



Preliminary studies on therapeutic effect of ethanolic extract of *Tylophora villosa* leaves against paracetamol-induced hepatotoxicity in mice

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ABSTRACT

This study intended to investigate the therapeutic effect of ethanolic extract of *Tylophora villosa* leaves (E2TL) against paracetamol (PC)-induced hepatotoxicity (PCIH) in mice (*Mus musculus*). PCIH were generated using daily 250 mg/kg body weight (bw) PC administration by gavage for seven days, and then daily 27.5; 55.0; 82.5; 110.0; or 220.0 mg/kg bw E2TL were treated by gavage for seven or fourteen days. Meanwhile, the controls were given solvent only in the same manner. Mortality, blood glucose, and condition (color, weight, volume) of the livers were observed on day 15 (D15). Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were examined on D15, D22, and D30, and then malondialdehyde (MDA) was determined on D15. Results of this study revealed that on D15, the dosage of 110.0 mg/kg bw E2TL most effectively decreased MDA due to PCIH, from $6.78 \pm 1.70 \mu\text{mol/L}$ to $3.45 \pm 0.43 \mu\text{mol/L}$, approaching the control condition ($2.45 \pm 0.05 \mu\text{mol/L}$). PC administration was really toxic dosage and caused 13.3 % mortality. Blood glucose, weight, and volume of the liver decreased as the effect of PC administration, and then 220.0 mg/kg bw E2TL treatment could recover the condition as well as the controls. Color of the liver indicated a similar recovery by E2TL treatment. SGPT and SGOT increased significantly by PC administration, and this PCIH facts could be recovered gradually near the controls according to the dosages (55.0; 110.0; or 220.0 mg/kg bw) and duration (seven or fourteen days) of E2TL treatment. It could be concluded that E2TL showed therapeutic effect against PCIH in *M. musculus*.

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1. Introduction

Hepatotoxicity is a prevalent problem in Sumatra, Indonesia. Drug-induced hepatotoxicity (DIH) is a complication that occurs in the human liver which impacts the function of the liver caused by drugs. It is very likely to occur in the liver because the organ is the central of drugs metabolism. The disease is related to the way the toxin of a drug reacts to the body so that DIH is the main reason of drug withdrawal from markets.¹ The cause of DIH is non-steroidal

anti-inflammatory drugs (NSAIDs), such as paracetamol (PC). PC is a commonly used as analgesic and antipyretic drug.² It is safe at therapeutic levels, but at high doses it can lead to undesirable side effects such as PC-induced hepatotoxicity (PCIH³);). In PCIH the amount of reactive oxygen species (ROS) will increase in mitochondria and impact the emergence of oxidative stress (OS). OS causes damage to cell membranes by morphological and biochemical changes, followed by the malfunctioning cells and it is ended by hepatocyte death that leads to apoptosis and necrosis of

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liver cells. Morphological changes that is preceded by the OS induces lipid peroxidation and malondialdehyde (MDA) as the final product.^{4,5} The presence of hepatocellular injury can also be detected by measuring the level of two hepatic enzymes, namely serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT).^{6,7}

Developing new drugs requires concept and methodology of natural products and traditional medicine through study of relationship between natural products, traditional medicines, and modern medicines.⁸ The use of herbal medicines for treating various diseases, including liver disease, is currently very popular.⁹ A number of reports are available which have shown hepatoprotective effects of natural plant products against toxic substances including PC.^{10,11} Natural antioxidant products are increasingly being used to treat various pathological liver injuries

because of the role of oxidative stress in their pathogenesis. Aqueous extract of *Auricularia polytricha* (AEAP), for example, has been used as food or medicine due to its antioxidant activity. PC increased significantly the SGPT and SGOT; furthermore, this PC-induced hepatotoxicity could be attenuated significantly by AEAP administration. These facts indicate that the AEAP has significant protective effect against PCIH due to its potent antioxidant activity.¹² It was reported that flavonoids which play a role as antioxidants in biological systems are natural phenolic compounds present in fruit and vegetable species.¹³ The Flavonoids have many useful effects that include their being antioxidants and having antibacterial, anti-cancer, anti-mutagenic, and anti-inflammatory properties.^{14,15}

Residents of Lubuklinggau, South Sumatra traditionally use Nangka Kuning (*Tylophora villosa* Blume; <http://www.theplantlist>.

Table 1
Experimental design to investigate therapeutic effect of ethanolic extract of *Tylophoravillosa* leaves (E2TL) against PC-induced hepatotoxicity (PCIH) of mice (*Mus musculus*). Both control and treated animals obtained *ad libitum* standard food and drink.

Stage of research	Group of experimental animals	N	Schedule research activity, on day (D)						
			D1-7 (T)	D8-14 (T)	D15 (O)	D15-21 (T)	D22 (O)	D22-29 (T)	D30 (O)
General condition A	A0: Control, normal.	15	Solvent	Solvent	Mortality. Blood glucose. Colour, weight, volume of the liver.				
	A1: 250 mg/kg bw PC.	15	PC [G]	Solvent	Mortality. Blood glucose. Colour, weight, volume of the liver.				
	A2: 250 mg/kg bw PC, 220 mg/kg bwE2TL	15	PC [G]	E2TL [G]	Mortality. Blood glucose. Colour, weight, volume of the liver.				
Serum parameters B	B0: Control, Normal	1	Solvent						
	B1: 250 mg/kg bw PC	1	PC [G]						
	B2: 250 mg/kg bw PC, 55.0 mg/kg bwE2TL	9 (9)	PC [G]	E2TL [G]	SGPT & SGOT (3)	E2TL [G]	SGPT & SGOT (3)	E2TL [G]	SGPT & SGOT (3)
	B3: 250 mg/kg bw PC, 110.0 mg/kg bwE2TL	9 (9)	PC [G]	E2TL [G]	SGPT & SGOT (3)	E2TL [G]	SGPT & SGOT (3)	E2TL [G]	SGPT & SGOT (3)
	B4: 250 mg/kg bw PC, E2TL 220.0 mg/kg bw	9 (9)	PC [G]	E2TL [G]	SGPT & SGOT (3)	E2TL [G]	SGPT & SGOT (3)	E2TL [G]	SGPT & SGOT (3)
Lipid peroxidation C	C0: Control, normal	4	Solvent	Solvent	MDA				
	C1: 250 mg/kg bwPC	4	PC [G]	Solvent	MDA				
	C2: 250 mg/kg bbPC, 27.5 mg/kg bwE2TL	4	PC [G]	E2TL [G]	MDA				
	C3: 250 mg/kg bbPC, 55.0 mg/kg bwE2TL	4	PC [G]	E2TL [G]	MDA				
	C4: 250 mg/kg bbPC, 82.5 mg/kg bwE2TL	4	PC [G]	E2TL [G]	MDA				
	C5: 250 mg/kg bbPC, 110.0 mg/kg bwE2TL	4	PC [G]	E2TL [G]	MDA				
The total number of experimental animals		98							

