Evaluation of the Acute Toxicity of Two Extracts of *Chisochetonmacrophyllus* Seeds in Female Wistar Rats

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Abstract
Chisochetonmacrophyllus seed extract contains four bioactive compounds, i.e. dysobinine, 7-methoxy-3-sitosterol, 24-hydroxy dammara-25,26-dien-3-on and dammarandiennon, shown to have antimalarial activity against Plasmodium falciparum and the activity was stronger than chloroquine. This study aims to evaluate the acute toxicity of ethyl acetate and n-hexane extracts of C. macrophyllusseedsin female Wistar rats. Acute oral toxicity test was based on OECD 425:2006 guidelines with a limit test at 5000 mg/kg BW and main test consist of ten treatments including control with four replications each. The results showed decreasing the weight gain along with increasing doses of the both tested substance. The relative weight of organ i.e. liver, kidney, heart and spleen of the extract-treated animals only affected at higher doses, except the stomach which showed higher relative weight at all dosages compared with control (p<0.05).

The LD50 value estimated by Probit analysis was19700.13 mg/kg BW for ethyl acetate extract and 12253.14 mg/kg BW for n-hexane extract categorized as relatively harmless and practically non-toxic respectively. Histopathological examination showed an increasing of necrotic cells as well as disruption of tissue architectural of all organ in a dose-dependent manner. This study showed that n-hexane extract of C. macrophyllus seeds showed higher acute toxicity than ethyl acetate extract in female Wistar rats.

Keywords: acute toxicity, *Chisochetonmacrophyllus*, *n*-hexane, ethyl acetate, female Wistar rats.

Introduction

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. In 2015, 91 countries and areas had ongoing malaria transmission. Malaria is preventable and curable and increased efforts are dramatically reducing the malaria burden in many places. Between 2010 and 2015, malaria incidence among populations at risk (the rate of new cases) fell by 21% globally. In that same period, malaria mortality rates among populations at risk fell by 29% globally among all age groups and by 35% among children under 5. The WHO African Region carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 90% of malaria cases and 92% of malaria deaths.[32]

The *Chisocheton* genus, a member of the Meliaceae family, consists of approximately 50 species that are distributed mainly in India, Thailand, Malaysia and Indonesia[3].The genus *Chisocheton* belongs to the subtropical and tropical plant family widely known for its insecticidal limonoid constituents[23]. Previous phytochemical studies on *Chisocheton* species have yielded a number of interesting compounds including limonoids[16], antifungal meliacin-type compound[3], dammarane triterpenoids[9] and spermidine alkaloids[28].

As part of our studies on novel compounds from Indonesian Meliaceae plants[5], we carried out a study on *Chisochetonmacrophyllus* seeds. *C. macrophyllus* is a higher plant found growing in the rain forest in the northern part of Sulawesi Island, Indonesia[6]. The plant is known as Ma-aa in Indonesia and the seed oil from this plant is used in Indonesia for lighting[30]. *Chisocheton macrophyllus* seeds extract contains four bioactive compounds i.e. dysobinine, 7-methoxy-3-sitosterol, 24-hydroxy dammara-25,26-dien-3-on and dammarandiennon shown to have antimalarial activity against *Plasmodium falciparum* and the activity was stronger than chloroquine.[18]

Medicines obtained from natural sources have become the basis for pharmaceutical drugs. Traditional herbal medicines are naturally occurring plant derived substances; these have been used for treatment and cure of various diseases and as nutraceuticals. Toxicological research and testing help to live safely and predict benefit from synthetic and natural substance while avoiding harm. The toxicity study is done for data profiling and safety of the herbal drugs[25].The information generated by the test is used in hazard identification and risk management of the drugs. Pre-clinical studies of herbal drugs provide scientific justification for their traditional use and prove that they are safe and efficacious.[31]

Acute oral systemic toxicity testing is conducted to determine the hazard potential of a single oral exposure to various chemicals and products in which single dose of the tested substance is used in each animal on one occasion to evaluate general toxic signs and LD50 (median lethal oral dose). It is usually the initial assessment and evaluation of

the toxic characteristics and toxic manifestations of a substance, thus, providing information on health hazards likely to arise from short-term exposure to the substance. Generally, the smaller is the LD50 value, the more toxic the substance is and vice versa. Acute toxicity data can also be the basis of the classification and labelling of a substance, as well as used to establish dose levels and designing further toxicity tests.[8,21] The study aims to evaluate the acute toxicity levels of *Chisocheton macrophyllus* seeds in ethyl acetate and n-hexane extract solvent systems. This acute toxicity testing is conducted to get information regarding its safety and further evaluate biological activity and mechanism of action of the tested substances.

Material and Methods

Collection and extraction of plant materials: The seeds of *Chisocheton macrophyllus* were collected from Bogor Botanic Garden (Centre for Plant Conservation), Bogor West Java Province. Indonesia. The plant was identified in Plant Taxonomy Laboratory, School of Life Science and Technology, InstitutTeknologi Bandung.

