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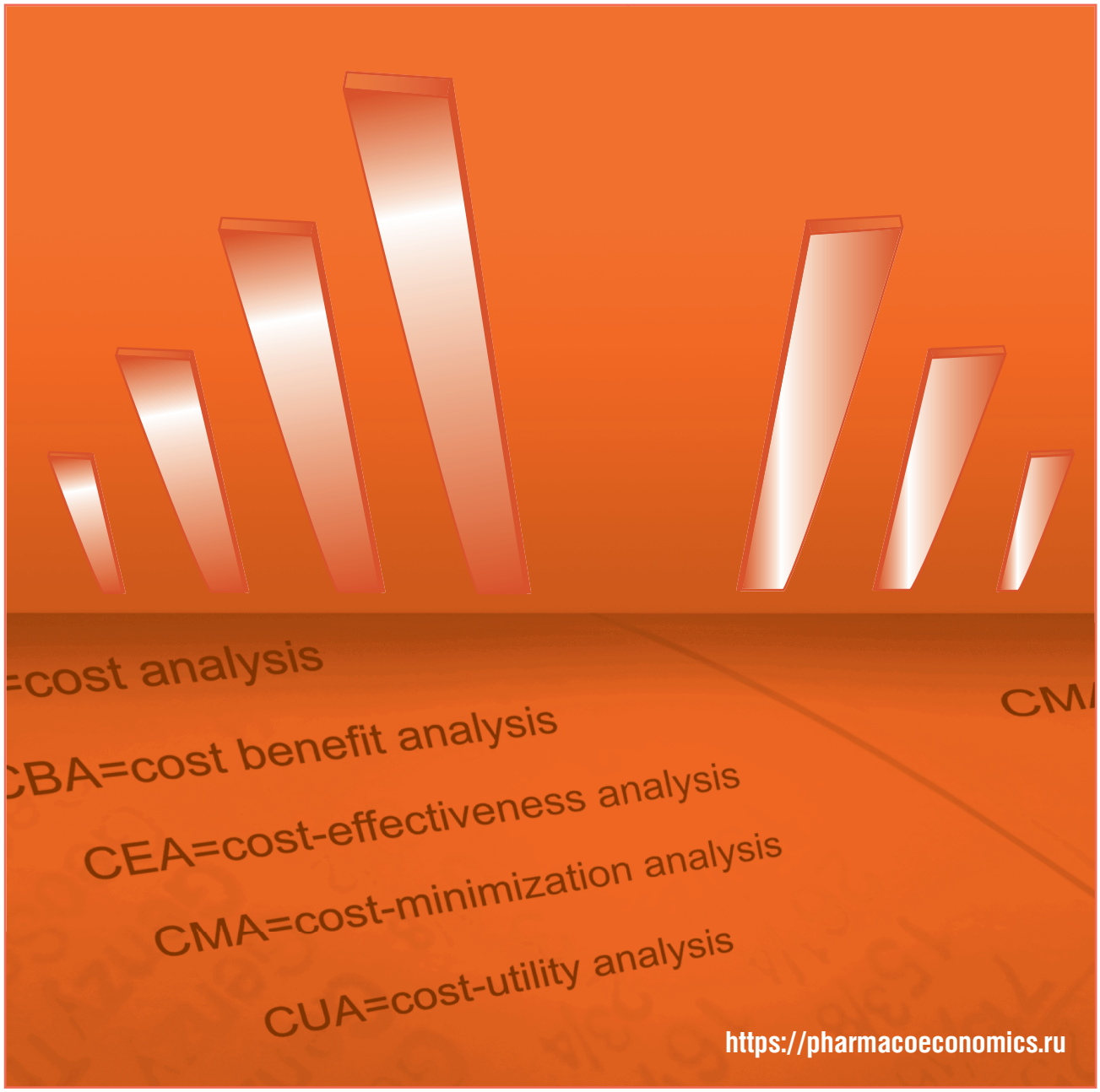

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The influence of the combination of methanolic extract *Scurrula atropurpurea* (Blume) and *Dendrophthoe pentandra* on rat liver function and structure

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SUMMARY

Background. Tea mistletoe (*Scurrula atropurpurea* (Blume)) and mango mistletoe (*Dendrophthoe pentandra*) have been known as a potential medicine for several diseases.