Note: bw=body weight; PC=paracetamol; E2TL=ethanolic extract of *Tylophora villosa* leaves; SGPT=serum glutamate pyruvate transaminase; SGOT=serum glutamate oxaloacetate transaminase; MDA=malondialdehyde; T=treatment; O=observation [G]=administrated by gavage

org/tpl/record/tro-2602757) as drug to treat diseases associated with impaired function of the liver (<http://mogasehat.blogspot.co.id/2013/12/drug-natural-liver-disease-or-hepatitis.html>). Since it has been widely accepted that *T. villosa* is an ethnomedicinal plant, traditionally known for curing several ailments such as liver diseases, the plant is not only taken from nature, but it has also been planted in the garden as an ornamental plant. This fact is an interesting challenge for researchers to immediately start collecting scientific data on this candidate medicinal plant. It was reported that leaves of *T. villosa* contain alkaloids, flavonoids, and triterpenoids and this is the reason why this plant can be used as an ethnomedicinal plant. Meanwhile, identification results using spectroscopy and ¹H-NMR spectroscopy indicated that ethanolic extract of *T. villosa* leaves (E2TL) contains the compounds of tylophorine (C₂₄H₂₇NO₄). Furthermore results of toxicity tests using *Artemia salina* Leach by the same researchers showed that E2TL has a LC₅₀ at 124 ppm.¹⁶ There are still some things that should be examined from E2TL. Based on these considerations we believe that preliminary studies on therapeutic effect of E2TL against PCIH in mice (*Mus musculus*) should be performed.

2. Materials and methods

2.1. Preparation of crude extract

Leaf sample of *T. villosa* (<http://www.theplantlist.org/tpl/record/tro-2602757>; plant identification was verified with the help of Research of Biology, Indonesian Institute of Sciences; <http://lipi.go.id/>) as research plant materials were collected from the surroundings of Lubuklinggau, South Sumatra. Selected leaves located at the base of the stem were then washed and cut into small pieces. *T. villosa* fresh leaves were wind-dried for 7 days to get 250 g of dried leaves which then were crushed into fine powder. The leaf powder was macerated in 200 mL ethanol (80%) for 24 h at room temperature, and the resulting filtrate was concentrated by rotary evaporator¹⁷ so that the concentrated extract, the ethanolic extract of *T. villosa* leaves (E2TL), was ready to be used as test material of this research.¹⁸

2.2. Phytochemical tests

Phytochemical tests were conducted on E2TL. The crude extract samples were tested to determine their contents of terpenoids, flavonoids, steroids, alkaloids, saponins, and tannins using the standard procedures.¹⁹

2.3. Tested chemicals

Paracetamol (PC) in the form of Paracetamol Afi Farma 500 mg Tablet was purchased from PT AFI FARMA (<https://www.goapotik.com/paracetamol-afi-farma-500-mg-tablet.html>), Kediri, Indonesia.

Table 2

Results of different phytochemicals present in ethanolic extract of *Tylophora villosa* leaves (E2TL).

No.	Phytochemical test	Results
1	Alkaloids	Positive (+)
2	Flavonoids	Positive (+)
3	Saponins	Negative (-)
4	Triterpenoids	Positive (+)
5	Steroids	Negative (-)

2.4. Dosages of investigation

It has been reported that PC lethal dosage in mice is 400 mg/kg body weight (bw; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4917664/>), and PC toxicity were generated in this research using daily 250 mg/kg bw PC administration by gavage for seven days. *T. villosa* leaf is traditionally used as a herbal medicine for liver dysfunction at a dosage of 15 g per day for an adult weighing 50 kg,²⁰ so an adult weighing 70 kg is recommended to consume 21 g. Since the conservation factor from 70 kg human to 20 g mice is 0.0026,²¹ the dosage for mice 0.0026 × 21 g is 55 mg. Based on the calculations, this study used five doses, namely 27.5; 55.0; 82.5; 110.0; or 220.0 mg/kg bw E2TL were treated by gavage for seven or fourteen days.

2.5. Experimental animals

Swiss Webster mice (*Mus musculus*) were used as experimental animals with regard to local regulations established by the Institutional Animal Care and Use Committee (IACUC) of Bengkulu University (Komisi Bioetika Universitas Bengkulu). This study paid attention to the ethical use of animals including aspects of the humane treatment of animals, in accordance with the principle of 5F (Freedom), namely; (a) free from hunger and thirst, (b) free from discomfort, (c) free from pain, injury and disease, (d) free from fear and the long-term stress, (e) freely express behavior naturally, given the space and appropriate facilities.²² The animals were reared in a room at 23–27 °C, and 83 % humidity. Both food and water were given *ad libitum*.^{23,24} Total number of male *M. musculus* aged 6–8 weeks with 25–35 g body weight (bw¹⁸;) used in PC detoxification on the liver of *M. musculus* by E2TL administration was 98 male animals.²⁵ The male animals were used in this research to avoid individual variation derived from *Mouse Estrous Cycle*. This research was conducted in three stages of research (A, B and C; Table 1).

Table 3

Mortality of *M. musculus* before day 15 (D15), which previously, on D1-7, received daily 250 mg/kg bw PC (A1), on D1-7 received daily 250 mg/kg bw PC and on D8-14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0).

Group of experimental animals	N (%)	Mortality of <i>M. musculus</i> before D15	
		Mortality (%)	Life (%)
A0: Control, normal.	15 (100)	0 (0.00)	15 (100)
A1: 250 mg/kg bw PC.	15 (100)	2 (13.33)	13 (86.67)
A2: 250 mg/kg bw PC, 220 mg/kg bw E2TL.	15 (100)	1 (6.67)	14 (93.33)

Note: PC=Paracetamo; E2TL = ethanolic extract of *Tylophora villosa* leaves; $\chi^2 = 2.14$; There are significant differences in mortality on A0, A1, and A2 ($p < 0.05$).

Table 4

Body weight of *M. musculus* on day 15 (D15), which previously, on D1-7, received daily 250 mg/kg bw PC (A1), on D1-7 received daily 250 mg/kg bw PC and on D8-14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0).

Group of experimental animals	N	Body weigh ± SD (g) on D15
A0: Control, normal.	15	30.00 ± 2.30 ^a
A1: 250 mg/kg bw PC.	13	25.00 ± 1.36 ^b
A2: 250 mg/kg bw PC, 220 mg/kg bw E2TL.	14	31.93 ± 2.29 ^a

Note: PC=Paracetamo; E2TL = ethanolic extract of *Tylophora villosa* leaves; Rates followed by the same letters (^a, ^b) are not significantly different data addressing the same column ($p < 0.05$).

2.6. General condition (A)

The forty-five experimental animals were divided into three groups (A0, A1, and A2), each consisting of fifteen male *M. musculus*. On day 1–7 (D1–7), 250 mg/kg bw PC was administered daily by gavage on A1 group. On D1–7, 250 mg/kg bw PC was administered daily by gavage, and then on D 8–14 220 mg/kg bw E2TL was given daily by gavage in A2 group. A0 group as the controls of investigation were administered solvent only in the same manner. Mortality of *M. musculus* was observed before D15, blood samples were collected on D15 from the tails for blood glucose determination using the Glucose test (multiCarein; http://www.biosys.it/en/pr_multicarein.html), and then both control and treated animals were sacrificed by cervical dislocation (CD) on D15 (Table 1). The animals were dissected immediately, and then their liver was separated from other organs to determine its color, weight, and volume.