Source and housing conditions of tested animals: The experiments were performed using healthy young adult female Wistar rats (10-12 weeks), nulliparous and non-pregnantwith weighing 170-190 g provided by Biosystem Laboratory of Biology Department, Universitas Padjdjaran. Female rats were chosen because they are generally slightly more sensitive than the male. Animals were randomly assigned to control and treated groups. They were housed under standard environmental conditions of room temperature with a constant relative humidity under 12 h dark-light cycle. The animals were fed with a standard laboratory pellet diet with tap water *ad libitum*. The animals were acclimated to holding facilities for one week prior to dosing.

Acute Toxicity Test Procedure: Acute toxicity test procedure was based on OECD 425 Guidelines for The Testing of Chemicals: Acute Oral Toxicity – Up and Down Procedure. Acute toxicity test was performed using animals of female Wistar rats. The animals were fasted from the food supply, but no water 12 h prior to treatment. The ethyl acetate extract was dissolved in 1% dimethyl sulfoxide (DMSO) whereas *n*-hexane extract was dissolved in 0.5 % Carboxyl Methyl Cellulose (CMC). The extract solution was administered orally by gavage using a ball-tipped stainless-steel feeding needle and the volume must not exceed than 2 mL/kg.

Main test consists of single ordered dose progression in which animals are dosed, one at a time, at a minimum of 48-hour interval and was done to determine the Median Lethal Dose 50 (LD50) value from each of the extract. The first animal receives a dose a step upper of the limit test dose based on the dose progression flow chart of annex 2 in the guidelines. If the animal survives, the dose for the next animal is increased; if it dies, the dose for the next animal is decreased by a similar dose progression. In the main test, we used the dose at 5500, 6500, 7300, 8200, 9100, 12000, 12600, 17500 and 22500 mg/kg BW as well as 0 mg/kg BW by which vehicle solution is given as a control group. The treatment was given to maximum five animals each. After the substance has been administered, food may be withheld for further 3-4 hours.

The animals were observed individually after dosing at least once during the first 30 minutes periodically during the first 24 hours with special attention given during the first 4 hours and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. All observations are systematically recorded with individual records being maintained for each animal. Surviving animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period.

On the 15th day, after an overnight fast, the survived animals were sacrificed by cervical dislocation. Gross necropsies was done to randomly selected animals, including those that die during the test or are removed from the study for animal welfare reasons. The positions, shapes, sizes and colors of internal organs were evaluated. Liver, kidneys, heart, spleen and stomach were removed from all animals to visually detect gross lesions. All tissues were fixed in Bouin's solution for histopathological examination. The tissues were embedded in paraffin and then thin-sectioned (5 µm), stained with hematoxylin and eosin and then were examined microscopically.

Data collection and analysis: Mortality (number of death rats) was counted in each group and recorded. The median lethal dose(LD_{50}) that killed 50% of the tested animals was determined using Probit analysis. Weight gain, relative weight of the organ and percentage of necropsies cells data were expressed as mean ± standard deviation (S.D). The data were analyzed using Paired sample *t*-test at 95% confidence level by software Statistical Package for Social Sciences (SPSS) and *P* values less than 0.05 were considered significant.

Results

Acute toxicity of the ethyl acetate and n-hexane extract of C. macrophyllus seeds on female Wistar rats: Limit test at 5000 mg/kg BW of ethyl acetate and n-hexane extracts of *C. macrophyllus* seeds result in no death or signs of toxicity on the treated animals up to 14-days observation, therefore the LD50 value was higher than the tested dose. Based on Globally Harmonised Classification System (GHS) in OECD 423 guideline, the LD50 value is more than 5000 mg/kg BW categorized as unclassified toxicity.

In main test, no mortality and toxicity signs were observed in any animals treated by dose of 5500, 6500, 7300 and 8200 mg/kg BW of the ethyl acetate extract as well as by dose of 5500, 6500, 7300, 8200 and 9100 mg/kg BW respectively of the n-hexane extract. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation and sleep of the treated as well as the control animals were found to be normal. The animals treated with the ethyl acetate extracts at dose of 9100, 12000, 12600, 17500 and 22500 mg/kg BW respectively the n-hexane extract at dose of 12000, 12600 and 17500 mg/kg BW showed toxicity symptoms i.e. decreasing motoric activity, convulsions and salivations up to 72 h after the extract administration. In addition, toxic signs i.e. tremors, lethargy, diarrhea and coma did not occur in any of the animal (table 1).

Mortality ratio of the tested animals after oral administration of ethyl acetate extract at dose of 17500 mg/kg BW was 1/4 after 72 h of observation as well as at dose of 22500 mg/kg BW with the mortality ratio was 1/4, 1/4 and 2/4 after 24, 48 and 72 h of observation respectively. Whereas in animals treated with n-hexane extract, mortality ratio which has been observed at dose of 12000 mg/kg BW was 1/4 after 48 h of observation and at dose of 12600 mg/kg BW with the mortality ratio was 1/4 each after 24 and 48 h of observation, as well as at dose of 17500 mg/kg BW with the mortality ratio was 2/4, 1/4 and 1/4 after 24, 48 and 72 h of observation respectively (table 1). The n-hexane at dose of 22500 mg/kg BW was not given to the tested animals because at dose of 17500 mg/kg BW it showed mortality more than 50% in rats' population.

