Comparison of Immunomodulatory Effects of Phyllanthus niruri L. and Toman Fish Albumin Formulations with Phyllanthus urinaria L. and Toman Fish Albumin through Anti-Hepatotoxic Cytokine Regulation Against Male Rattus norvegicus Experiencing CCl4 Toxicity

Tutut Puji Lestari, Mochamad Sasmito Djati, Muhaimin Rifa'i

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, 65145, Malang, Jawa Timur, Indonesia
Email: immunobiology@ub.ac.id

Abstract

Impaired liver function can be seen from increased levels (SGOT-SGPT) due to proinflammatory cytokines TNF-α, (iL-1) and (iL-6) which inhibit hormone production (PDGF) in the process of liver tissue regeneration. The formulation of Phyllanthus niruri L. and Phyllanthus urinaria L. combined with toman fish albumin is a combination that can work synergistically in suppressing the production of proinflammatory molecules, because the combination contains compounds that are antioxidants. A total of 24 male Rattus norvegicus were divided into four treatment groups, namely: negative control (healthy), CCl4-induced positive control, dose 1 formulation and dose 2 formulation. Male rattus norvegicus induced CCl4 of 3 ml/kg intraperitoneally, to describe impaired liver function. Male rattus norvegicus was given formula (Phyllanthus niruri L. with Toman fish albumin) and (Phyllanthus urinaria L. and Toman fish albumin) orally at a dose of 1 (620 mg/200 g body weight: 62 mg/200 g body weight), and dose 2 (1240 mg/200 g body weight: 124 mg/200 g body weight) for 14 days. SGOT-SGPT levels were analyzed with a flowcytometer on the first day after acclimatization and day 14, and analysis of proinflammatory cytokines (TNF-α + iL-1 + iL-6) was performed with flow cytometry. This study reported that the combination of Phyllanthus niruri L formula with toman fish albumin (PNA) and Phyllanthus urinaria L formula with toman fish albumin (PUA) was able to show striking hepatoprotective effects through a significant decrease in SGOT-SGPT levels in the blood of male Rattus norvegicus and decreased proinflammatory cytokine profiles in Rattus norvegicus liver cells males exposed to CCl4. In addition, the antioxidant defenses of PNA and PUA formulas almost completely prevent the occurrence of degeneration, necrosis, fibrosis and steatosis in liver cells. So that the combination of PNA and PUA formulas given has the potential to be used as an alternative to hepatitis drugs.

Keywords: g Phyllanthus niruri L., Phyllanthus urinaria L., Channa mikropeltes, Albumin, SGOT, SGPT, TNF-α, iL-1, iL-6.

The liver is an important organ that has a central role in human metabolic processes, such as in the process of protein synthesis, hormone production, and the process of detoxification of toxic substances from the bloodstream (Kodoli et al., 2021). However, various factors such as exposure to toxins, viral infections, alcoholism, and genetic disorders can cause impaired liver function. One common liver disease is hepatitis, which is usually caused by viral infections, especially hepatitis B. Many studies use male Rattus norvegicus as a disease model animal to illustrate the concept of impaired liver function and related processes, because these mice have physiological similarities with humans. Therefore, in describing liver damage such as patients with hepatitis B, this study used male Rattus norvegicus induced liver damage using the chemical carbon tetrachloride (CCl4).

Carbon tetrachloride is a toxic chemical compound and can cause serious liver damage if exposed for long periods of time (Sihaloho, 2022). This CCl4 display starts from the metabolic process in the liver by cytochrome P450 enzymes, especially P450 2E1 (CYP2E1). This process produces highly reactive trichloromethyl (CCl3) radicals. CCl3 radical compounds can combine with oxygen molecules (O2), and form trichloromethylperoxy radical compounds (CCl3OOH) (Mishra et al., 2020).

Trichloromethylperoxy is a highly reactive compound and can cause oxidative stress in cells (Mehendale, 2010). Trichloromethylperoxy compounds can induce oxidative stress by initiating lipid peroxidation on hepatocellular membranes. Lipid peroxidation produces free radicals and other oxidation products, which damage the structure of cell membranes. Lipid peroxidation and oxidative stress can disrupt calcium homeostasis in hepatocytes.

This disorder causes the release of Ca2+ by cellular organelles, especially by the endoplasmic reticulum (Mishra et al., 2020). The accumulation of free radicals and changes in calcium homeostasis can lead to necrosis (cell death) of hepatocytes. This necrosis occurs mainly in the perihepatic area. Then in response to hepatocyte necrosis, SGOT-SGPT previously present in liver cells will be released into the blood. So that the level of SGOT-SGPT in the blood of male Rattus norvegicus has increased. In addition, an increase in free radicals can also cause an inflammatory response in the liver by activating macrophages to produce tumor necrosis factor-α (TNF-α) and other proinflammatory cytokines (Irawati., 2018). Therefore, an increase in antioxidant signaling pathways is an important mechanism in liver protection during acute oxidative stress. It can be obtained using formulas (Phyllanthus niruri L. with Toman fish albumin) and (Phyllanthus urinaria L. with Toman fish albumin).

Phyllanthus niruri L. plants are known to contain flavonoid compounds that act as antioxidants and play a role in hepatoprotective activity (Sujono et al., 2015). Flavonoid compounds found in Phyllanthus niruri are types of niruriflavones (Risnawati et al., 2021). In addition, in other studies also showed that the plant Phyllanthus niruri contains other flavonoid compounds such as quercetin, kaempferol and Rutin (Mediani et al., 2017). Flavonoid compounds are known to have antioxidant and anti-inflammatory properties that can help protect liver health and increase body immunity (Risnawati et al., 2021). Flavonoid compounds can also help inhibit the activity of cyclooxygenase enzymes (COX), so as to reduce inflammation (Alyidrus et al., 2019). This property of cyclooxygenase (COX) enzymes plays an important role in the mechanism of liver damage when exposed CCl4.

Phyllanthus urinaria contains flavonoid compounds of quercetin and kaempferol types (Wu et al., 2013). These flavonoid compounds have the potential to provide health benefits, especially in protecting the liver and treating diseases such as hepatitis and liver damage (Du et al., 2018). In addition, Phyllanthus urinaria also contains other compounds such as gentisic

acid 4-O-b-d-glucopyranoside and syringin (Du et al., 2018). The flavonoid compounds present in Phyllanthus urinaria have been shown to have strong antioxidant activity, which can be beneficial in protecting the liver from damage and inflammation (Husnunnisa et al., 2022).

Toman fish (Channa micropeltes) is rich in albumin which is a high-quality protein (Firlianty, et.al 2013). Toman fish contains various proteins such as β-galactosidase, Glutamate dehydrogenase, Albumin, Carbonic anhydrase, Myoglobin, Lysozyme and Aprotinin which are essential for human nutrition (Nurilmala et al., 2021). Albumin in Toman fish has antioxidant properties that can help speed up the healing process. This can reduce the impact of oxidation caused by Reactive Oxygen Species (ROS) modulating inflammation, thereby promoting effective wound healing (Carabelly et al., 2019).