2.7. Serum parameters (B)

The twenty-nine experimental animals were divided into five groups (B0, B1, B2, B3, and B4), B0 and B1, each consisting of one, while B2, B3, and B4, each consisting of nine male *M. musculus*. On D1–7, 250 mg/kg bw PC was administered daily by gavage in B1 group. On D1–7, 250 mg/kg bw PC was administered daily by gavage, and then on D 8–29, 55.0 mg/kg bw E2TL was given daily by gavage on B2 group. On D1–7, 250 mg/kg bw PC was administered daily by gavage, and then On D 8–29, 110.0 mg/kg bw E2TL was given daily by gavage in B3 group. On D1–7, 250 mg/kg bw PC was administered daily by gavage, and then On D8–29, 220.0 mg/kg bw E2TL was given daily by gavage in B4 group. B0 group as the controls of investigation were administered solvent only in the same manner. *M. musculus* were sacrificed by CD on D15, D22, or D30 (Table 1). The animals were dissected immediately, and blood

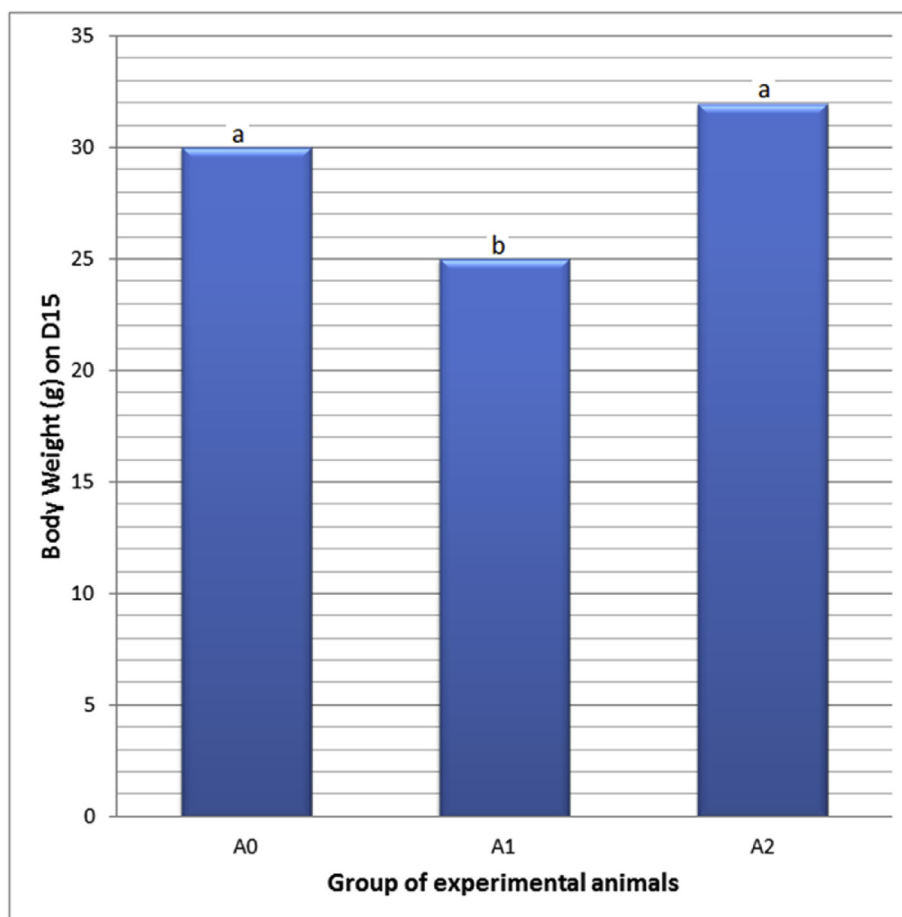


Fig. 1. Body weight of *M. musculus* on day 15 (D15), which previously, on D1–7, received daily 250 mg/kg bw PC (A1), on D1–7 received daily 250 mg/kg bw PC and on D 8–14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0). Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column ($p < 0.05$).

Table 5

Blood glucose levels of *M. musculus* on day 15 (D15), which previously, on D1–7, received daily 250 mg/kg bw PC (A1), on D1–7 received daily 250 mg/kg bw PC and on D 8–14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0).

Group of experimental animals	N	Blood glucosa $\pm$ SD (mg/dL) on D15
A0: Control, normal	15	112.66 $\pm$ 0.58 ^a
A1: 250 mg/kg bw PC	13	69.26 $\pm$ 3.30 ^b
A2: 250 mg/kg bw PC, 220 mg/kg bw E2TL	14	136.20 $\pm$ 6.30 ^c

Note: PC=Paracetamo; E2TL = ethanolic extract of *Tylophora villosa* leaves; $\alpha = 0.05$ Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column ($p < 0.05$).

samples were collected from the hearts. Briefly, the blood was allowed to stand for sometime followed by centrifugation at 2400 rpm for 20 min. Clear supernatant so obtained was designated as serum. Various serum parameters, namely serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT), were measured on Autoanalyzer (Erba Mannheim XL-640) using kits (Erba Mannheim XL System Packs²⁶).

2.8. Lipid peroxidation (C)

The twenty-four experimental animals were divided into six groups (C0, C1, C2, C3, C4, and C5), each consisting of four males *M. musculus*. On D1-7, 250 mg/kg bw PC was administered daily by gavage in C1 group. On D1-7, 250 mg/kg bw PC was administered daily by gavage, and then on D 8-14 27.5 mg/kg bw E2TL was given

daily by gavage in C2 group. On D1-7, 250 mg/kg bw PC was administered daily by gavage, and then on D 8-14, 55.0 mg/kg bw E2TL was given daily by gavage in C3 group. On D1-7, 250 mg/kg bw PC was administered daily by gavage, and then on D 8-14, 82.5 mg/kg bw E2TL was given daily by gavage in C4 group. On D1-7, 250 mg/kg bw PC was administered daily by gavage, and then on D 8-14, 110.0 mg/kg bw E2TL was given daily by gavage in C5 group. C0 group as the controls of investigation were administered solvent only in the same manner. *M. musculus* were sacrificed by CD on D15 (Table 1). The animals were dissected immediately, and then their liver was separated from other organs to determine the MDA. The generation of MDA was determined as the indicator of lipid peroxidation using a Bioxytech MDA-586 Kit (Oxis Research, Portland, OR, USA). Briefly, the supernatants of liver homogenate were mixed with probucol, N-methyl-2-phenylindole, and hydrochloric acid to