The body weights of almost all the rats were increased after the oral administration of either the ethyl acetate or the n-hexane extract, except for the rats treated with the ethyl acetate extract at dose of 12000, 12600 and 17500 mg/kg BW respectively which showed decreasing body weight in the end of treatment period. The weight gain of all the extract-treated groups was lower than the control group (table 2). Furthermore, the relative weight of organ i.e. liver, kidneys, heart and spleen showed a fluctuate response and not different with control group, except for the liver and spleen of tested animals treated with higher dosage of the ethyl acetate extract. The stomach of all treated animals showed an increasing relative weight along with increasing the dosages and significantly different with control group ($p<0.05$) (table 3).

Histopathological examination of the liver, kidneys, heart, spleen and stomach of *C. macrophyllus* seeds extracts treated rats showed significantly histological differences, mainly increasing of necropsies cells along with increasing the dosage treatment and significantly different with the control group ($p<0.05$) (table 4). In liver, parenchymal tissue showed vacuolization in form of hydropic or lipid degeneration and necrotic nuclei in hepatocytes whereas sinusoids and central vein show normal, only noting the infiltration of the inflammatory cells (fig 1A and 2F). In kidneys, glomerulus nuclei and Bowman's capsule were normal, whereas the proximal tubules cells showed both hydropic and fatty degenerations, as well as necrotic nuclei (fig. 1B and 2G).

Cardiac myocytes also showed a necrosis cell (fig. 1C and 2H). Spleen shows normal red and white pulp as well as centrum germinativum within the white pulp which was occupied mainly by lymphocytes at various stages of maturation and reticular cell (fig. 1D and 2I). Stomach histological architectures in the extracts treated rats also showed an impairment by occurrence of epithelial desquamation and eruption in mucosa layer, but muscular is external shows normal (fig. 1E and 2J).

Table 1
Mortality and toxic signs of female Wistar rats in the acute toxicity test of the ethyl acetate and n-hexane extracts from *C. macrophyllus* seeds

Extract Dose (mg/kg BW)	Number of tested animal	Ethyl acetate		n-Hexane	
		Number of death	Toxic signs[#]	Number of death	Toxic signs[#]
(control)	4	0	-	0	-
5000	4	0	-	0	-
5500	4	0	-	0	-
6500	4	0	-	0	-
7300	4	0	-	0	-
8200	4	0	-	0	-
9100	4	0	+ (1/4)	0	-
12000	4	0	+ (1/4)	1	+ (1/4)
12600	4	0	+ (1/4)	3	+ (1/4)
17500	4	1	+ (1/4)	4	+ (1/4)
22500	4	4	+ (1/4)	-	-

Note: Toxic signs observes were decreasing motoric activity, convulsions and salivations with ratio number of test animal showed toxic signs per total tested animal in a group.

Table 2
Body weight of female Wistar rats in the acute toxicity test of the ethyl acetate and n-hexane extracts from *C. macrophyllus* seeds