Based on this, we hypothesize that the PNA formula (Phyllanthus niruri L. with Toman fish albumin) and the PUA formula (Phyllanthus urinaria L. with Toman fish albumin) will be more effective in producing immunomodulatory effects in controlling inflammatory responses in impaired liver function, especially in regulating anti-inflammatory cytokines and SGOT-SGPT levels. In addition, the potential role of anti-hepatotoxic cytokines from Phyllanthus niruri L., Phyllanthus urinaria L. and toman fish albumin as prevention of the development of liver damage is still poorly studied. Therefore, this study aimed to evaluate the levels of SGOT-SGPT and proinflammatory cytokines (TNF-, IL-1 and IL-6) after administration of PNA and PUA in male Rattus norvegicus exposed to CCl4.

MATERIALS AND METHODS

Description of Experimental Animals and Sampling Techniques of Experimental Animals

The experimental animals used in this study were male mice (Rattus norvegicus) with genetic variations of the swiss Webster strain, aged 8-12 weeks and weighing 200 grams. Rats were obtained from Murine Farm, Singosari, Malang, East Java, Indonesia in healthy conditions. Rats are kept in chambers and acclimatized for 7 days with cycles (light/dark) for 12 hours, humidity ranging from 60% with a temperature of ± 22 oC and get food or drink ad libitum. The technique used for sampling is a complete random design. The rat population that meets the criteria will be taken as many as 24 heads, then randomly grouped into 4 treatment groups, so that each treatment group consists of 6 mice.

Preparation of Phyllanthus niruri L. Extract Formulation with Toman Fish Albumin and Phyllanthus urinaria L. with Toman Fish Albumin

Plants Phyllanthus niruri L. and Phyllanthus urinaria L. obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicine of Tawangmangu Central Java in the form of fresh plants. Plant extraction is carried out by weighing fresh plants, then washing, draining and drying with a 45oC oven for 48 hours. The dried sample is then powdered and sifted on a sieve with a sieve hole diameter of 1 mm. Phyllanthus niruri L. and Phyllanthus urinaria L. powders were each added with 70% ethanol, then stirred for 30 minutes and allowed to stand for 24 hours. After letting stand for 24 hours, the sample is filtered 3 times so that pulp and filtrate are obtained.

The filtrate is evaporated with a vacuum rotary evaporator and a waterbath heater with a temperature of 70oC so that thick extracts of Phyllanthus niruri L. and Phyllanthus urinaria L. The thick extract is poured into a porcelain dish, then heated with a waterbath heater while continuing to stir so that extracts of Phyllanthus niruri L. and Phyllanthus urinaria are obtainedL. Albumin is obtained from IFALMIN products produced by PT. Ismut Fitomedika, Makassar, Indonesia. IFALMIN is a product that contains extracts from Toman fish.

The extraction process of Toman fish is carried out by cleaning the body parts of the Toman fish and cutting off the fins and removing the scales. The gills and organs of the toman fish

are discarded for meat. The extraction of Toman fish meat was carried out at a temperature of 50 ° C using water as a solvent and the extract was dried using a freeze dryer. Phyllanthus plant extract will be combined with albumin with two different doses, which is then called Phyllanthus niruri L. with albumin (PNA) and Phyllanthus urinaria L. with albumin (PUA).

The dosage of plants Phyllanthus niruri L. and Phyllanthus urinaria L. is based on the reference Rachmawati et al. (2010) that before the extract is applied to experimental animals, it is necessary to pay attention to the conversion of plant doses to humans which ranges between 500 mg/kg body weight and 1000 mg/kg body weight, while the dose of albumin (INFALMIN) is 500 mg/capsule. Therefore this conversion will be applied according to the human dose into animal equivalent dose (AED) according to the food and drug administration (USFDA, 2005), resulting in a conversion dose for PNA and PUA formulations with the following modifications: dose 1 (620 mg/200 g body weight: 62 mg/200 g body weight), and dose 2 (1240 mg/200 g body weight: 124 mg/200 g body weight). The combination of the extract with albumin will later be stored at 4oC before use.

CCl4 Induction and Measurement of SGOT-SGPT Levels in Rat Blood

Induction to obtain a picture of liver damage in hepatitis in humans, using CCl4 solution treated in experimental animals male Rattus norvegicus wistar strain based on protocol (Rohmatin et al., 2015) with modifications to obtain mouse models of hepatitis. CCl4 induction was performed on male Rattus norvegicus wistar strain intraperitoneally, at a dose of 3 ml/kg/BB. CCl4 induction was carried out 2 x 1 week with a span of 2-3 days and was carried out for two weeks. Measurement of SGOT-SGPT levels was carried out on the first day after acclimatization of male Rattus norvegicus and the 14th day after CCl4 induction. Rat's blood serum collection was carried out in the orbital sinuses in the rat's eyes using capillary pipes and inserted into the EDTA tube.

Blood is transferred into a microtube and centrifuge at a speed of 3,000 rpm for 15 minutes, so that serum is obtained from the blood. The serum that has been obtained is taken using an ependrove pipette, then separated into another tube. Next, take blood serum of 100 µl and mix it with 100 µl of SGOT and SGPT reagents. Then perform the analysis in a flowcytometer at a wavelength of 560 nm and a temperature of 37oC.

Immunostaining and Flow Cytometry

Antibody staining was carried out based on the Rifa'i and Widodo (2014) protocol, starting with the rat stage in each treatment group dislocated neck and dissected using tweezers. The spleen and liver parts to be analyzed are separated into containers. Furthermore, the liver and spleen organs are crushed in a solution of 5 ml of PBS once. Then the grinding results are transferred into a 15 ml propylene tube. Then the suspension of liver and spleen cells was centrifuged at a speed of 2500 rpm, with a temperature of 10°C for 5 minutes. Thus producing supernatants (solution on top of pellets) and pellets (cell deposits).

Centrifuged pellets are resuspended with 1 ml of PBS and homogenized. Then, after homogeneity, the pellets are divided into microtubes as much as 50 µl. Then the extracellular antibody staining process is carried out on the surface cells first. Cells in the sample were incubated with 50 µl of specific extracellular antibodies, such as CD4 or CD8. Incubation is carried out for 20 minutes inside the icebox (4°C) to facilitate the binding of antibodies with cellular targets.