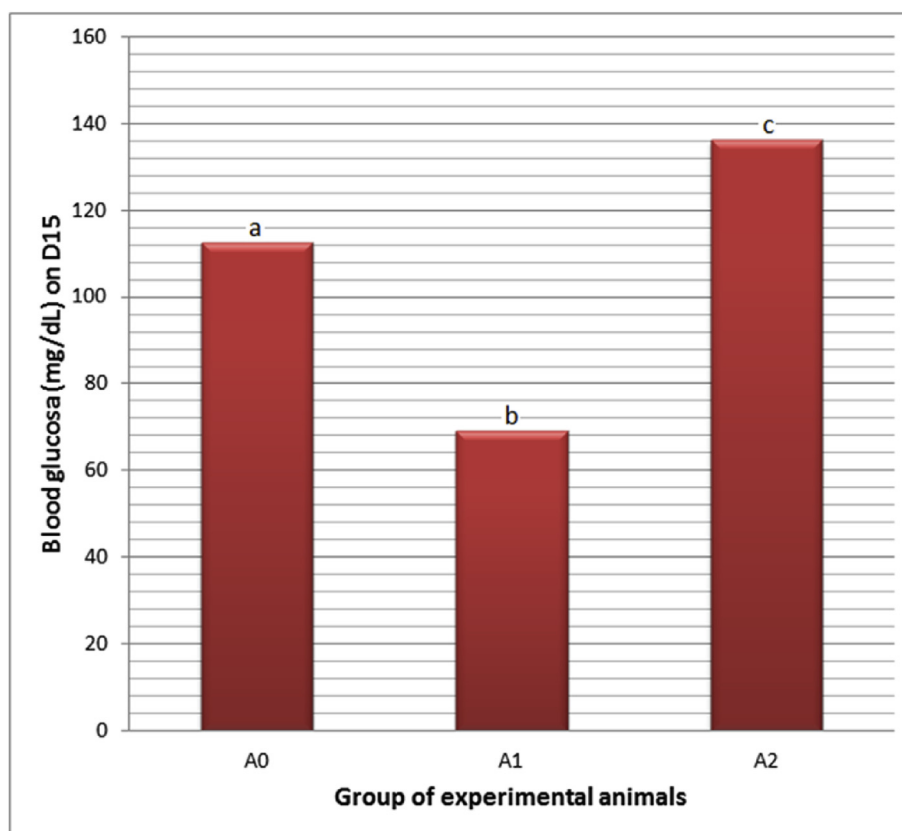


Fig. 2. Blood glucose levels of *M. musculus* on day 15 (D15), which previously, on D1-7, received daily 250 mg/kg bw PC (A1), on D1-7 received daily 250 mg/kg bw PC and on D 8-14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0). Rates followed by the same letters (^a, ^b) are not significantly different data addressing the same column ($p < 0.05$).

Table 6

Colour of the *M. musculus* liver on day 15 (D15), which previously, on D1-7, received daily 250 mg/kg bw PC (A1), on D1-7 received daily 250 mg/kg bw PC and on D 8-14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0).

Group of experimental animals	N (%)	Colour of the liver of <i>M. musculus</i> (%) on D15			
		Brown red	Ruby red	Oxide red	Coral red
A0: Control, normal.	15 (100)	8 (53.33)	5 (33.33)	2 (13.34)	0 (0)
A1: 250 mg/kg bw PC.	13 (100)	5 (38.46)	0 (0)	0 (0)	8 (61.54)
A2: 250 mg/kg bw PC, 220 mg/kg bw E2TL	14 (100)	7 (50.0)	1 (7.14)	0 (0)	6 (42.86)

Note: PC=Paracetamo; E2TL = ethanolic extract of *Tylophora villosa* leaves. $\chi^2 = 18.65$; There are significant differences in color of the liver on A0, A1, and A2 ($p < 0.05$).

produce a stable dye. After centrifugation, the absorbance of the clear supernatant was measured using a spectrometry at 586 nm. The concentration of MDA was plotted against a standard curve and expressed in n mole per milligram of wet tissue.²⁷

3. Results

3.1. Phytochemicals

Phytochemical test results in this study showed that E2TL contained alkaloids, flavonoids, and triterpenoids (Table 2), and the result was the same as it had been done before.¹⁶ These reproducible results were found because the E2TL were produced through the same protocol.

3.2. The liver of *M. musculus*

A condition of PC toxicity was generated by 250 mg/kg bw PC daily administration for seven days (D1-7), and the administration caused the death of two mice (13.33%). Meanwhile, the same PC administration which was followed by 220 mg/kg bw E2TL daily treatment during seven days (D8-14) could reduce mortality into

one mouse (6.67%; Table 3). The facts showed that the PC administration stimulated the condition of PC toxicity in mice, and then level of the toxicity could be decreased by E2TL treatment. In general it revealed that E2TL indicates potential therapeutic effect against PC toxicity. The PC administration decreased body weight (25.00 ± 1.36 g), lower than the controls' (30.00 ± 2.30 g). Meanwhile, when the PC administration was followed by treatments with E2TL for seven days, the body weight increased again (32.00 ± 2.29 g), approaching the control's (Table 4). This fact is further evidence that E2TL is able to restore weight loss due to the PC administration towards the normal conditions (Fig. 1).

The PC administration reduced blood glucose (70.00 ± 3.30 mg/dL), lower than the controls' (120.00 ± 0.58 mg/dL). Meanwhile, when the PC administration was followed by E2TL treatments for seven days, they raised blood glucose (130.00 ± 6.30 mg/dL), close to control (Table 5). These facts revealed that E2TL was able to recover a decrease in blood glucose due to PC administration to the normal circumstances (Fig. 2).

The PC administration also changed a percentage of brown red heart (5; 38.46%; A1), which was lower than the control's (8; 53.33%; A0). Meanwhile, when the PC administration was followed by E2TL treatments for seven days, they could increase the

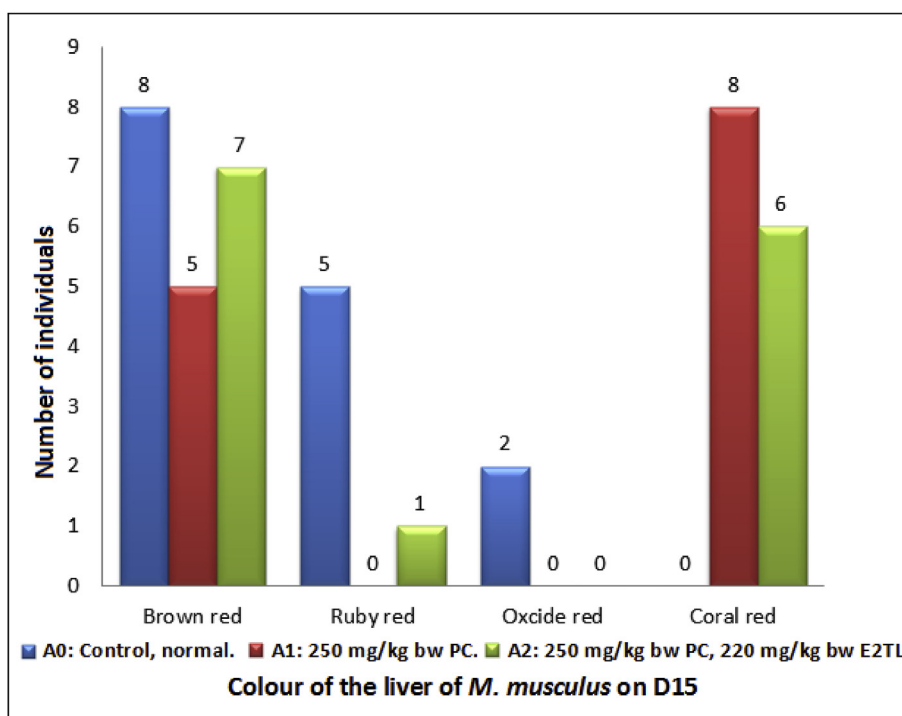


Fig. 3. Colour of the *M. musculus* liver on day 15 (D15), which earlier on D1-7 received daily 250 mg/kg bw PC (A1), on D1-7 received daily 250 mg/kg bw PC and on D 8-14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0). $\chi^2 = 18.65$; There are significant differences in color of the liver on A0, A1, and A2 ($p < 0.05$).