Objective: to investigate the effect of the combination of methanolic extract *Scurrula atropurpurea* (Blume) and *Dendrophthoe pentandra* (MESA-DP) on rat liver function and structure using serological and histopathological analysis.

Material and methods. This study was experimental during 28 days using 20 rats divided into four groups (Group 1 as a control, while Groups 2, 3, and 4 were given MESA-DP at doses 250, 500, and 1,000 mg/kg of body weight, respectively). The liver histopathological structure was observed using hematoxylin-eosin staining. The liver function assessment included total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein, albumin, globulin, cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels. Data were analyzed using a one-way ANOVA analysis (ANalysis Of VAriance) and performed via SPSS Statistics 17.0 (IBM, USA).

Results. The administration of MESA-DP did not show significant differences at all doses for the liver rat function in total bilirubin, SGOT, SGPT, albumin, cholesterol and triglycerides ($p>0.05$), while globulin, total protein, HDL and LDL showed significant results ($p<0.05$). The liver histopathological structure showed the number of pyknotic, karyorrhectic and karyolytic cells in rats after MESA-DP administration compared to controls, which grew with increasing dose.

Conclusion. The liver function in rats after being exposed to MESA-DP was not affected in terms of total bilirubin, SGOT, SGPT, albumin, cholesterol, and triglyceride levels. However, using MESA-DP increased the necrotic liver cells. It may be beneficial for the liver health of experimental animals taking into account the correct dosage.

KEYWORDS

Dendrophthoe pentandra, Scurrula atropurpurea (Blume), histopathology, methanolic extract, liver, *Rattus norvegicus*, serological analysis.

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Воздействие комбинации метанольного экстракта *Scurrula atropurpurea* (Blume) и *Dendrophthoe pentandra* на функцию и структуру печени у крыс

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РЕЗЮМЕ

Актуальность. Омела чайная (*Scurrula atropurpurea* (Blume)) и омела манго (*Dendrophthoe pentandra*) известны как потенциальные лекарственные средства от ряда заболеваний.

Цель: изучить эффект комбинации метанольного экстракта *Scurrula atropurpurea* (Blume) и *Dendrophthoe pentandra* (MESA-DP) на функцию и структуру печени у крыс с помощью серологического и гистопатологического анализа.

Материал и методы. Исследование проводилось в течение 28 дней с использованием 20 крыс, разделенных на четыре группы: 1-я группа служила контролем, а крысы во 2-й, 3-й и 4-й группах получали MESA-DP в дозах 250, 500 и 1000 мг/кг массы тела соответственно. Гистопатологическую структуру печени наблюдали с помощью окрашивания гематоксилином и эозином. Функцию печени оценивали по сывороточным уровням общего билирубина, глутамат-щавелевоуксусной трансаминазы (ГЩТ), глутамин-пировиноградной трансаминазы (ГПТ), общего белка, альбумина, глобулина, холестерина, триглицеридов, липопротеинов высокой плотности (ЛПВП) и липопротеинов низкой плотности (ЛПНП). Данные проанализированы с использованием однофакторного анализа ANOVA (англ. ANalysis Of VAriance) и программы SPSS Statistics 17.0 (IBM, США).

Результаты. Введение MESA-DP при всех дозах не привело к достоверным различиям в функции печени у крыс по показателям общего билирубина, ГЩТ, ГПТ, альбумина, холестерина и триглицеридов ($p>0,05$), в то время как уровни глобулина, общего белка, ЛПВП и ЛПНП показали значимые различия ($p<0,05$). Изучение гистопатологической структуры печени продемонстрировало повышенное количество пикнотических, кариоректических и кариолитических клеток у крыс, получавших MESA-DP, по сравнению с контролем, которое росло с увеличением дозы.