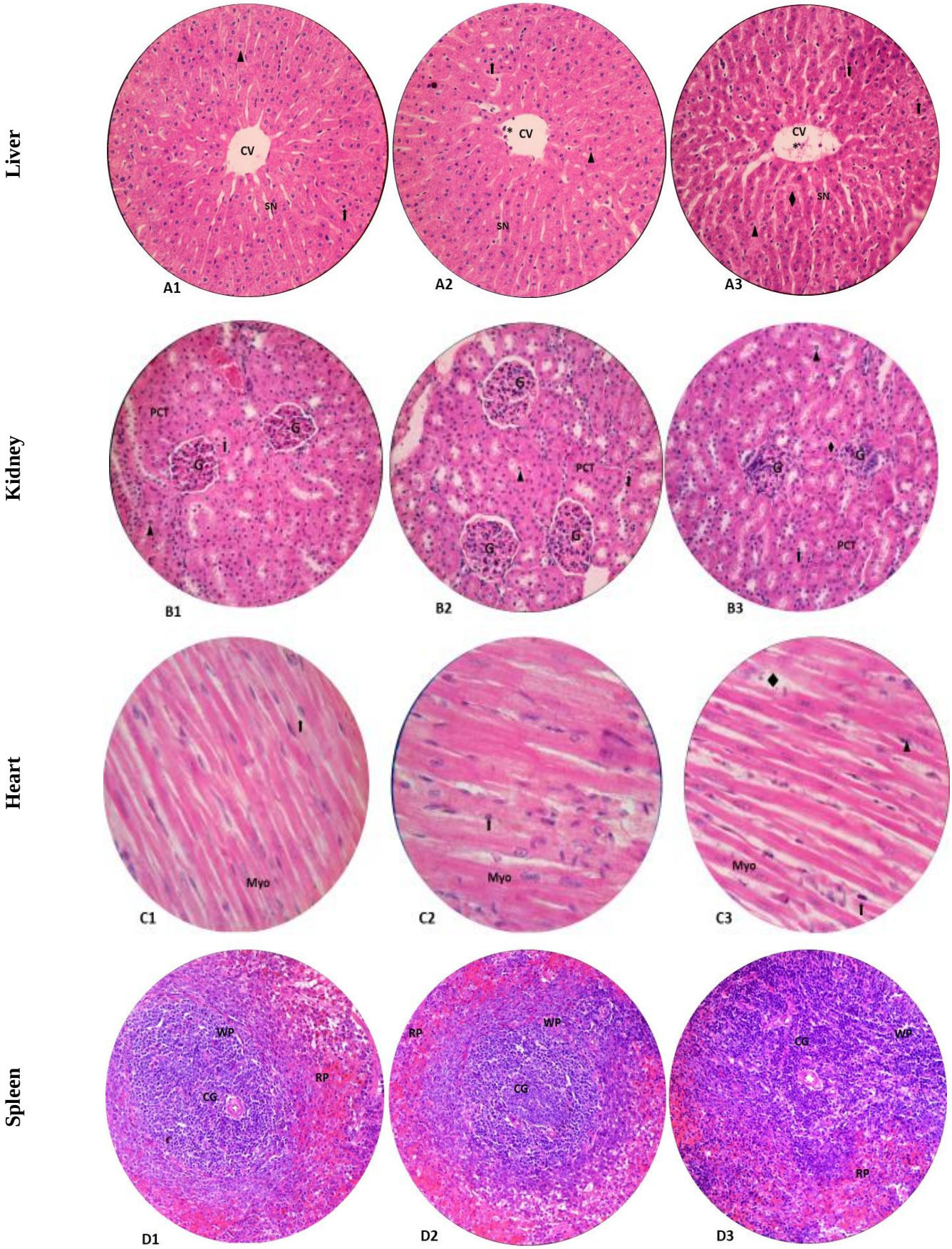
Extract Dose (mg/kg BW)	Ethyl Acetate			n-Hexane		
	Body weight (g)		Weight Gain (g)	Body weight (g)		Weight Gain (g)
	Day 1	Day 14		Day 1	Day 14	
0 (control)	182.1 ± 1.76	205.7 ± 4.08	23.6 ± 4.71	179.5 ± 3.65	209.9 ± 1.56	30.4 ± 2.99
5000	183.7 ± 4.46	192.9 ± 4.23	9.2 ± 0.82	180.7 ± 3.69	200.1 ± 5.95	19.4 ± 3.83
5500	179.7± 2.18	197.2 ± 4.40	17.6 ± 3.26	182.7 ± 3.70	205.3 ± 7.94	22.6 ± 6.39
6500	181.4 ± 2.91	201.7 ± 3.32	20.3 ± 0.44	175.5 ±3.85	193.7 ± 6.34	18.2 ± 2.77
7300	185.3 ± 1.95	203.1 ± 7.46	17.8 ± 7.86	181.8 ±3.03	196.1 ± 3.75	14.2 ± 1.70
8200	178.7 ± 3.75	192.1 ± 2.87	13.5 ± 6.62	177.2 ± 3.30	187.4 ± 0.96	10.3 ± 3.88
9100	182.0 ± 3.83	191.4 ± 3.58	9.4 ± 7.11	179.1 ± 5.25	183.3 ± 3.08	4.2 ± 2.21
12000	184.1 ± 3.49	178.3 ± 3.60	-5.8 ± 6.78	183.6 ± 2.33	185.6 ± 1.81	2.0 ± 2.21
12600	179.7 ± 3.02	168.8 ± 2.28	-10.9 ± 0.95	181.9 ± 2.31	-	-
17500	185.5 ± 3.22	169.2 ± 2.57	-16.3 ± 4.33	-	-	-
22500	179.9 ± 2.88	-	-	-	-	-

Note: Data are expressed as mean ± S.D (n=4).

Table 3
Relative weight of organ of female Wistar rats in the acute toxicity test of the ethyl acetate and n-hexane extract from *C. macrophyllus* seeds