Table 7

Weight and volume of the liver of *M. musculus* on day 15 (D15), which previously, on D1-7, received daily 250 mg/kg bw PC (A1), on D1-7 received daily 250 mg/kg bw PC and on D 8-14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0).

Group of experimental animals	N	The liver of <i>M. musculus</i>	
		Weight $\pm$ SD (g)	Volume $\pm$ SD (mL)
A0: Control, normal.	15	1.86 ± 0.33^a	1.85 ± 0.36^a
A1: 250 mg/kg bw PC.	13	1.78 ± 0.30^b	1.60 ± 0.29^b
A2: 250 mg/kg bw PC, 220 mg/kg bw E2TL.	14	1.96 ± 0.29^c	1.87 ± 0.26^a

Note: PC=Paracetamo; E2TL = ethanolic extract of *Tylophora villosa* leaves. Rates followed by the same letters (^a, ^b) are not significantly different data addressing the same column ($p > 0.05$).

percentage of brown red heart (7; 50.0%; A2), approaching the control condition (Table 6). These facts showed that E2TL was able to recover the percentage of brown red heart as additional information due to PCIH to the normal circumstances (Fig. 3).

This research revealed that the PC administration decreased weight and volume of the liver (1.78 ± 0.30 g and 1.60 ± 0.29 mL, compared the control which was 1.86 ± 0.33 g and 1.85 ± 0.36 mL). Meanwhile, when the PC administration was followed by E2TL treatment for seven days, the weight and volume of the liver increased (1.96 ± 0.29 g and 1.87 ± 0.26 mL), approaching the control condition (Table 7). These facts indicated that E2TL was able to recover the weight and volume of liver in PCIH to the normal circumstances (Figs. 4 and 5).

3.3. SGPT and SGOT

Serum parameter observation revealed the daily 250 mg/kg bw PC administration for seven day (D1-7) which was followed by daily 55.0 E2TL treatments (B2) for seven (D8-14), fourteen (D8-21), twenty-one (D8-29) days decreased SGPT levels to 294.88 ± 13.91 (D15), 225.06 ± 2.73 (D22), 163.77 ± 2.81 (D30) U/L respectively, lower than 318.25 U/L (B1; Positive control). Furthermore, the decline of SGPT levels also occurred in line with two dosages (110; 220 mg/kg bw) E2TL treatment and three time periods (D8-14; D8-21; D8-29) treatment. The highest dosage (220 mg/kg bw) and the longest period of time (D8-29) of E2TL treatment revealed the lowest levels of SGPT, 89.94 ± 1.70 U/L, approaching the value of

60.00 U/L (B0; Control; Table 8). This SGPT observation revealed that E2TL had therapeutic effect against PCIH in retaining SGPT level of *M. musculus* (Fig. 6).

Other serum parameter observation revealed that the daily 250 mg/kg bw PC administration for (D1-7) which was followed by daily 55.0 E2TL treatments (B2) for seven (D8-14), fourteen (D8-21), twenty-one (D8-29) days decreased SGOT levels to 498.75 ± 4.68 (D15), 395.34 ± 4.33 (D22), 231.82 ± 7.80 (D30) U/L respectively lower than 510.15 U/L (B1; Positive control). Furthermore, the decline of SGOT levels also occurred in line with two dosages (110; 220 mg/kg bw) E2TL treatment and three time periods (D8-14; D8-21; D8-29) treatment. In the highest dosage (220 mg/kg bw) and the longest period time (D8-29) of E2TL treatment revealed the lowest levels of SGOT, 135.03 ± 2.00 U/L, approaching the value of 100.00 U/L (B0; Control; Table 9). This SGOT observation revealed that E2TL had therapeutic effect against PCIH in recovering SGOT level of *M. musculus* (Fig. 7).

3.4. MDA

A level of MDA in the liver of mice treated daily by 250 mg/kg bw PC for seven days (6.78 ± 1.70 mol/L; C1) was significantly higher than the controls (2.45 ± 0.05 mol/L; C0), however when PC toxicity conditions was given daily therapy at 27.5 mg/kg bw E2TL for seven days the levels of MDA slightly decreased (5.53 ± 0.22 μ mol/L; C2). Therapy with daily dosage of 55.0, 82.5, and 110.0 mg/kg bw E2TL for seven days into the toxicity PC condition (6.78 ± 1.70 μ mol/L;

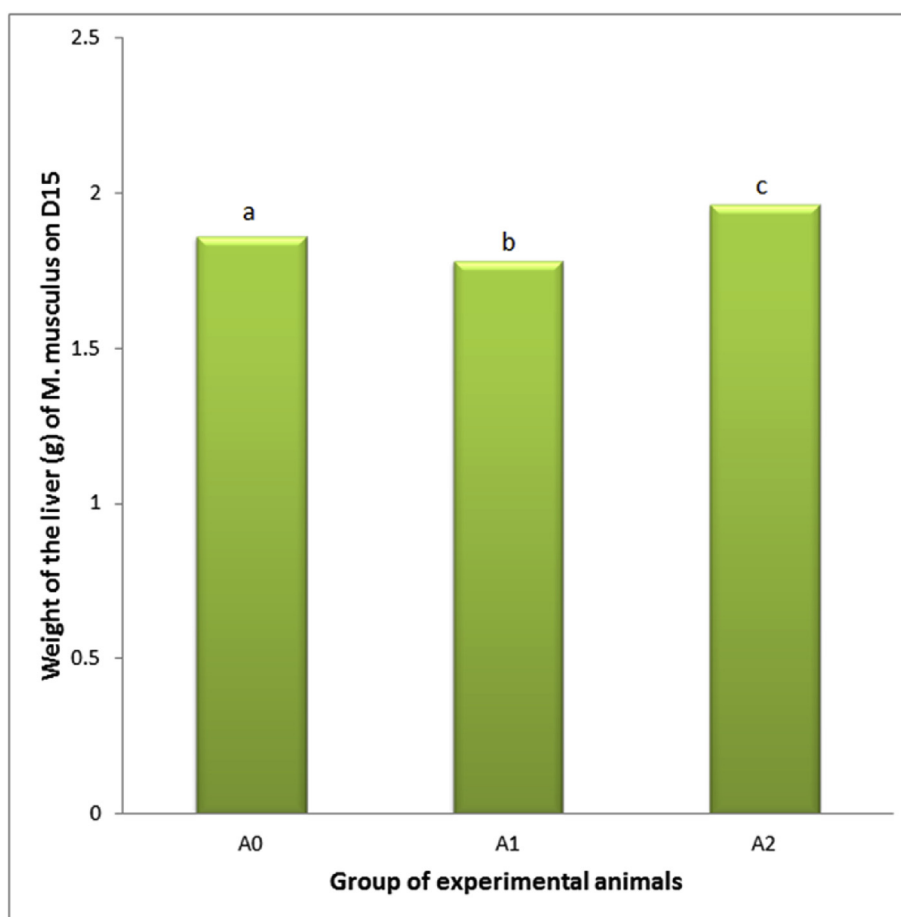


Fig. 4. Weight of the liver of *M. musculus* on day 15 (D15), which previously, on D1-7, received daily 250 mg/kg bw PC (A1), on D1-7 received daily 250 mg/kg bw PC and on D 8-14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0). Rates followed by the same letters (^a, ^b) are not significantly different data addressing the same column ($p < 0.05$).

