oral dose (2000 mg/kg BW) did not cause mortality in 72 h and during 14 days of observation. These results indicated that acute exposure to the ethanol extract of *F. deltoidea* leaves did not cause toxic effects, and the value of LD_{50} was considered to be >2000 mg/kg BW in mice that are harmless in acute doses [36].

During this subchronic test, all animals were active and responded positively to stimuli. There were no mortality, behavioral changes and clinical signs of localized or systemic toxic effects were observed. In addition to mortality as a clear sign of toxicity, there are other variables that may exhibit side effects such as BW during clinical treatment and signs of toxicity [15]. Changes in BW function as an indication of sensitive animal health status are usually used as a tool, indicator, and marker of side effects in toxicological investigation [37], while the weight of the organs is relative to indicate pathological changes in the disturbed organs [38]. The current results in subchronic toxicity studies revealed that BW showed no significant changes after administration of the extract for 28 days in comparison to the control group. Meanwhile, relative weight gain also did not show a significant difference, except in mice that treat with ethanolic extract of *F. deltoidea* leaves at the dose of 125 and 1000 mg/kg BW which relative weight gain was found a significant decrease. The present finding is supported by the previous research stated that the leaves of methanolic extract of *F. deltoidea* at a dose of 200 mg/kg did not cause abnormal changes in liver and kidney function in subchronic toxicity study [39]. This is likely due to some external factors affecting this group in particular, such as the struggle for domination (the struggle for social hierarchy, the characteristics of a male beast), or the monotonous of a diet that can lead to decreased feed intake. Furthermore, most of the relative organ weight seems to be unaffected by the extract treatment except at the relative hepatic weight of the extract at the dose of 125 and 500 mg/kg BW which decreased in comparison to the control. The difference in weight of internal organs may be due to variations in the size of internal organs and/or BW of animals [40].

To evaluate the symptoms of organ disorders, various hematological and biochemical blood parameters are also measured in male mice. Repeated administration of a substance to subchronic toxicity can cause temporary or permanent damage to the hematopoietic system. Evaluation of hematological parameters is one of the systems that are very sensitive to toxic compounds and can be used as an indicator to determine the effect of toxic substances and provide information about the pathophysiology of mammals [41], [42].

The current results showed that there was an increase Ht but decreased in MCV of mice treated with 250 mg/kg BW dose. This condition is likely irrelevant because they were in the normal physiological range [43]. These changes were not dose dependent because it was only observed in groups treated with 250 mg/kg BW dose, while at higher dosages were not found. Since no corresponding changes were observed in other parameters, significant changes in HCT and MCV were not related to the treatment. Further, there was no regularity between increasing the dose and the increase in toxic effects. These results are similar to the previous findings, conducted by Bo Li *et al*. [44] who found that some blood hematology parameters do not have a consistent pattern between increasing the dose with the change in hematological parameters and the time of measurement. This unfixed pattern is due to some variations of a small number of animal in one group and cannot be claimed that the test sample provided a toxic effect on the referred parameter.

To investigate systemic toxicity or target organs inflicted by the substances being tested, blood serum biochemical test is also needed for further assessment. Serum biochemical test on experimental animal studies aims to determine the toxic effects, especially on the kidneys and liver [45], [46]. In toxicity study, the assessment of liver and kidney function is very important because these two organs are the primary internal organs in the body that has some important functions for the survival of organisms [47]. The results of the present study appeared that AST and ALP blood serum of the mice in the group 1000 mg/kg/BW was significantly higher than control. Balogunand and Ashafa [48] revealed that

the increase AST and ALP at a high-dose extract might be having a hepatotoxic effect, while an increase in ALP may occur obstruction of the bile duct in the liver. In addition to these two parameters, it seems that there were slightly biochemical differences that were not constant in all doses tested versus control and inconsistent between the dose and the observation period, considering as an incidental. Moreover, some parameters for each animal were found slightly increase or decrease in the treatment group compared to the control group, including the total protein, total bilirubin, urea, creatinine, uric acid, TG, glucose, and LDH. Meanwhile, the ALT, ALB, and cholesterol parameters were found no significantly different compared to the controls. Regardless of its significance, these data indicated that there was no apparent dose–response relationship, and all small fluctuations in these parameters were within the normal range of testing laboratories. Therefore, these variations were no relevance to the clinical significance or toxic effects of extracts but rather than the physiological variability of the animal itself. In addition, the effect was not dose dependent, and it is not accompanied by clinical signs or symptoms [49].

Conclusion

This study shows that acute and subchronic oral administration of the ethanol extract of *F. deltoidea* leaves for male mice does not induce clinical symptoms of toxicity or cause mortality. The LD_{50} from the ethanol extract of *F. deltoidea* leaves for mice >2000 mg/kg is considered as practically non-toxic based on the BW, relative BW, hematological, and blood biochemical analysis.

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