Заключение. Функция печени после воздействия MESA-DP у крыс не изменилась по показателям общего билирубина, ГЩТ, ГПТ, альбумина, холестерина и триглицеридов. Однако применение MESA-DP привело к увеличению количества некротических клеток печени. Таким образом, оно может оказывать положительный эффект на печень подопытных животных с учетом правильного дозирования.

КЛЮЧЕВЫЕ СЛОВА

Dendrophthoe pentandra, *Scurulla atropurpurea* (Blume), гистопатология, метанольный экстракт, печень, *Rattus norvegicus*, серологическое исследование.

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Highlights

What is already known about the subject?

- Mango mistletoe (*Dendrophthoe pentandra*) is one of the semi-parasitic plants used in traditional therapy because of its great efficacy
- Tea mistletoe (*Scurrula atropurpurea* (Blume)) and *Dendrophthoe pentandra* combination have been known as a potential medicine for several diseases

What are the new findings?

- The administration of a combination of mistletoe extract with doses of 250, 500, and 1,000 mg/kg of body weight in rats did not show differences in total bilirubin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, albumin, cholesterol, and triglycerides serum levels, while serum levels of globulins, total protein, high-and low-density lipoproteins showed significant differences ($p<0.05$)
- It was shown that an increase in the dose of the mistletoe combination leads to the increase in number of pycnotic, karyorectic and karyolytic cells in the histopathological structure of rat liver

How might it impact the clinical practice in the foreseeable future?

- This study results might help to develop the optimal combination of methanolic extract *Scurrula atropurpurea* (Blume) and *Dendrophthoe pentandra* to maintain liver health

Основные моменты

Что уже известно об этой теме?

- Омела манго (*Dendrophthoe pentandra*) – одно из полупаразитических растений, которое используется в традиционной терапии, показывая высокую эффективность
- Комбинация омелы чайной (*Scurrula atropurpurea* (Blume)) и *Dendrophthoe pentandra* известна как потенциальное лекарственное стредство от ряда заболеваний

Что нового дает статья?

- При введении комбинации экстракта омелы в дозах 250, 500 и 1000 мг/кг массы тела у крыс не выявлено значимых различий в сывороточных уровнях общего билирубина, глутамат-щавелевоуксусной и глутамин-пировиноградной трансаминаз, альбумина, холестерина и триглицеридов, в то время как изменения уровней глобулина, общего белка, липопротеинов высокой и низкой плотности были достоверны ($p<0,05$)
- Показано, что с увеличением дозы комбинации омелы в гистопатологической структуре печени крыс повышается количество пикнотических, кариоректических и кариолитических клеток

Как это может повлиять на клиническую практику в обозримом будущем?

- Результаты исследования могут помочь разработать оптимальный состав комбинации метанольного экстракта *Scurrula atropurpurea* (Blume) и *Dendrophthoe pentandra* для поддержания здоровья печени

INTRODUCTION / ВВЕДЕНИЕ

Tea mistletoe (*Scurrula atropurpurea* (Blume)) from *Loranthaceae* family is a parasitic plant or mistletoe which lives on the tea plant [1]. It has been known to have wide potential as medicine including leaves, stems, and pea-sized berries [2]. Several studies have reported on the *in vitro* role of methanolic extract *Scurrula atropurpurea* (MESA) leaves in reducing the contractility of the separated rat tail arteries [1]. It also has the potential to lower blood pressure by improving oxidative stress and endothelial dysfunction [3–8]. Moreover, according to previous research, MESA can be used as a cure for hypertension [6, 9–10].