After incubation with extracellular antibodies, cells were added 50 µl of cytofix/cytoperm fixative solution and incubated for 20 minutes in an icebox. The purpose of this step is to remediate the cell so that intracellular antibodies can enter the cell. Then after the incubation process is completed, 300-500 µl of waspherm is added to clean the remnants of the

fixative solution and unbound antibodies. Next, the cell suspension is centrifuged again at a speed of 2500 rpm, at 10°C for 5 minutes to separate the cells and pellets. The pellets formed were then stained with 50 µl of intracellular antibodies and incubated for 20 minutes in an icebox. Cells stained with extracellular and intracellular antibodies were added with PBS of 300-500 µl and transferred to cuvettes for analysis by flow cytometry. The flow cytometry program is regulated as acquisition and gating is carried out according to the type of cell to be identified and analyzed, namely proinflammatory cytokines.

Histological Preparation Preparation

The stage of making histological preparations is made sequentially based on the protocol according to Kiernan (2008) for Hematoxylin-Eosin (HE) staining which includes sample collection by means of mice in each treatment dislocated aseptically and dissected using tweezers. Then the liver to be analyzed is separated into a container and tissue fixation process is carried out by soaking the liver in a 10% formalin neutral buffer (BNF) solution for 24 hours. After 24 hours, samples are taken and sliced (trimming) with a size of 1 x 5 cm. After that the sample is placed on a network cassette and labeled written in pencil bearing the specimen code.

Organs that have been included in the cassette tissue are then closed and placed into the tissue processing basket. Specimens that have been trimmed are then continued into the next process in the tissue processor. In a tissue processor, the specimen will be soaked first in formalin solution, then continued at graded alcohol (70% alcohol, 80% alcohol, 90% alcohol, and 96% alcohol), then soaked in toluene 1 and toluene 2 for 2 hours each. Then the tissue is put into a liquid paraffin solution with a temperature of 56oC for 2 hours twice.

The next process of tissue is taken with tweezers and a blocking process (embedding) is carried out using an embedding center tool and material in the form of paraffin that has been heated. The processed tissue is then heated, then taken and removed from the cassette tissue and then soaked in liquid paraffin. The tissue is covered with cassette tissue and labeled. After that the organ is cooled, and the next stage is the cutting process using microtome tools and microtome knives with a thickness of 4-5 µm. The cut tissue is developed over the water in a waterbath, then captured with the object's glass and labeled with diamond pens. Then proceed with staining with Hematoxylin-Eosin (HE).

Pewarnaan hematoxil-eosin (HE)

Liver organs obtained in Rattus norvegicus surgery, washed with sterile PBS once and put in 4% formalin for histological analysis. The stages of Hematoxylin-Eosin staining using the Harris method start from: incision slide on the glass of the object soaked in xylol I for 5 minutes, then continued with xylol II, and xylol III which are each soaked for 5 minutes. After that, the preparation is soaked in the first 100% alcohol and the second 100% alcohol with a period of 5 minutes, then the preparation is put into aquades and continued soaked in Harris Hematoxylin for 15 minutes.

The next stage of the preparation is dipped in aquades by lifting and lowering it. The preparation is then dipped in 1% acid alcohol for 7-10 dips, and soaked in aquades for 15 minutes, then continued soaking in eosin for 2 minutes. Next, the preparation was soaked in 96% alcohol I and II for 3 minutes each, 100% alcohol I and II for 3 minutes each, and in xylol IV and V for 5 minutes each. The preparation is dried and mounted using a bundle. The preparation is examined under a microscope for examination of histopathological changes. Histopathological analysis in Hematoxylin-Eosin staining was used to see the effect of Phyllanthus niruri L. plants with albumin and Phyllanthus urinaria L. plants with albumin on liver tissue which included features of nuclear structure, vacuole degeneration, necrosis tissue, steatosis, and fibrosis compared with each treatment in this study.

Observation of rat liver organ tissue was carried out after making liver preparations with

Haematoxylin-Eosin (HE) staining, and observed using a Nikon brand microscope with a magnification of 400x, after which an assessment of the level of damage that occurred to the liver organ was carried out. The histological structure of mice from each treatment was compared with negative controls. The determination of damage scores to organs was carried out according to the criteria of Mordue et al. (2001) with the provisions described in Table 1 Scoring of Hepatic Degeneration Damage, and Table 2 Scoring of Hepatic Necrosis Damage.

Table 1. Degeneration Damage Scoring

DEGENERASI	SCORE
No degeneracy was found in one observed field of view	0
1-20% degeneration was found in one observed field of view	1
21-50% degeneration was found in one observed field of view	2
found 51-75% degeneration in one observed field of view	3
It was found >75% degeneration in one observed field of view	4

Table 2. Necrosis Damage Scoring

NEKROSIS	SCORE
No necrosis was found in one observed field of view	0
1-20% necrosis was found in one observed field of view	1
21-50% necrosis was found in one observed field of view	2
found 51-75% necrosis in one observed field of view	3
>75% necrosis was found in one observed field of view	4

The method of scoring organ damage assessment using the scoring method by Mordue (2001) which has been modified is by observing one field of view divided into four parts, if one part of one field of vision experiences degeneration or necrosis, then the observed part is given a score of 1, if two parts of one field of vision experience degeneration or necrosis then the part is given a score of 2, if all three parts of one field of vision experience degeneration or necrosis, then the part is given a score of 3, if all four parts of one field of view experience degeneration or necrosis, then the part is given a score of 4 (Arsya et al., 2019). Meanwhile, the scoring assessment on pathological NASH (Non-Alcoholic Steatohepatitis) includes states of steatosis, degeneration and fibrosis measured by scores in Table 3 NASH (Non-Alcoholic Steatohepatitis) scores

Table 3. NASH (Non-Alcoholic Steatohepatitis) Scores

STEATOSIS		DEGENERASI		FIBROSIS	
0	Normal	**0**	None	**0**	None
1	Light	**1**	Exist	**1**	Perisinusoidal
2	Keep	**2**	Signed	**2**	Peroportal
3	Bad			**3**	*Bridging fibrosis*
				4	Syrosis

Data Analysis

The flow cytometry data was analyzed using BD cellquest ProTM software. The data from the analysis is continued with statistical analysis. The statistical analyses used were one way ANOVA parametric analysis ($p \leq 0.05$), and an independent T test. The application used for statistical analysis is SPSS version 26 for windows

RESULTS AND DISCUSSION

Decreased SGOT-SGPT Levels in Rattus norvegicus Model of Hepatitis after

Giving a combination of Phyllanthus niruri L. – Albumin (PNA) and Phyllanthus urinaria – Albumin (PUA)

The results of the study of SGOT levels before and after treatment are presented in Table 4. for the CCl 4 + PNA treatment induction group (Phyllanthus niruri L. – Albumin) and Table 5 for the CCl4 + PUA treatment (Phyllanthus urinaria L. – Albumin) induction group. The results of the PNA group treatment analysis,

showed that CCl4 exposure caused an increase in SGOT levels on day 14 by 162.59 ± 14.25 U/L compared to negative controls of 112.84 ± 21.72 U/L. Then the administration of PNA treatment had a significant effect on SGOT levels shown in the treatment dose 1 PNA worth 88.59 ± 4.37 or 45.51% compared to SGOT levels in positive controls and dose 2 treatment worth 86.22 ± 7.31 U/L or 46.97% compared to SGOT levels in positive controls.