Extract Dose (mg/kg BW)	Liver (%)	Left Kidney (%)	Right Kidney (%)	Heart (%)	Spleen (%)	Stomach (%)
Ethyl acetate						
0 (control)	3.42 ± 0.142	0.44± 0.063	0.51± 0.021	0.54 ± 0.054	0.46 ± 0.048	1.91 ± 0.160
5000	3.56 ± 0.136*	0.46± 0.019	0.53± 0.051	0.51 ± 0.028	0.50 ± 0.035	2.11 ± 0.094
5500	3.45 ± 0.163	0.46 ± 0.058	0.45± 0.006*	0.59 ± 0.027	0.50 ± 0.009	2.17 ± 0.142*
6500	3.45 ± 0.256	0.42± 0.023	0.45± 0.043	0.49 ± 0.056	0.47 ± 0.019	2.20 ± 0.210
7300	3.62 ± 0.180	0.44± 0.010	0.45± 0.019*	0.44 ± 0.026	0.51 ± 0.053*	2.54 ± 0.166*
8200	3.53 ± 0.091	0.51± 0.018	0.46± 0.018	0.56 ± 0.059	0.53 ± 0.034	2.86 ± 0.323*
9100	3.67 ± 0.197	0.53± 0.014	0.50± 0.041	0.57 ± 0.056	0.54 ± 0.038	3.10 ± 0.150*
12000	4.04 ± 0.217*	0.52± 0.052	0.50± 0.018	0.51 ± 0.024	0.55 ± 0.016	3.44 ± 0.185*
12600	4.19 ± 0.125*	0.56± 0.018	0.55± 0.027	0.45 ± 0.043*	0.55 ± 0.049*	4.32 ± 0.296*
17500	4.45 ± 0.178*	0.54 ± 0.035*	0.53± 0.009	0.46 ± 0.018	0.68 ± 0.045*	4.56 ± 0.113*
n-Hexane						
0 (control)	3.67 ± 0.045	0.46± 0.043	0.51± 0.039	0.54 ± 0.058	0.49 ± 0.049	1.55 ± 0.069
5000	3.61 ± 0.193	0.46± 0.016	0.51± 0.046	0.48 ± 0.086	0.52 ± 0.063	1.80 ± 0.126*
5500	3.40 ± 0.240	0.43± 0.012	0.49± 0.104	0.53 ± 0.033	0.46 ± 0.036	1.90 ± 0.174
6500	3.48 ± 0.201	0.49± 0.012	0.53± 0.030	0.57 ± 0.033	0.53 ± 0.051*	1.94 ± 0.121*
7300	3.77 ± 0.256	0.46± 0.043	0.53± 0.041	0.46 ± 0.026	0.52 ± 0.069	2.19 ± 0.096*
8200	3.71 ± 0.259	0.53± 0.028	0.49± 0.022	0.53 ± 0.091	0.49 ± 0.052	2.23 ± 0.069*
9100	3.71 ± 0.277	0.61± 0.022*	0.52± 0.028	0.56 ± 0.067	0.56 ± 0.051	2.55 ± 0.031*
12000	3.82 ± 0.071	0.55± 0.052	0.52± 0.101	0.45 ± 0.013	0.54 ± 0.055	2.63 ± 0.042*

Note: Data are expressed as mean ± S.D (n=3). * Significantly different from control (**p**<0.05) by Paired simple *t*-test.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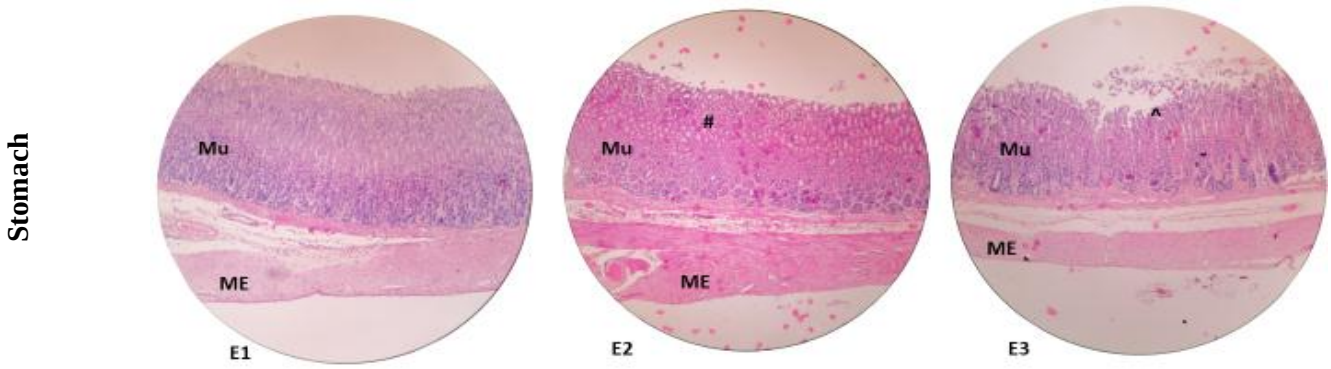
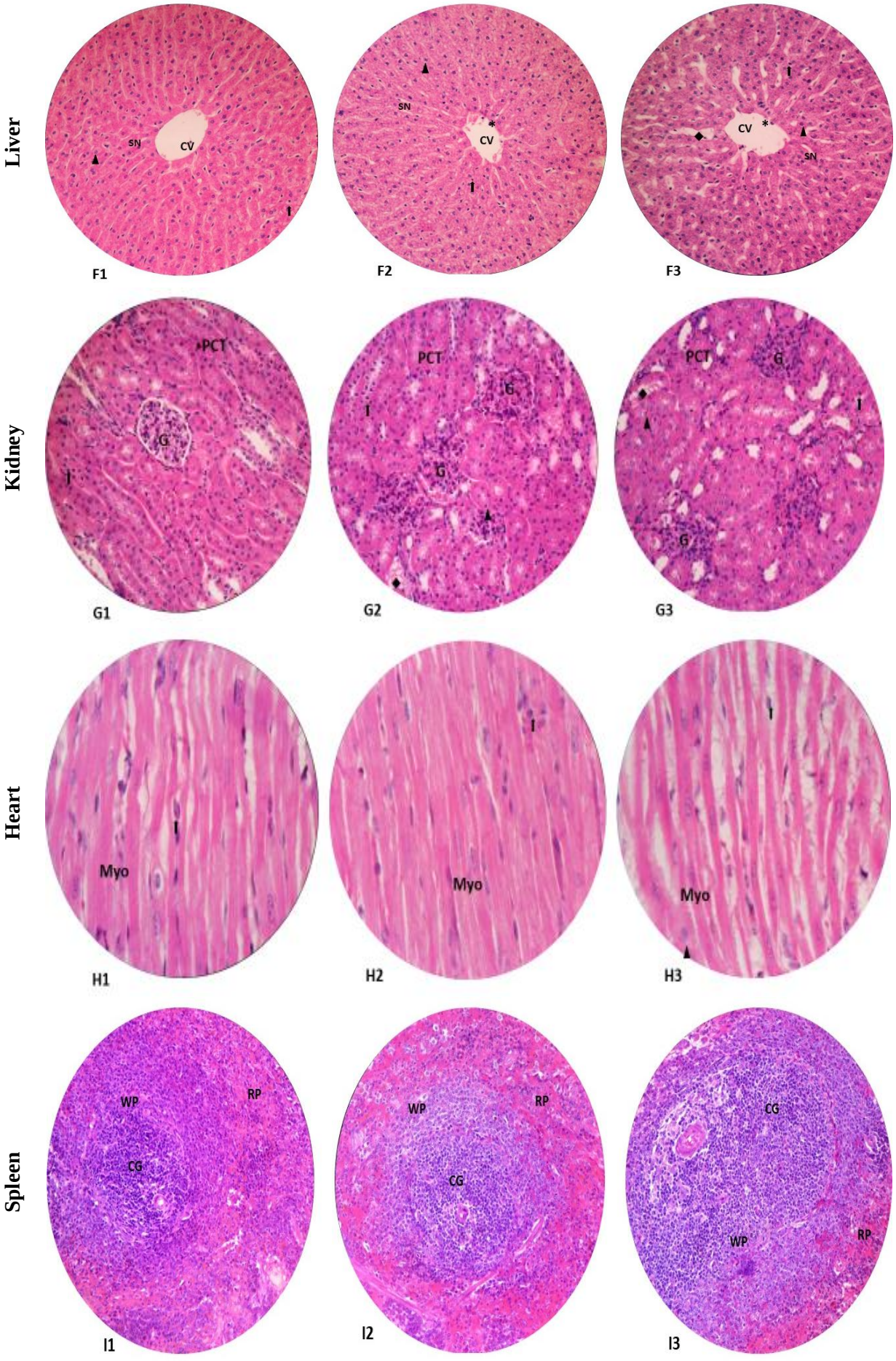