Mango mistletoe (*Dendrophthoe pentandra*, DP) belongs to one family with tea mistletoe. It is a semi-parasitic plant that grows on mango trees as a host plant. It is widely found in tropical rainforests at lowland plantations including Indonesia. Many previous studies reported that its leaves, stems, and flowers had potential for medicine [9]. Mango mistletoe leaves extract is known as a chemotherapeutic agent for cervical cancer [11] and has anti-inflammatory properties [12]. The study showed that the combination of mango mistletoe and 5-Fluorouracil can increase the number of apoptosis cells and p21 expression as well as reduce the surviving expression of HeLa cells [11]. Mango mistletoe treatment significantly inhibited the population

of $CD4^+$ T-cells, interleukin (IL) 17 cells and the IL-17 concentration in the supernatant. It decreased Th17-associated cytokines IL-17, whereas the production of IL-10 of regulatory T cells in mesenteric lymph nodes of colonic tissue was enhanced by mango mistletoe extract-treated mice [12].

The tea mistletoe leaves contain several secondary metabolites such as tannins, flavonoids, quercetin, glycosides, alkaloids, saponins, and inulin. These active substances have been reported to have roles in hypertension [6]. On the other hand, mango mistletoe leaves were also reported to be rich in tannins, saponins, flavonoids, alkaloids, acetoacetic acid, citramalic acid, linolenic acid, ethylene glycol, monoacetate, and beta-sitosterin [13]. Recently, *in vitro* and *in vivo* studies on the pharmacological activity of mango mistletoe leaves extracts have found various efficacy, namely anticancer, immunomodulatory, cardiac, antidiabetic, antihyperglycemic, hepatoprotective, neuropharmacological, antibacterial, and antifungal [14, 15].

Thus, the preparation of tea mistletoe leaves will have commercial opportunities when combined with mango mistletoe leaves. However, the toxicity effects of tea mistletoe when combined with mango mistletoe have never been studied. The current research included toxicity tests, histopathological studies [9], *in vitro* and *in vivo* tests for combination of methanolic extract of *Scurrula atropurpurea* (Bl.) and *Dendrophthoe pentandra* (MESA-DP), and resulted that these extracts are safe and can be used as health drinks to prevent hypertension [6].

Objective: to investigate the effect of MESA-DP combination on rat liver function and structure using serological and histopathological analysis.

MATERIAL AND METHODS / МАТЕРИАЛ И МЕТОДЫ

Study design / Дизайн исследования

The experimental method used in this study was a completely randomized design (CRD). In the true experimental study, 20 female Wistar rats (*Rattus norvegicus*) were divided into three treatments and one control (Group 1 as a control, while Groups 2, 3, and 4 were given MESA-DP at doses 250, 500, and 1,000 mg/kg of body weight (BW), respectively) based on Frederer formula. Each treatment had five replications. The tea mistletoe and mango mistletoe combination was administered for 28 days. The clinical biochemical examination of the liver function and the histopathological observations of the rat livers were conducted [16].

Experimental animals / Экспериментальные животные

The inclusion criteria were female white rats (*R. norvegicus*) aged 6–8 weeks with body weight 100–200 g. Each rat except the control was administered with MESA-DP combination 5 times a week for 28 days (sub-chronic study) [9].

The rats were acclimatized in the animal house laboratory of the Faculty of Medicine, Islamic University of Malang for 1 week under room temperature ±24 °C with approximately 50–60% humidity, protected from industrial fumes and other pollutants. On the 7^{th} day of the acclimatization process, the rats were weighed for pre-condition body weight. The weighing continued once every 7 days during the research.

Plants material and extraction / Растительный материал и его экстракция

Scurrula atropurpurea (Blume) and *Dendrophthoe pentandra* leaves were obtained from Kepanjen, Malang, Indonesia then identified and determined in Balai Materia Medica Laboratory (Batu, East Java, Indonesia). The leaves used were dry simplicia leaves which were clean and not rotten. Each leaf was heated at 40–60 oC in the oven until the water content was lost. Once dried, the leaves were then cut until crushed. Then, they were mashed by a blender to form a powder (simplicia powder) [7].