Furthermore, the results of the analysis in the PUA treatment group showed that CCl4 exposure also increased SGOT levels on day 14 by 155.94 ± 14.03 U/L compared to negative controls by 70.18 ± 13.40 U/L, and PUA treatment had a significant effect on SGOT levels shown in dose 1 PUA treatment worth 66.77 ± 6.15 or by 57.15% compared to SGOT levels in positive controls and dose 2 treatment worth 66.94 ± 7.61U/L or by 57.07% compared to SGOT levels in positive controls. Based on this, it can be seen that the administration of treatment shows a significant difference in the effect of SGOT levels in CCl4-induced male Rattus norvegicus.

Table 4. SGOT Levels in Male Rattus norvegicus CCl4 Induction Group + Treatment PNA (Phyllanthus niruri L. – Albumin)

Treatment Group	Mean ± SD Kadar SGOT (IU/L)		Effect of PNA on SGOT Levels Compared to Positive Control at Day 14
	H0	H14	
Control (-) Healthy	115.01 ± 15.32	112.84 ± 21.72b	-
Control (+) CCl_4 Induction	98.72 ± 7.86	162.59 ± 14.25c	-
CCl_4 Induction + Treatment Dose 1 PNA	106.56 ± 15.00	88.59 ± 4.37ab	45,51%
CCl_4 Induction + Treatment Dose 2 PNA	117.75 ± 5.26	86.22 ± 7.31a	46,97%

Table 5. SGOT Levels in Male Rattus norvegicus CCl4 Induction Group + Treatment PUA (Phyllanthus urinaria L.– Albumin)

Treatment Group	Mean ± SD Kadar SGOT (IU/L)		Effect of PUA on SGOT Levels Compared to Positive Control at Day 14
	H0	H14	
Control (-) Healthy	103.01 ± 15.32	70.18 ± 13.40b	-
Control (+) CCl_4 Induction	86.56 ± 7.73	155.94 ± 14.03c	-
CCl_4 Induction + Treatment Dose 1 PUA	94.56 ± 15.00	66.77 ± 6.15a	57,15%
CCl_4 Induction + Treatment Dose 2 PUA	109.09 ± 9.73	66.94 ± 7.61ab	57,07%

Information:
H0 : average SGOT level day 0
H14 : average SGOT level day 14

Increased levels of SGOT in the blood of male rats are caused by exposure to CCl4. This can be seen from the results of measuring SGOT levels in the positive control group, where it was shown that SGOT levels from both treatment groups had levels above normal determined by BPOM (2021), which ranged from 45-100 U/L. The increase in SGOT levels by exposure to CCl4 was caused by the hepatic metabolic process of cytochrome P450 enzymes, specifically P4502E1 (CYP2E1). This process produces highly reactive trichloromethyl (CCl3) radicals. CCl3 radical compounds can combine with oxygen molecules (O2) to form trichloromethylperoxy radical compounds (CCl3OOH) (Mishra et al., 2020).

Trichloromethylperoxy is a highly reactive compound that can cause oxidative stress in cells (Mehendale, 2010). Trichloromethylperoxy compounds can induce oxidative stress by causing lipid peroxidation in liver cell membranes. Lipid peroxidation produces free radicals and other oxidation products that damage the structure of cell membranes. Lipid peroxidation and oxidative stress can disrupt the homeostasis of calcium hepatocytes.

This disruption triggers the release of Ca2+ by organelles, specifically the endoplasmic reticulum (Mishra et al., 2020). The accumulation of free radicals and changes in calcium homeostasis can lead to necrosis (cell death) of hepatocytes. This necrosis occurs mainly in the perihepatic area. Then in response to hepatocyte necrosis, SGOTs previously present in cells are released into the blood. So that SGOT levels in the blood of male Rattus norvegicus have increased.

Research on SGPT levels in male Rattus norvegicus was conducted before and after treatment. The measurement of SGPT levels pays attention to the range of normal levels in rats with a range of 10-50 IU/L (BPOM, 2021). The results of SGOPT levels before and after treatment are presented in Table 6 for the CCl4 + PNA treatment (Phyllanthus niruri L. – Albumin) induction group and Table 7 for the CCl4 + PUA treatment (Phyllanthus urinaria L. – Albumin) induction group.

Table 6 SGPT Levels in Male Rattus norvegicus CCl4 Induction Group + Treatment PNA (Phyllanthus niruri L. – Albumin)

Treatment Group	Mean ± SD Kadar SGPT (IU/L)		Effect of PNA on SGPT Levels Compared to Positive Control at Day 14
	H0	H14	
Control (-) Healthy	54.50 ± 7.66	48.50 ± 7.66b	-
Control (+) CCl_4 Induction	46.28 ± 3.86	79.96 ± 3.89c	-
CCl_4 Induction + Treatment Dose 1 PNA	50.28 ± 7.50	46.12 ± 2.72ab	42,32%
CCl_4 Induction + Treatment Dose 2 PNA	55.87 ± 2.63	40.87 ± 5.96a	48,88%

Table 7 SGPT Levels in Male Rattus norvegicus CCl4 Induction Group + Treatment PUA (Phyllanthus urinaria L. – Albumin)

Treatment Group	Mean ± SD Kadar SGPT (IU/L)		Effect of PUA on SGPT Levels Compared to Positive Control on Day 14
	H0	H14	
Control (-) Healthy	48.50 ± 7.66	23.91 ± 12.22a	-
Control (+) CCl_4 Induction	40.28 ± 3.86	87.25 ± 8.76c	-
CCl_4 Induction + Treatment Dose 1 PUA	44.28 ± 7.50	39.28 ± 3.89b	54,97%
CCl_4 Induction + Treatment Dose 2 PUA	554 ± 4.86	30.80 ± 10.96ab	64,69%

Information:
H0 : average SGPT level day 0
H14 : average SGPT level day 14

Exposure to CCl_4 in male Rattus norvegicus can increase blood levels of SGPT (alanine aminotransferase) which is an indicator of liver damage (Teschke et al., 1983). This process occurs due to the metabolism of CCl_4 in liver cells involving cytochrome P450 enzymes, resulting in free radicals that can damage liver cells. The cellular mechanism in the liver due to CCl_4 exposure begins with CCl_4 metabolism, where CCl_4 is converted by cytochrome P450

enzymes into free radical compounds such as trichloromethyl (CCl_3) and trichloromethylperoxide (CCl_3OOH) (Mishra et al., 2020).