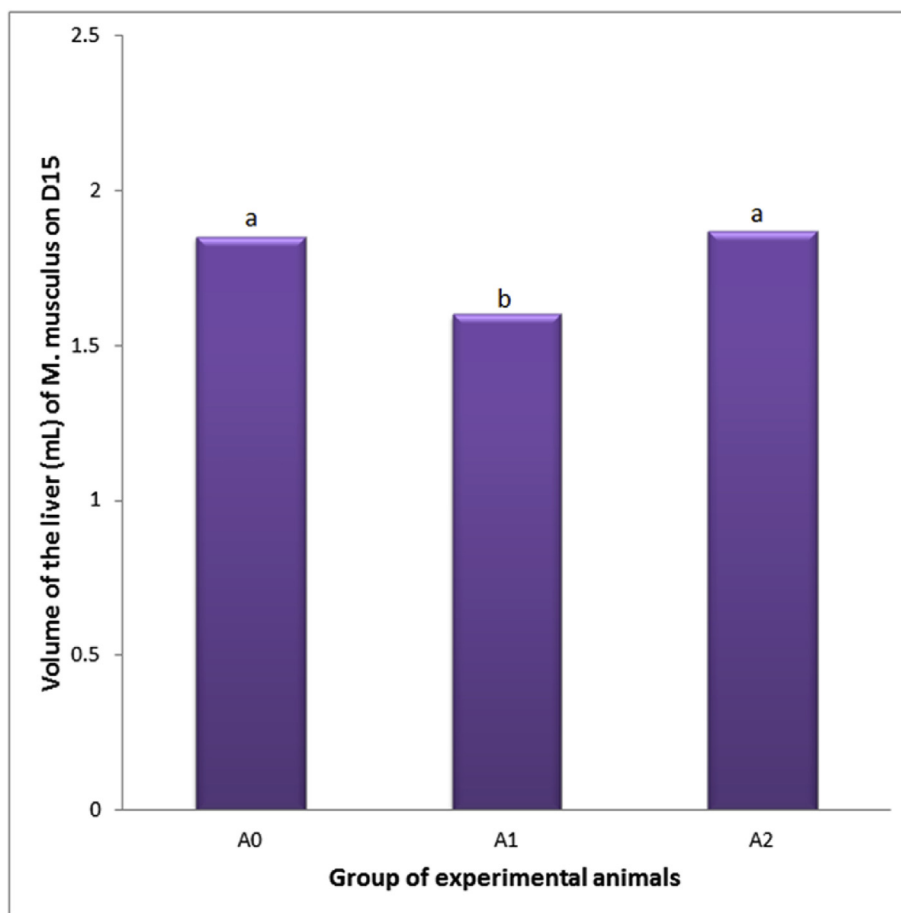


Fig. 5. Volume of the liver of *M. musculus* on day 15 (D15), which previously, on D1–7, received daily 250 mg/kg bw PC (A1), on D1–7 received daily 250 mg/kg bw PC and on D 8–14 were given daily 220 mg/kg bw E2TL (A2), while the controls received only the solvent in the same manner (A0). Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column ($p < 0.05$).

Table 8

SGPT levels of *M. musculus* on day 15, 22, and 30 (D15, D22, and D30) which previously, on D 1–7, received daily 250 mg/kg bw PC (B1), on D1–7 received daily 250 mg/kg bw PC and on D8–29 were given daily 55 (B2), 110 (B3), and 220 (B4) mg/kg bw E2TL respectively, while the controls received only the solvent in the same manner (B0).

Group of experimental animals	N	SGPT concentration in the blood of <i>M. musculus</i> (U/L), on		
		D15	D22	D30
B0: Control, normal.	1	60.00		
B1: 250 mg/kg bw PC.	1	318.25		
B2: 250 mg/kg bw PC, 55 mg/kg bw E2TL.	3	294.88 ± 13.91 ^a	225.06 ± 2.73 ^d	163.77 ± 2.81 ^g
B3: 250 mg/kg bw PC, 110.0 mg/kg bw E2TL.	3	230.73 ± 4.62 ^b	158.03 ± 4.75 ^e	119.66 ± 3.80 ^h
B4: 250 mg/kg PC, 220 mg/kg bw E2TL.	3	197.18 ± 5.73 ^c	100.03 ± 2.42 ^f	89.94 ± 1.70 ⁱ

Note: PC=Paracetamo; E2TL = ethanolic extract of *Tylophora villosa* leaves; SGPT = serum glutamate pyruvate transaminase; Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column and the same line ($p < 0.05$).

C1) appeared to reduce the level of MDA 4.82 ± 0.29 , 4.17 ± 0.70 , and 3.45 ± 0.43 $\mu\text{mol/L}$ respectively. Furthermore it revealed that therapy with the dosage of 110.0 mg/kg bw E2TL for seven days into the toxicity PC condition was able to recover the levels of MDA (4.17 ± 0.7 $\mu\text{mol/L}$; C4), similar to the control condition (2.45 ± 0.05 $\mu\text{mol/L}$; C0; Table 10). The facts showed that E2TL had therapeutic effect against PCIH by inhibition of lipid peroxidation.

4. Discussions

Lubuklinggau community use *T. villosa* as the traditional drug to treat diseases associated with impaired function of the liver. Some testimonies explained that *T. villosa* is the medicinal plant for

hepatitis, so this claim needs to be tested scientifically. Meanwhile, Lubuklinggau communities also commonly use PC for their health purposes. PCIH remains the key factor limiting the clinical application of PC, and herbs are the important sources for isolation of compounds preventing PCIH.²⁸ This study tried to gather data to verify whether this traditional practice can be classified into empirical and rational phenomena or just a psychological suggestion in the culture of society. PCIH in several group of experimental animals were treated by E2TL, and then this therapeutic phenomenon was analyzed starting from the levels of morphology to biochemical and enzymatic aspects as presented below.