The mistletoe extraction was conducted using maceration. Extraction began after simplicia powder was formed; each of the tea mistletoe leaves and mango mistletoe leaves was weighed at 100 g and then put into a 1.5-liter bottle. The simplicia powder was soaked in 90% methanol as much as 1 liter and was shaken for 60 minutes until the solution was homogenous. Then, the shaken simplicia powder was allowed to stand and precipitate for 24 hours to break the cell walls of tea mistletoe leaves and mango mistletoe leaves, and the active substances in the leaves could be drawn by methanol. The soaking of simplicia powder for 24 hours result in the formation of two layers in which the top layer was called supernatant while the bottom layer was natant. This supernatant was collected and extracted using a rotary evaporator. The supernatant was the active substance of mistletoe leaves in methanol [5–7].

The administration of extracts / Введение экстрактов

The doses used in this study were three doses of methanolic extracts of the combination of MESA-DP. The doses in Groups 2, 3, 4 were 250, 500, and 1,000 mg/kg BW, respectively [7]. Of the dosage administered to the rats, the maximum volume of the fluid administered at one time depends on the weight of the rats. Administration by oral gavage was carried out according to the given dosage and volume of the fluid to each rat. Before the rats were administered the combination of tea and mango mistletoes, they were not given any food or drink for 14–18 hours (fasting conditions). Food and drink were given again 3–4 hours after oral gavage. The treatment in this study was each dose given 5 times a week for 28 days (sub-chronic toxicity test) orally [7]. Weighing of the rats' body weight was conducted once a week to determine the volume of the fluid to be administered to the rats, which was 1 ml/100 g BW.

Sample isolation / Изоляция образцов

After 28 days of observation and treatment on the female Wistar rats, the incision was carried out and blood samples were taken according to the order of the treatment [7]. Liver and blood were then isolated. Blood was separated from serum plasma at 3,000 rpm (600 g) for 10 minutes using Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany) to separate sera. The liver was placed in kalium cloride (KCl) and phosphate-buffered saline (PBS) 25 mM, then kept in 40% formaldehyde buffered neutral at room temperature for histological preparations.

Hematoxylin-eosin staining / Окрашивание гематоксилином и зозином

Liver organs were placed in 25 mM KCl and PBS buffer, then stored in 40% neutral buffered formaldehyde at room temperature. Hematoxylin and eosin (~5 μm) parts were prepared for histopathological measurement. The parts were photographed at ×400 magnification using Olympus (Tokyo, Japan). Microscope lighting, focus, and plane selection were optimized to differentiate cell boundaries. The images were opened and, after setting the threshold, they were analyzed.

Blood serum analysis / Анализ сыворотки крови

Blood serum was used to examination the levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total bilirubin, albumin, cholesterol, triglycerides, globulins, total protein, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). This process was performed at Bromo Clinic (Malang, Indonesia).

Data analysis / Анализ данных

The data were expressed as mean ± standard error. The mean values and standard errors were calculated using Excel 2007 (Microsoft, USA). Data were analyzed using a one-way ANOVA analysis (ANalysis Of VAriance) and performed via SPSS Statistics 17.0 (IBM, USA). Histopathological analysis of the liver was carried out descriptively.

RESULTS / РЕЗУЛЬТАТЫ

Blood biochemical profile / Биохимический профиль крови

In this study, the three doses of the herbal combination of MESA-DP were safe, hence they did not cause an increase in total bilirubin level compared to the control group (**Table 1**). The administration of the herbal combination at the third dose (Group 4) did not cause erythrocyte abnormality, which was indicated by an increase in bilirubin level.

Furthermore, there were also no significant differences in the effect of the administration of the three doses of the MESA-DP on the SGOT and SGPT serum levels compared to the control group (see Table 1). The first possibility might be because the three doses of this herbal combination were safe, hence they did not cause liver dysfunction, which was indicated by an increase in hepatic transaminase serum levels due to hepatic enzyme leakage. The secondary metabolites here are those of tea mistletoe and mango mistletoe.