Free radicals produced by CCl_4 metabolism can inhibit enzymes in mitochondria, including enzymes involved in energy production, causing oxidative stress and mitochondrial damage (Teschke et al., 1983). The resulting free radicals can also cause a chain reaction of lipid peroxidation on the liver cell membrane, damaging the structure of the cell membrane and producing oxidation products that can damage liver cells (Khan et al., 2012). As a result, lipid peroxidation and oxidative stress occur which cause damage to hepatocytes, including organelles such as mitochondria and endoplasmic reticulum, as well as the release of SGPT enzymes into the blood.

The results of the PNA group treatment analysis showed that CCl4 exposure caused an increase in SGPT levels on day 14 of 79.96 ± 3.89 U/L compared to negative controls of 48.50 ± 7.66 U/L, and PNA treatment had a significant effect on SGPT levels shown in the treatment dose 1 PNA worth 46.12 ± 2.72 or 42.32% compared to SGPT levels in positive control and dose 2 treatment worth 40.87 ± 5.96 U/L or 48.88% compared to SGPT levels at positive controls.

Furthermore, the results of the analysis in the PUA treatment group showed that CCl4 exposure also increased SGPT levels on day 14 by 87.25 ± 8.76 U/L compared to negative controls by 23.91 ± 12.22 U/L, and the administration of PUA treatment had a significant effect on SGOT levels shown in the treatment dose 1 PUA worth 39.28 ± 3.89 or by 54.97% compared to SGPT levels in positive controls and dose 2 treatment worth 30.80 ± 10.96 U/L or by 64.69% compared to SGPT levels in positive control. Based on this, it can be seen that the administration of treatment shows a significant difference in the effect of SGPT levels in CCl4-induced male Rattus norvegicus.

The treatment has an effect because the Phyllanthus niruri plant is known to have strong antioxidant activity. The plant extract is able to capture free radicals and inhibit lipid peroxidation, thereby protecting liver cells from oxidative stress. Oxidative stress can cause cell damage and release liver enzymes into the bloodstream, including SGPT. The antioxidant properties contained in the plant Phyllanthus niruri, are known to inhibit the process of lipid peroxide, which is one of the main mechanisms of liver cell damage.

This suggests that Phyllanthus niruri plant extract can help maintain the integrity of liver cell membranes and prevent the release of SGOT-SGPT in the blood (Ezzat et al., 2020). In addition, the Phyllanthus niruri plant also has hepatoprotective effects, potentially protecting liver cells from damage caused by various factors, including exposure to CCl_4 (Ezzat et al., 2020). The mechanism involves regulation of liver enzymes and inflammatory responses at the cellular level, as well as modulation of inflammatory pathways within the liver, which can reduce inflammation, cell damage, and SGOT-SGPT levels (Da'i et al., 2016). Plant antioxidant compounds can help the recovery and repair of the liver, thus causing a decrease in SGOT-SGPT levels in the blood (Ezzat et al., 2020). This suggests that the Phyllanthus niruri plant has potential therapeutic benefits for liver health, specifically in protecting against oxidative stress and liver damage caused by a variety of factors, including CCl_4 exposure.

Phyllanthus urinaria L. is known to have hepatoprotective effects against liver damage caused by CCl4 exposure in rats. The potential hepatoprotective effects of the plant Phyllanthus urinaria L. are known to weaken hepatotoxicity stemming from CCl4 exposure through regulation of L-carnitine, taurocolic acid, and amino acid metabolism, which may be promising targets for the treatment of liver toxicity (GUO et al., 2017). Research studies show that Phyllanthus urinaria L. contains a variety of compounds with potential pharmacological

effects. Some of the compounds identified include flavonoids, alkaloids, lignans, and tannins (Geethangili & Ding, 2018).

Phyllanthus urinaria contains flavonoid compounds of quercetin and kaempferol types(Wu et al., 2013). These two flavonoid compounds are known to have potential as antioxidants that provide a scavenging effect on ROS. Therefore, it is hypothesized that flavonoids in Phyllanthus urinaria are able to work to control ROS released from mitochondria, thus protecting liver cells and in turn can guard against cell inflammation.

The plant has traditionally been used in various cultures due to its potential hepatoprotective, antiviral, anti-inflammatory, and antibacterial properties (Oktarina et al., 2021).Phyllanthus urinaria extract, which has antioxidant properties, can capture free radicals produced by CCl_4 and inhibit lipid peroxidation. So the protection of the plant Phyllanthus urinaria L can help protect liver cells from damage and release of liver enzymes, including SGOT-GPT, into the blood.

Albumin is a protein that has a molecular weight of 65,000 daltons (Prawiro, 2016). Albumin compounds are found in the blood at normal concentrations of 3.5 to 5.0 grams of albumin per 100 ml (Hidayat, 1991). Albumin has many sulfhydryl groups (-SH) which can act as free radical binders (Kusumaningrum, 2013). Proteins rich in –SH groups will have the ability to bind harmful metals as well as compounds with antioxidant effects (Prawiro, 2016).

Fish albumin can be used as an alternative to meet the needs of albumin in the body, but it can also be used as a substitute for human serum albumin (HSA) which is still relatively expensive to help meet the needs of albumin in the wound healing process (Ishida et al., 2014). Toman fish (Channa micropeltes) has a fairly high albumin content, with an albumin content of (5.35%) (Suprayitno et al., 2013). In addition to albumin, toman fish also contains several components such as zinc, omega-6 and omega-3 (Firlianty et al., 2013).

The content of Omega-6 found in toman fish has a derivative called arachidonic acid (AA) which plays an important role in the development of neutrophils during the wound healing period (Diana et al., 2013 and Serhan, 2007). Neutrophils can produce normal amounts of ROS that can kill and digest bacteria during phagocytosis (Standring, 2016). In addition, arachidonic acid (AA) compounds can be converted into lipoxin during the inflammatory phase, which plays a role in suppressing inflammation (Serhan, 2007).

The sulfhydryl group in toman fish is an antioxidant that is known to protect cells from damage such as that caused by ROS (Sudha et al. 2010). Albumin can destroy ROS formed during oxidation, by breaking down hydrogen peroxide radicals, so as to neutralize Reactive Oxygen Species (Pieniazek et al., 2018). Therefore, albumin from toman fish can help protect cells from damage due to ROS (Fitriyani, 2018).

Based on this, the application of a combination of PNA treatment (Phyllanthus niruri L. + Albumin) and a combination of PUA treatment (Phyllanthus urinaria L. + Albumin) against CCl4-induced male Rattus norvegicus can have a real effect on SGOT-SGPT levels in the blood, because of the antioxidant properties in the treatment combination that can function to provide hepatoprotective effects and accelerate the healing process. The histopathological picture of liver cells exposed to carbon tetraclorida (CCl4) and the effect of the combination treatment of Phyllanthus niruri L.+ Albumin (PNA) and Phyllanthus urinaria L. + Albumin (PUA) is shown in Figure 1 Histopathology of Hepar Rattus norvegicus PNA Group and Figure 2 Histopathology of Hepar Rattus norvegicus Group PUA.