Fig. 1: Photomicrograph of tissue cross section of female Wistar rats after acute toxicity test of the ethyl acetate extract from *C. macrophyllus* seeds from (1) normal, (2) medium and (3) highest severity. Hematoxylin-Eosin stain. M. 100×. Note: Central Vein (CV), Sinusoids (SN) in liver (A); Glomerulus (G), Proximal Convoluted Tubules (PCT) in kidney (B); Myocyte in heart (C); Centrum Germinativum (CG), White Pulp (WP), Red Pulp (RP) in spleen (D); Mucosa (Mu), Muscularis Mucosae (MM) in stomach (E). Necropsies cell (arrow), vacuolization (arrowhead), infiltration of inflammatory cell (*), epithelial desquamation (#) and epithelial eruption (^).

Table 4
Percentage of necropsies cell of female Wistar rats in the acute toxicity test of the ethyl acetate and n-hexane extract from *C. macrophyllus* seeds

Extract Dose (mg/kg BW)	Liver (%)	Kidneys (%)	Heart (%)	Spleen (%)	Stomach (%)
Ethyl acetate					
0 (control)	9.67 ± 5.03	8.90 ± 2.65	7.07 ± 2.52	8.77 ± 3.21	9.30 ± 3.61
5000	9.27 ± 4.51*	7.97 ± 7.09	6.30 ± 4.58	8.23 ± 6.03	13.50 ± 6.56*
5500	9.37 ± 3.51	8.13 ± 6.81	6.87 ± 2.52	8.67 ± 4.04	14.70 ± 8.00*
6500	9.10 ± 2.00	9.07 ± 4.16	7.20 ± 3.61	8.57 ± 6.66	15.37 ± 4.93*
7300	10.40 ± 7.55	11.33 ± 5.69*	7.07 ± 3.51	9.37 ± 5.51	16.53 ± 6.11*
8200	11.23 ± 7.23	13.33 ± 5.86*	8.20 ± 6.56	10.40 ± 6.56*	16.43 ± 4.73*
9100	13.70 ± 4.58*	14.20 ± 4.00*	9.73 ± 5.13*	10.80 ± 3.61*	17.17 ± 4.51*
12000	15.33 ± 4.73*	15.13 ± 6.66*	10.93 ± 4.16*	12.17 ± 3.51*	17.70 ± 6.00*
12600	15.40 ± 6.24*	16.00 ± 3.61*	13.27 ± 4.51*	13.67 ± 5.69*	18.03 ± 3.21*
17500	16.63 ± 6.43*	16.87 ± 4.04*	14.43 ± 7.09*	15.17 ± 3.51*	18.90 ± 4.00*
n-Hexane					
0 (control)	8.23 ± 15.50	6.90 ± 4.58	8.13 ± 5.51	8.13 ± 5.03	8.70 ± 4.00
5000	6.97 ± 7.09	6.80 ± 4.00	8.13 ± 1.53	7.23 ± 6.51	8.60 ± 2.00
5500	7.43 ± 5.03	7.60 ± 2.65	8.73 ± 3.79	7.73 ± 4.16	8.90 ± 2.00
6500	7.73 ± 4.16	8.07 ± 3.51	9.33 ± 5.69	9.27 ± 4.93	10.37 ± 6.43*
7300	9.70 ± 8.00	8.30 ± 4.00	10.50 ± 6.56*	11.30 ± 8.89*	10.90 ± 6.56*
8200	11.33 ± 5.86	10.27 ± 9.07	11.57 ± 7.09*	11.37 ± 9.45*	12.13 ± 3.51*
9100	12.87 ± 4.16*	12.37 ± 5.86	12.83 ± 3.51*	11.93 ± 3.51*	11.97 ± 3.06*
12000	13.13 ± 6.66*	13.60 ± 8.18	13.77 ± 4.51*	12.50 ± 4.58*	12.93 ± 2.52*