The interaction between the components in the two herbs might be mutually neutralizing or antagonistic, thereby reducing their toxic effects. The second possibility was that the deviations of the SGOT and SGPT levels were almost above 20%, resulting in being not significantly different when the data were analyzed statistically ($p>0.05$) (see Table 1).

The effects of MESA-DP administration at three doses did not show any significant difference in the serum albumin level compared to the control group but were different for globulin level and total protein, which tend to decrease ($p>0.05$) (see Table 1). The three doses of the herbal combination were safe. Hence, they did not cause liver dysfunction, which was indicated by the decrease in serum albumin level.

The three doses of the herbal combination were safe. Therefore, they did not cause an increase in the levels of total cholesterol and serum triglyceride compared to the control group ($p>0.05$). The administration of the three doses did not cause disruption of lipid metabolism in the liver, which was indicated by an increase in the levels of total cholesterol and triglyceride. The level of serum LDL decreased significantly compared to the control group due to the administration of the herbal combination ($p<0.05$), hence further research could be carried out on this herbal combination as an anti-hyperlipidemia (see Table 1). It was presumed that the herbal combination decreased serum LDL levels through the mechanism of increasing the number of LDL receptors. On the other hand, there were a significant decrease in HDL levels compared to the control group.

Histopathology / Гистопатологическое исследование

The histopathological examination was conducted by counting the damaged liver cells. The liver cells were observed by the microscope under ×400 magnification (**Fig. 1**). The normal structure of liver cell tissue will have clear nuclei. On the other hand, abnormal liver tissues have necrotic nuclei which are pyknotic, karyorrhectic, or karyolysis. Based on the microscopic observation of the liver, administration of excessive MESA-DP could affect the structure of liver cell tissues in the form of pyknotic, karyorrhectic, and karyolysis cells.

Damaged liver cells in the female rats treated by MESA-DP at a dose 250 mg/kg BW showed fewer necrotic cells compared to those treated using doses 500 and 1,000 mg/kg BW (**Table 2**). This dose was applied with the expectation of not causing toxicity to the liver cells.

DISCUSSION / ОБСУЖДЕНИЕ

The liver is one of the organs which has an important role as a toxin neutralizer and is responsible for the biotransformation of toxic substances into non-toxic substances. Therefore, this process causes liver cells to easily experience damage both in the form of damage to the cell structure as well as liver dysfunction [17]. Several herbal compounds which enter the body will undergo absorption, distribution, metabolism, and excretion processes [18]. The liver is the main organ of metabolism, which is often damaged by the compound itself or the accumulation of metabolites. Compounds will be metabolized in the

Table 1. Results of the rats' blood biochemical profile after the administration of the combination of tea and mango mistletoe

Таблица 1. Результаты биохимического анализа крови после введения комбинации чайной омелы и омелы манго у крыс

Parameter / Параметр	Group 1 (control) / 1-я группа (контроль)	Group 2 (250 mg/kg BW) // 2-я группа (250 мг/кг МТ)	Group 3 (500 mg/kg BW) // 3-я группа (500 мг/кг МТ)	Group 4 (1,000 mg/kg BW) // 4-я группа (1000 мг/кг МТ)
Total bilirubin, mg/dl // Общий билирубин, мг/дл	0.0825±0.020	0.0940±0.023	0.0920±0.016	0.0720±0.016
SGOT, U/l // ГЩТ, Ед/л	210.0±10.21	205.0±32.44	220.6±53.40	205.2±67.56
SGPT, U/l // ГПТ, Ед/л	112.4±8.53	120.4±37.20	133.4±32.68	91.8±9.33
Albumin, g/dl // Альбумин, г/дл	3.545±0.120	3.584±0.179	3.458±0.057	3.544±0.093
Globulin, g/dl // Глобулин, г/дл	5.250±0.173	5.062±0.314*	4.614±0.171*	4.600±0.393*
Total protein, g/dl // Общий белок, г/дл	8.795±0.076	8.652±0.351*	8.058±0.167*	8.378±0.130*
Cholesterol, mg/dl // Холестерин, мг/дл	68.75±10.35	62.40±5.50	51.80±8.40	58.60±12.05
Triglyceride, mg/dl // Триглицериды, мг/дл	62.25±6.48	55.00±12.94	66.00±29.30	56.20±5.58
HDL, mg/dl // ЛПВП, мг/дл	63.72±7.80	59.72±3.81**	52.70±3.81**	52.68±6.42**
LDL, mg/dl // ЛПНП, мг/дл	40.02±2.98	40.08±11.42**	33.50±2.05**	35.62±1.49**