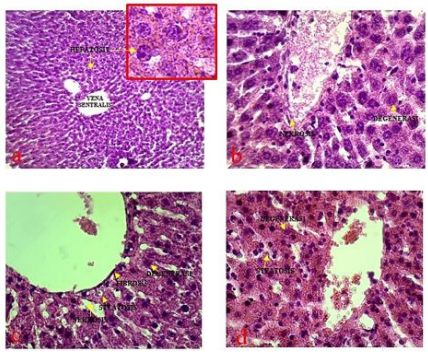

Figure 1. Liver Histopathology of Rattus norvegicus PNA Group (a) Normal, (b) CCl4 Induction, (c) CCl4 Induction + 1 PNA Dose, (d) CCl4 Induction + 2 PNA Dose

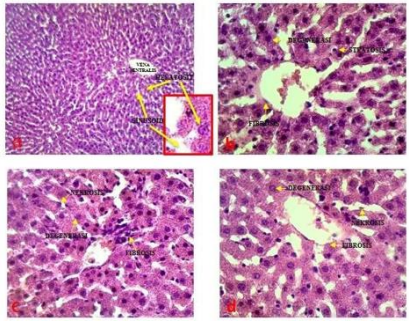

Figure 2. Histopathology of Hepatic Rattus norvegicus PUA Group (a) Normal, (b) CCl4 Induction, (c) CCl4 Induction + 1 PUA Dose, (d) CCl4 Induction + 2 PUA Dose

CCl4 metabolites that bind to the lipid membrane cause the membrane to leak so that the plasma membrane is damaged. Therefore, it causes ATP production to decrease due to reduced energy, causing sodium and air ions to enter the cell and potassium ions to exit the cell, followed by an increase in osmosis pressure which causes a lot of air to flow into the cell (Istikhomah, 2015). This continues to cause dysfunction of the endoplasmic reticulum in synthesizing protein membranes so that cells experience hydropic degeneration. Then the cell organelles also absorb air and swell, resulting in the cytoplasm appearing granulated and the cell experiencing parenchymatous degeneration (McGavin, 2006). The research study produced a degeneration score on hepatocyte histology shown in Table 8.

Table 8 Hepatic Degeneration Scoring

Types of Treatment	Average Scoring of Hepatic Cell Degeneration ± SD	
	Treatment *Phyllanthus niruri* + Albumin (PNA)	Treatment *Phyllanthus urinaria* + Albumin (PUA)
Control (-) Healthy	53 ± 0.23	20 ± 0.11
Control (+) CCl_4 Induction	3.46 ±0.23	3.33 ± 0.30
CCl_4 Induction + Treatment Dose 1	33 ± 0.30	1.80 ± 0.20
CCl_4 Induction + Treatment Dose 2	2.07 ± 0.50	2.07 ± 0.30

Research studies show that there are changes in the structure of liver cell degeneration. Treatment on male Rattus norvegicus showed different rates of degeneration. The results of the analysis of the average liver degeneration in the PNA treatment group, showed that CCl4 exposure caused an increase in the average hydropic degeneration of 3.46 ± 0.23 compared to negative controls of 53 ± 0.23, and the administration of PNA treatment had a significant effect on the average hydropic degeneration shown in the treatment dose 1 PNA worth 33 ± 0.30 and treatment dose 2 worth 2.07 ± 0.50.

Furthermore, the results of the analysis in the PUA treatment group showed that CCl4 exposure also increased the average hydropic degeneration in liver cells by 3.33 ± 0.30 compared to negative controls worth 20 ± 0.11, and the administration of PUA treatment had a significant effect on the average degeneration shown in the treatment dose 1 PUA worth 1.80 ± 0.20 and treatment dose 2 by 2.07 ± 0.30. Based on this, it can be seen that the administration of treatment shows a significant difference in the effect of degeneration in male Rattus norvegicus induced CCl4.

Necrosis is cell death often caused by injury or exposure to toxic substances. Male Rattus norvegicus hepatocyte cells exposed to carbon tetrachloride (CCl4), can cause pathological liver cells to experience necrosis. The characteristics of hepatocellular necrosis include cell swelling, loss of cell nuclear structure, and rupture of cell membranes (Susatyo et al., 2018). The mechanism of necrosis that occurs in hepatocytes due to CCl4 exposure includes oxidative processes that damage cell membranes and organelles and disrupt cell function (Mulina, 2013). Exposure to CCl4 can cause oxidative stress leading to hepatocyte damage and necrosis (Susatyo et al., 2018). The research study resulted in scoring necrosis in hepatocyte histology shown in Table 9.

Table 9. Hepatic Necrosis Scoring

Types of Treatment	**Average Scoring of Hepatic Cell Necrosis ± SD**	
	Treatment *Phyllanthus niruri* + Albumin (PNA)	**Treatment *Phyllanthus urinaria* + Albumin (PUA)**
Control (-) Healthy	6 ± 0.20	1 ± 0.23
Control (+) CCl_4 Induction	3.46 ±0.11	3.33 ± 0.30
CCl_4 Induction + Treatment Dose 1	40 ± 0.20	53 ± 0.41
CCl_4 Induction + Treatment Dose 2	2.60 ± 0.40	2.33 ± 0.57

The results showed that there were changes in liver cells due to CCl4 exposure, resulting in liver cell pathology into necrosis. Liver cells that undergo necrosis will show several pictures of the cell nucleus such as pycnose, karyorexia and karyolysis. The pycnosesis stage, describes the nucleus of a liver cell undergoing shrinkage due to cytoplasmic homogenization and eosinophilia. In addition to pycnosesis, cell nuclei that undergo necrosis can also show a state of destruction or tearing, and leave chromatin fragments scattered throughout the cell, which is called karyorexia. Then the cell nucleus that dies at the time of necrosis will lose its ability to stain or disappear, which is called karyolysis (Price et al., 2006).

Histopathological features of necrosis with characteristics of cell nuclei appear smaller and darker (pycnose), shown in Figure 1 point (c) liver histology of Rattus norvegicus with treatment (CCl4 induction + PNA Treatment Dose 1) and Figure 2 point (c) liver histology of Rattus norvegicus with treatment (CCl4 induction + PUA Treatment Dose 1). Then the histopathological picture of necrosis with the characteristics of the cell nucleus damaged and spread to all parts of the cell (karyorexis), shown in Figure 1 point (b) histology of the liver of Rattus norvegicus with treatment (CCl4 induction). Furthermore, the histopathological picture of necrosis with the characteristics of the cell nucleus is missing and not visible in the cell (karyolysis), shown in Figure 2 point (d) histology of the liver of Rattus norvegicus with treatment (CCl4 induction + PUA Treatment Dose 2). Research studies show that the rate of cell death (necrosis) is more pronounced in the positive control treatment group (CCl4 induction), because this is due to exposure to CCl4 which has the ability to cause liver cell damage. While treatment with Phyllanthus niruri L + Albumin (PNA) treatment and Phyllanthus urinaria L + Albumin (PUA) treatment can

reduce cell death due to CCl4 exposure compared to the positive control group.