Note: Data are expressed as mean ± S.D (n = 3). * Significantly different from control (**p**<0.05) by Paired simple *t*-test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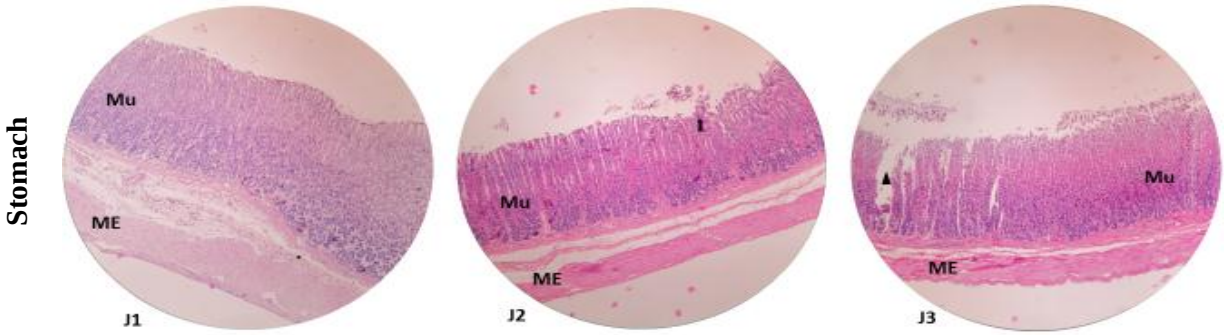

Fig. 2: Photomicrograph of tissue cross section of tissue female Wistar rats after acute toxicity test of the n-hexane extract from *C. macrophyllus* seeds from (1) normal, (2) medium and (3) highest severity. Hematoxylin-Eosin stain. M. 100×. Note: Central Vein (CV), Sinusoids (SN) in liver (F); Glomerulus (G), Proximal Convoluted Tubules (PCT) in kidney (G); Myocyte in heart (H); Centrum Germinativum (CG), White Pulp (WP), Red Pulp (RP) in spleen (I); Mucosa (Mu), Muscularis Mucosae (MM) in stomach (J). Necropsies cell (arrow), vacuolization (arrowhead), infiltration of inflammatory cell (*), epithelial desquamation (#) and epithelial eruption (^).

Discussion

Chisocheton plants are known to produce various types of toxic limonoids. For example, erythrocarpines A–E isolated from *C. Erythrocarpus*barks[1] and five limonoids isolated from *C. siamensis* seeds are found to have antimalarial, antimycobacterial and cytotoxic activities[14]. Our previous study also showed that *C. macrophyllus* seeds extract contains four bioactive compounds, which are dysobinine, 7-methoxy-3-sitosterol, 24-hydroxy dammara-25,26-dien-3-on and dammarandiennon. All compounds showed antimalarial activity against *Plasmodium falciparum* and the bioactivity was found to be higher than that for chloroquine. To further explore the bioactivity of the seed extract of *C. macrophyllus*, toxicity assay was carried out and studied in this present report. Some related studies have been reported by Khan et al[11] that evaluated acute oral toxicity of antimalarial phyto-medicine using methanolic leaf extract of *Nepatacateria* and by Vale et al[29] that studied toxicity of barks extract of *Himatanthusarticulates*.

The selection of extracting solvents is important in this study. It significantly determines total phytochemical content of the plants. The selection is generally based on solvent polarity. For example, the higher is the polarity, the better is the solubility of compounds extracted from the plants[20]. In this study we used ethyl acetate and *n*-hexane to extract the seeds of *Chisochetonmacrophyllus* seeds. This option was based on a report from Nurlelasari et al[18] showing that extracts of ethyl acetate and *n*-hexane have antimalarial activity against *Plasmodium falciparum* and their bioactivity was higher than that of chloroquine.