M. musculus has a lethal dosage of PC of 400 mg/kg bw, while the sublethal dosage is 200 mg/kg bw.²⁹ At therapeutic dosages, PC will

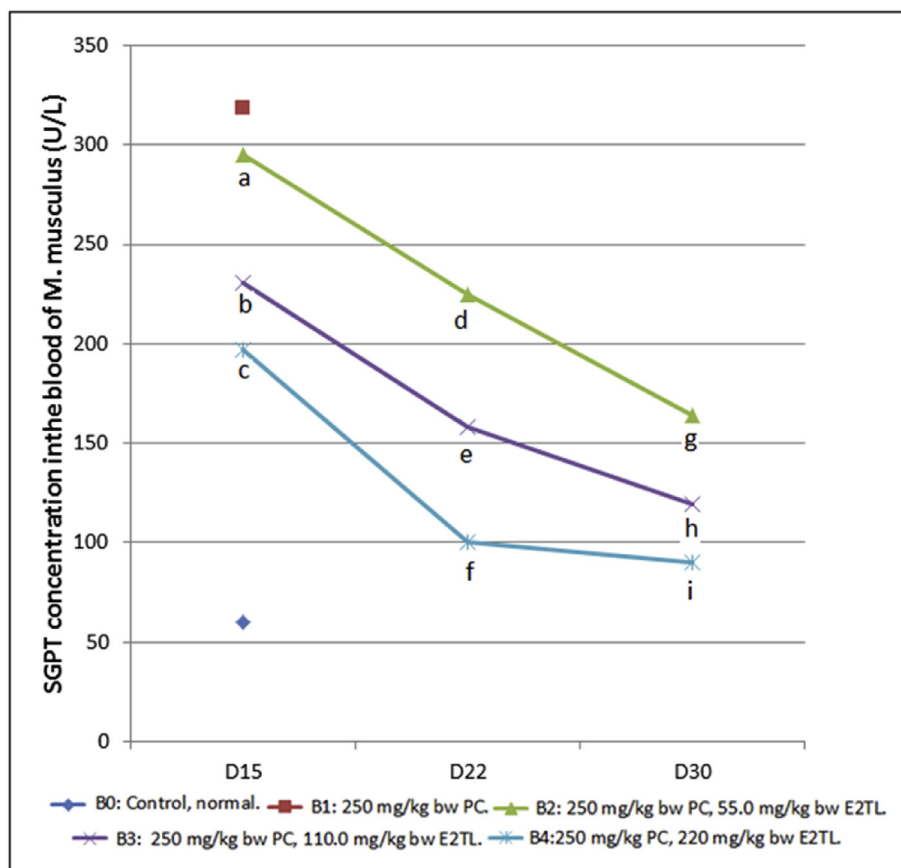


Fig. 6. SGPT levels of *M. musculus* on day 15, 22, and 30 (D15, D22, and D30) which previously, on D 1–7, received daily 250 mg/kg bw PC (B1), on D1–7 received daily 250 mg/kg bw PC and on D8–21 were given daily 55 (B2), 110 (B3), and 220 (B4) mg/kg bw E2TL respectively, while the controls received only the solvent in the same manner (B0). Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column and the same line ($p < 0.05$).

Table 9

SGOT levels of *M. musculus* on day 15, 22, and 30 (D15, D22, and D30) which previously, on D 1–7, received daily 250 mg/kg bw PC (B1), on D1–7 received daily 250 mg/kg bw PC and on D8–29 were given daily 55 (B2), 110 (B3), and 220 (B4) mg/kgbw E2TL respectively, while the controls received only the solvent in the same manner (B0).

Group of experimental animals	N	SGOT concentration in the blood of <i>M. musculus</i> (U/L), on		
		D15	D22	D30
B0: Control, normal.	1	100.00		
B1: 250 mg/kg bw PC	1	510.15		
B2: 250 mg/kg bw PC, 55 mg/kg bw E2TL	3	498.75 ± 4.68 ^a	395.34 ± 4.33 ^d	231.82 ± 7.80 ^g
B3: 250 mg/kg bw PC, 110 mg/kg bw E2TL	3	380.03 ± 5.42 ^b	289.34 ± 6.67 ^e	194.46 ± 0.55 ^h
B4: 250 mg/kg bw PC, 220 mg/kg bw E2TL	3	291.07 ± 6.28 ^c	190.03 ± 2.42 ^f	135.03 ± 2.00 ⁱ

Note: PC=Paracetamo; E2TL = ethanolic extract of *Tylophora villosa* leaves; SGOT = serum glutamate oxaloacetate transaminase. Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column and the same line ($p < 0.05$).

be eliminated mainly by conjugation with glucuronide or sulfate in hepatocytes. The remaining PC is metabolized by the cytochrome P450 system and converted into a highly toxic intermediate, N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is depleted by conjugation with glutathione (GSH) and the complex is excreted mainly into bile.³⁰ However at toxic dosage of PC, excessive NAPQI depletes the cellular storage of GSH and binds to cellular proteins covalently, resulting in mitochondrial dysfunction and DNA damage, which leads to hepatocyte necrosis and cell death.^{31,32} This study used the dosage of 250 mg/kg bw PC for seven days which caused two mortality (13.33%). This PC toxicity condition could be decreased by daily 220 mg/kg bw E2TL treatment for seven days which caused only one mortality (6.67%; Table 3). This fact indicated that E2TL was generally able to decrease the PC toxicity condition presumably because E2TL as a natural material is

overcast alkaloids (matrine³³), and flavonoids (quercetin³⁴; Table 2). It has been widely recognized that weight loss is usual in line with the decrease of blood glucose.³⁵ This phenomenon was similar with results of the study which indicated that in PC toxicity conditions caused weight reduction (Table 4) along with a decrease of blood glucose (Table 5). Both decrease of body weight (Fig. 1) and blood glucose (Fig. 2) as the effects of PC administration could be recovered by E2TL treatments as well as the control. Results of macroscopic observation of the liver of mice after treatment using PC for seven days revealed some changes in color (Table 5), weight (Table 6), and volume (Table 7) of the liver which were significantly different from those of the control. The occurrence of liver macroscopic changes was a clue for PCIH. The PCIH were subsequently turned out to be restored with daily 220 mg/kg bw E2TL treatment for seven days so that the color (Fig. 3), weight (Fig. 4),