Fibrosis is a disorder of liver function due to damaged liver parenchyma cells and excessive buildup of extracellular matrix (ECM) components, such as collagen, in an organ (Bataller & Brenner, 2005). Accumulation of collagen in the liver indicates deterioration of liver function and is one of the causes of liver fibrosis (Gerling et al., 1996; Tang et al., 2012). Fibrosis in the liver of male rattus norvegicus caused by CCl4 involves an overaccumulation of extracellular matrix (ECM) proteins, leading to the formation of scar tissue. The research study produced a fibrosis scoring on hepatocyte histology shown in Table 10.

Table 10. Hepatic Fibrosis Scoring

Types of Treatment	Average scoring of liver cell fibrosis ± SD	
	Treatment *Phyllanthus niruri* + Albumin (PNA)	Treatment *Phyllanthus urinaria* + Albumin (PUA)
Control (-) Healthy	53 ± 0.11	20 ± 0.11
Control (+) CCl_4 Induction	3.60 ± 0.20	3.40 ± 0.20
CCl_4 Induction + Treatment Dose 1	2.60 ± 0.69	30 ± 0.41
CCl_4 Induction + Treatment Dose 2	2.60 ± 0.20	60 ± 0.34

The results showed that there were changes in liver cells due to CCl4 exposure, resulting in liver cell pathology becoming fibrosis. Histopathological features of the liver that undergoes changes in damaged parenchyma cells and excessive buildup of extracellular matrix (ECM) components, shown in Figure 1 point (c) liver histology of Rattus norvegicus with treatment (CCl4 induction + PNA Treatment Dose 1), Figure 2 point (b) liver histology of Rattus norvegicus with treatment (CCl4 induction), Figure 2 point (c) liver histology of Rattus norvegicus with treatment (CCl4 induction + PUA Treatment Dose 1) and Figure 2 point (d) liver histology of Rattus norvegicus with treatment (CCl4 induction + PUA Treatment Dose 2). Research studies show that CCl4 exposure is able to describe the extent of liver cell fibrosis damage in male Rattus norvegicus compared to negative controls. PNA and PUA treatment showed liver cell improvement, although there was still damage compared to positive controls.

Hepatic steatosis is characterized by lipid dysregulation and fat buildup in the liver, which can cause oxidative stress in the liver (Dai et al., 2014). Mouse models with steatosis can be mediated by CCl4. One feature of CCl4 toxicity is the rapid accumulation of triglycerides (TG) in the liver, which is similar to the picture of human liver tissue undergoing steatotics (Pan et al., 2007; Lee et al., 2011). Liver injury due to CCl4 exposure will mediate cytochrome P450 enzymes, resulting in trichloromethyl radicals (CCl3) and trichloromethylperoxy radicals (CCl3OO-) being formed. In particular, the cytochrome P450 enzyme CYP2E1 catalyzes this reaction and forms free radical compounds which, when reacted with lipids, cause lipid peroxidation. This causes damage to the cell membrane resulting in changes in the structure and function of liver cells. The research study produced steatocyte scoring on hepatocyte histology shown in Table 11.

Table 11. Hepatic Steatocyte Scoring

Types of Treatment	Average Scoring of Hepatic Cell Steatocytes ± SD	
	Treatment *Phyllanthus niruri* + Albumin (PNA)	Treatment *Phyllanthus urinaria* + Albumin (PUA)
Control (-) Healthy	60 ± 0.20	50 ± 0.15
Control (+) CCl_4 Induction	3.33 ± 0.11	2.90 ± 0.30

CCl_4 Induction + Treatment Dose 1	2.00 ± 0.34	1.70 ± 0.30
CCl_4 Induction + Treatment Dose 2	2.60 ± 0.34	1.80 ± 0.20

The results showed that there were changes in liver cells due to CCl4 exposure, resulting in liver cell pathology into steatosis. Histopathological features of liver that undergoes cell changes in the form of fat accumulation, shown in Figure 1 point (c) liver histology of Rattus norvegicus with treatment (CCl4 induction + PNA Treatment Dose 1), Figure 1 point (d) liver histology of Rattus norvegicus with treatment (CCl4 induction + PNA Treatment Dose 2), Figure 2 point (b) liver histology of Rattus norvegicus with treatment (CCl4 induction). Research studies show that CCl4 exposure is able to describe the degree of fatty liver cell buildup in male Rattus norvegicus compared to negative controls. PNA and PUA treatment showed liver cell improvement, although there was still damage compared to positive controls.

Effect of administration of PNA (Phyllanthus niruri L with Albumin) and PUA (Phyllanthus urinaria L. with albumin) on Proinflammatory Cytokine Expression

Based on the results of flow cytometry analysis, the expression of proinflammatory molecules in male Rattus norvegicus exposed to CCl4 was significantly higher than in the male Rattus norvegicus of the negative control group significantly ($p \leq 0.05$). The proinflammatory molecular parameters expressed by CD4 T cells in this study included CD4+TNF-α+, CD11b+IL-1+, and CD11b+IL-6+. PNA treatment and PUA treatment have different effects on the development of proinflammatory molecules (TNF-α) in each treatment. Giving dose 1 PNA treatment is known to be able to reduce TNF-α levels compared to positive control treatment. While in the PUA treatment group showed that the administration of dose 2 PUA treatment had a more reducing effect on TNF-α levels slammed with positive control treatment. The results of proinflammatory cytokine analysis (TNF-α) are shown in Figure 3 Treatment of PNA Treatment (Phyllanthus niruri L. with Albumin) against TNF-α Reduction. Figure 4 Treatment of PUA (Phyllanthus urinaria L. with Albumin) on TNF-α Reduction.