Acute oral toxicity of the ethyl acetate extract was determined at dose of 22500 mg/kg BW which caused death of the tested animals within 24 h after the extract administration. Meanwhile, treatment at dose of 17500 mg/kg BW caused delayed death up to 72 h after the extract administration. For the *n*-hexane extract, the acute oral toxicity was found at dose of 12600 and 17500 mg/kg BW while treatment at dose of 12000 mg/kg BW caused delayed death up to 72 h after the extract administration.

The median lethal dose (LD_{50}) was estimated by Probit analysis where for ethyl acetate extract the value was 19700.13 mg/kg BW and for *n*-hexane extract it was 12253.14 mg/kg BW. Consequently, the ethyl acetate and *n*-hexane extracts were categorized as relatively harmless (>15000 mg/kg BW) and practically non-toxic (5000-15000 mg/kg BW), respectively according to Hodge and Sterner.[7]

Toxic signs in the tested animals were observed after administration of the higher extract dosages due to the side effects of the extracts. These signs were characterized by behavioural changes in the tested animals. The key clinical toxic signs were noted as decreasing motoric activity, convulsions and salivations indicating that the extracts affect the somato-muscular system, spinal integrity in central nervous system and autonomic nervous system respectively.[17,19]

The body weight changes are indicators of adverse side effects of drugs orchemicals, as the animals that survive cannot lose more than 10% of the initial body weight.[4,22] This study found that the body weight loss was almost 10% from the initial weight in higher dosages, particularly in the treatment using ethyl acetate extract. This indicates that the administration of the ethyl acetate extracts does affect the food intake and the growth of the animals. Reduction in the body weight is a valuable indicator in evaluating the toxicity as it is a sensitive indication of the general health status of animals.

However, the differences in bodyweight and body weight gain of the tested animals may have resulted from physiological variations such as food intake and

metabolism[10]. Our result agreed with Miller et al[15] who showed that at high level of exposure, the diets with limonoids caused problems with weight gain.

Organ weight is also an important index of physiological and pathological status in animals. The relative organ weight is a basic to diagnose whether the organ was exposed to the injury or not[24]. Treatments with ethyl acetate extract at higher dose mainly increased the relative weight of liver and spleen, while treatments with *n*-hexane did not affect the relative weight of the vital organ. Alterations in liver and spleen weight may suggest treatment-related changes including hypertrophy. The direct and indirect toxicity in the liver and spleen are related to the decomposition of thered blood cells[27,28].

The stomach was the organ that showed an increase in the relative weight after the extracts treatment, even in lower dosages. This is correlated with our necropsy findings that the ethyl acetate extract cannot be digested in the stomach. This may be due to characteristic (i.e. active compounds, polarity etc.) and volume of the extract itself. Hence for the *n*-hexane extract, the animals cannot digest the diet that may be due to stress after the oral treatment using a ball-tipped stainless-steel feeding need lethat was injected directly into the stomach.

Changes in organ weights should always be interpreted in conjunction with necropsy and histopathologic findings because of the inherent variability. Necropsy and histopathological examinations can indicate the major target organs for toxicity and identification of these may help to focus subsequent testing[2]. Necropsy and histopathology examinations confirmed whether the organs or tissue had been damaged or not. The necropsy examinations of the organs of animals treated with various doses of the extract did not show any changes in color and texture of all organs compared with the control group.

Histopathological examination of liver, kidneys, heart, spleen and stomach showed a disruption in tissue architecture of the treated animal, including the control group. Necrosis confirmed when dead cells or tissue are observed in histological lesion. On microscopic examinations, necrotic cells show various morphological appearance such as cytoplasmic swelling and karyolys is or pycnosis for oncosis necrosis, whereas cytoplasmic shrinkage and karyorrhex is for apoptotic necrosis[12].

Vacuolization in form of hydropic and fatty degeneration was sub lethal manifestation of cell damage, but there were reversible abnormalities[13], as well as the necrotic cells that remain viable for variable periods of time after injury, depending on the type of cell, tissue, or organ and depending on the type of injury[12]. This types of cell damage was common in cell with high rate of metabolism, such as in hepatocytes and proximal tubules cells[13]. The necropsy and histopathological examinations are paramount in linking the general and target organ specific toxic effects of phytomedicine.[19,21]

The findings showed significant acute toxic effects of ethyl acetate and *n*-hexane extracts of *C. macrophyllus* seeds on organ weight and histological appearance. Ethyl acetate extract was profoundly more toxic to the kidneys and stomach than the *n*-hexane extract while the *n*-hexane extract was more toxic to the heart and spleen of the tested animals than the ethyl acetate one.

Conclusion

The study concluded that ethyl acetate and n-hexane extract from *C. macrophyllus* seeds are safe to use are suitable to develop as antimalarial phyto-medicine. However, acute toxicity data are of limited clinical application since cumulative toxic effects do occur even at very low doses. Hence, multiple dose studies are usually helpful in evaluating the safety profile of phytomedicines. Sub-acute and chronic toxicity tests are recommended in order to determine the long-term effects of the extract.

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