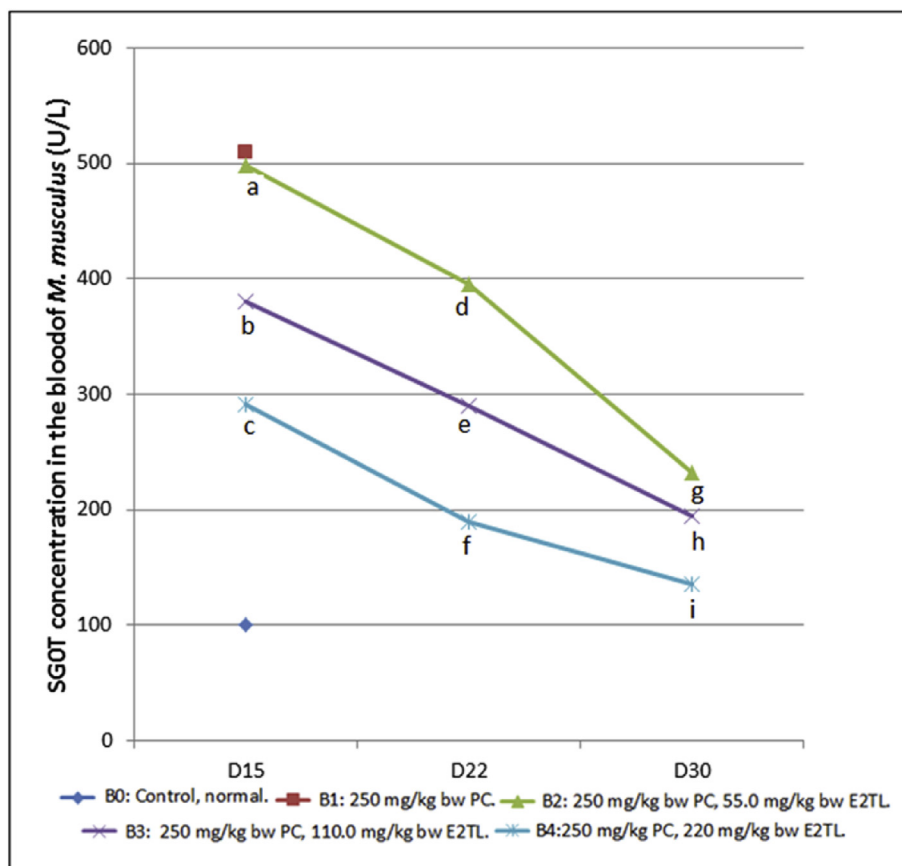


Fig. 7. SGOT levels of *M. musculus* on day 15, 22, and 30 (D15, D22, and D30) which previously, on D 1–7, received daily 250 mg/kg bw PC (B1), on D1–7 received daily 250 mg/kg bw PC and on D8–21 were given daily 55 (B2), 110 (B3), and 220 (B4) mg/kg bw E2TL respectively, while the controls received only the solvent in the same manner (B0). Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column and the same line ($p < 0.05$).

Table 10

MDA levels of *M. musculus* liver on day 15 (D15), which previously, on D1–7, received daily 250 mg/kg bw PC (C1), on D1–7 received daily 250 mg/kg bw PC, and on D8–14 were given daily 27.5 (C2), 55.0 (C3), 82.5 (C4), and 110 mg/kg bw E2TL respectively, while the controls only received the solvent in the same manner (C0).

Group of experimental animals	N	MDA concentration in the liver of <i>M. musculus</i> ($\mu\text{mol/L}$) on D15
C0: Control, normal.	4	2.45 ± 0.05^a
C1: 250 mg/kg bw PC.	4	6.78 ± 1.70^{bc}
C2: 250 mg/kg bw PC, 27.5 mg/kg bw E2TL.	4	5.53 ± 0.22^{cde}
C3: 250 mg/kg bw PC, 55.0 mg/kg bw E2TL.	4	4.82 ± 0.29^{df}
C4: 250 mg/kg bw PC, 82.5 mg/kg bw E2TL.	4	4.17 ± 0.70^{ade}
C5: 250 mg/kg bw PC, 110.0 mg/kg bw E2TL.	4	3.45 ± 0.43^{af}

Note: PC=Paracetamol; E2TL = ethanolic extract of *Tylophora villosa* leaves; MDA = malondialdehyde; Rates followed by the same letters (^{a, b}) are not significantly different data addressing the same column ($p < 0.05$).

and volume (Fig. 5) approached the control's. A similar phenomenon was shown by the study of the hepatoprotective activity of methanol extract of *M. malabathricum* leaves (MEMM) against PCIH.³⁶ E2TL and MEMM showed antioxidant activity due to both natural materials that contain flavonoids.

It is well known that the existence of hepatocellular injury can also be determined by measuring the level of two hepatic enzymes; SGPT and SGOT.^{6,7} This research indicated the same phenomenon of SGPT and SGOT. The daily 250 mg/kg bw PC administration for seven days (D1–7) which was followed by daily 55.0 E2TL treatments (B2) for seven (D8–14), fourteen (D8–21), twenty-one (D8–29) days decreased SGPT and SGOT levels to lower levels than the positive control (B1) respectively. Furthermore, the decline of SGPT and SGOT levels also occurred in line with two dosages (110; 220 mg/kg bw) E2TL treatment and three time periods (D8–14; D8–

21; D8–29) treatment. The highest dosage (220 mg/kg bw) and the longest period time (D8–29) of E2TL treatment resulted in the lowest levels of SGPT and SGOT, 89.94 ± 1.70 135.03 ± 2.00 U/L approaching the value of 60.00, 100.00 U/L (B0; control; Tables 8 and 9) respectively. These observations showed that E2TL had therapeutic effect against PCIH in recovering SGPT and SGOT level of *M. musculus* (Figs. 6 and 7). This therapeutic effects of E2TL was thought to have come from three phytochemical contents (alkaloids, flavonoids, and diterpenoids; Table 2). Some earlier reports stated that six derivatives of flavonoids such as baicalin,³⁷ luteolin,³⁸ galangin,³⁹ rutin, quercetin, hyperforin³⁴ are antioxidant which are able to recover back PCIH to normal conditions. This fact can be a preliminary indication that E2TL has the opportunity to be developed as a new herbal medicine.

The amount of ROS in mitochondria can be increased by

excessive PC administration, and impacts on the emergence of OS. Furthermore, OS causes cell membranes damage by morphological and biochemical changes followed by the malfunctioning cells and it is ended by hepatocyte death that leads to necrosis and apoptosis of liver cells. MDA is the final product of morphological changes that is preceded by the OS induces lipid peroxidation.^{4,5} This research revealed that the level of MDA in the liver of mice treated daily by 250 mg/kg bw PC for seven days (C1) was significantly higher than the controls' (C0). However, when PC toxicity conditions was given daily therapy 27.5 mg/kg bw E2TL for seven days the levels of MDA decreased slightly (C2). Therapy with daily dosage of 55.0, 82.5, and 110.0 mg/kg bw E2TL for seven days into the PCIH (C1) appeared to reduce the level of MDA. Furthermore, it revealed that therapy with the dosage of 110.0 mg/kg bw E2TL for seven days into the PCIH was able to recover the levels of MDA (C4), similar to the control condition (C0; Table 10). The facts showed that E2TL had therapeutic in PCIH by inhibition of lipid peroxidation. This therapeutic effect of E2TL was thought to have come from three phytochemical contents (alkaloids, flavonoids, and triterpenoids; Table 2). Some earlier reports stated that two derivatives of flavonoids such as genistein⁴⁰ and naringenin⁴¹ are antioxidant which were able to recover back PCIH to normal conditions. These facts can be additional preliminary indication that E2TL has the opportunity to be developed as the new herbal medicine.

5. Conclusion

The present experimental findings revealed that phytoconstituents of E2TL had therapeutic effect against PCIH of *M. musculus* by inhibition of radicals and lipid peroxidation.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Authors' contributions

AR conceived of the study, and participated in its design and coordination and helped to draft the manuscript. **BDS** participated in the preparing of experimental animals and serum parameters analysis. **EM** and **ZF** participated in the study of PC detoxification on blood and liver. **FA** and **SRP** carried out determination of lipid peroxidation. **AS** carried out extract preparation and phytochemical test. All authors read and approved the final manuscript.

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