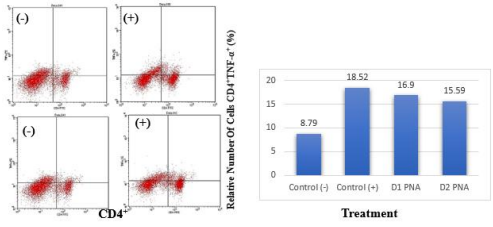

Figure 3. Treatment of PNA (Phyllanthus niruri L. with Albumin) on TNF-α Reduction

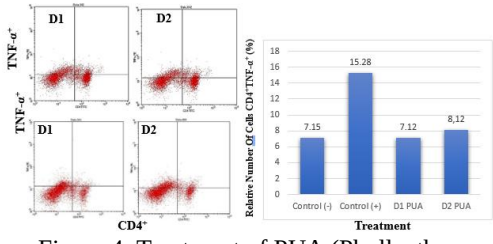

Figure 4. Treatment of PUA (Phyllanthus urinaria L. with Albumin) Treatment for TNF-α Reduction

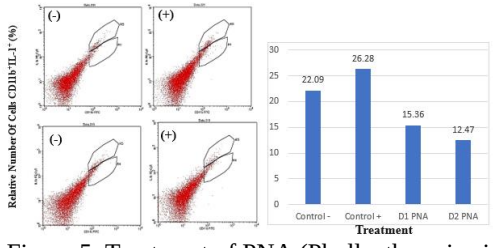

Figure 5. Treatment of PNA (Phyllanthus niruri L. with Albumin) on IL-1 Reduction

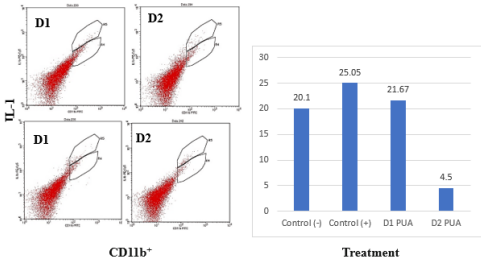

Figure 6 Treatment of PUA (Phyllanthus urinaria L. with Albumin) on IL-1 Reduction

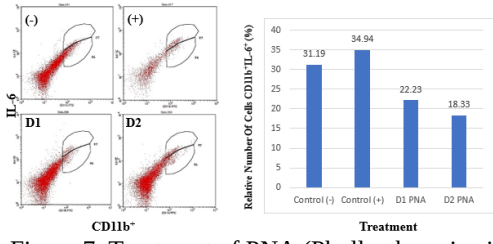

Figure 7. Treatment of PNA (Phyllanthus niruri L. with Albumin) on IL-6 Reduction

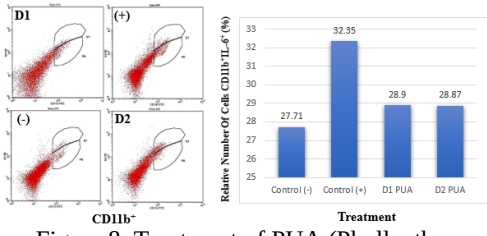

Figure 8. Treatment of PUA (Phyllanthus urinaria L. with Albumin) Treatment for IL-6 Reduction

Toxic chemicals such as CCl4 are often used in experimental research as models to study the potential hepatoprotective effects of substances of natural or synthetic origin (Popović., 2019). This CCl4 display starts from the metabolic process in the liver by cytochrome P450 enzymes, especially P4502E1 (CYP2E1). This process produces highly reactive trichloromethyl (CCl3) radicals. CCl3 radical compounds can combine with oxygen molecules (O2), and form trichloromethylperoxy radical compounds (CCl3OOH) (Mishra et al., 2020).

Trichloromethylperoxy is a highly reactive compound and can cause oxidative stress in cells (Mehendale, 2010). Trichloromethylperoxy compounds can induce oxidative stress by initiating lipid peroxidation on hepatocellular membranes. Lipid peroxidation produces free radicals and other oxidation products, which damage the structure of cell membranes. Lipid peroxidation and oxidative stress can disrupt calcium homeostasis in hepatocytes.

At the molecular biochemical level, CCl4 can disrupt the balance between prooxidant and antioxidant markers that normally cause permanent damage to cell membranes and intracellular structures of hepatocytes. CCl4 free radicals (especially trichloromethyl radicals) and Reactive Oxygen Species (ROS) can activate Kupffer cells, stellata cells, sinusoidal endothelial cells, and induce proinflammatory cytokines (TNF-α, IL-1β, IL-6) as well as mediate further damage to hepatocyte cells (Popović., 2019; Yu et al., 2021; Candlestick et al., 2017). In the pathogenesis of CCl4-induced (inflammatory) liver injury, it can cause liver damage, mainly through the process of its hepatotoxicity. In this context, TNF-α is known to play a role in stimulating or triggering inflammatory responses in the liver.

TNF-α contributes to the induction of the enzyme iNOS. iNOS is responsible for the production of nitric oxide (NO), a molecule that can play a role in various cellular functions, including the regulation of inflammation. The process of induction of iNOS by TNF-α can lead to the release of NO. NO, in this context, can contribute to nitrosative stress, which is a cellular response to an imbalance in nitrogen oxide levels. Excessive release of NO, especially if not properly regulated, can lead to nitrosative stress (Decicco et al., 1998; Beheshti et al., 2021). After 2 weeks of treatment with a combination of PNA and PUA, resulted in a significant decrease in proinflammatory cytokines compared to positive controls. This decrease is significant due

to the presence of antioxidants from plant extracts and Toman fish albumin.

Phyllanthus niruri plants are known to contain flavonoid compounds of quercetin, kaempferol, and rutin (Mediani et al., 2017). Flavonoid compounds are known to have antioxidant and anti-inflammatory properties that can help protect liver health and increase body immunity (Risnawati et al., 2021). Phyllanthus urinaria contains flavonoid compounds such as quercetin and kaempferol (Wu et al., 2013). The flavonoid compounds present in Phyllanthus urinaria have been shown to have strong antioxidant properties and may help protect the liver from damage and inflammation (Husnunnisa et al., 2022).

Thoman fish (Channa micropeltes) is rich in albumin, a high-quality protein (Firlianty, et.al 2013). Thoman fish contains various proteins that are important for human nutrition, such as β-galactosidase, glutamate dehydrogenase, albumin, carbonic anhydrase, myoglobin, lysozyme, and aprotinin (Nurilmala et al., 2021). Albumin found in toman fish has antioxidant properties that accelerate the healing process. This can reduce oxidative effects caused by reactive oxygen species (ROS) modulating inflammation, thereby promoting effective wound healing (Carabelly et al., 2019).

CONCLUSION

The results obtained in this study showed that the combination of Phyllanthus niruri L formula with toman fish albumin (PNA) and Phyllanthus urinaria L formula with toman fish albumin (PUA) was able to show striking hepatoprotective effects through a significant decrease in SGOT-SGPT levels in the blood of male Rattus norvegicus and decreased proinflammatory cytokine profiles in Rattus norvegicus liver cells males exposed to CCl4. In addition, the antioxidant defenses of PNA and PUA formulas almost completely prevent the occurrence of degeneration, necrosis, fibrosis and steatosis in liver cells. The determination of an effective combination for impaired liver function is more or less demonstrated in the administration of Phyllanthus niruri L treatment with toman fish albumin (PNA), but its clinical use is still poorly studied and requires further research to determine the maximum hepatoprotective and anti-inflammatory effects.

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