Note. *BW – body weight; SGOT – serum glutamic oxaloacetic transaminase; SGPT – serum glutamic pyruvic transaminase; HDL – high-density lipoproteins; LDL – low-density lipoproteins. * p<0.01. ** p<0.05.*

Примечание. *МТ – масса тела; ГЩТ – глутамат-щавелевоуксусная трансаминаза; ГПТ – глутамин-пировиноградная трансаминаза; ЛПВП – липопротеины высокой плотности ins; ЛПНП – липопротеины низкой плотности. * p<0.01. ** p<0.05.*

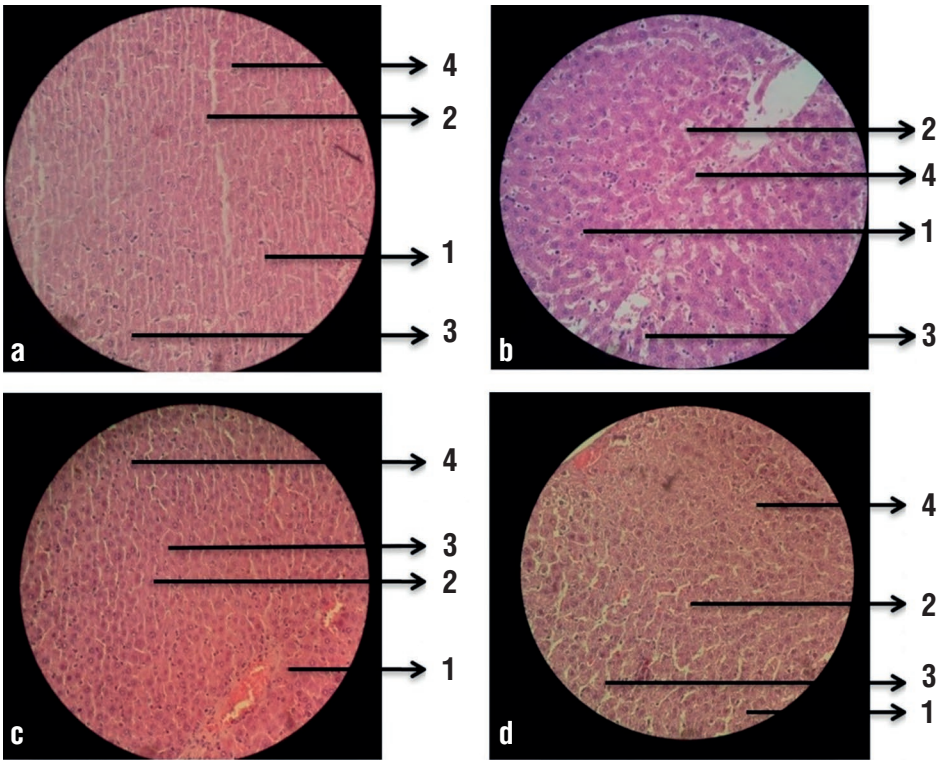

Figure 1. Histopathology of rat liver exposed to combination of tea and mango mistletoe for 28 days. There is no abnormality effect (hematoxylin-eosin staining, ×100):
a – control (without treatment);
b – combination at dose 250 mg/kg of body weight (BW); **c** – combination at dose 500 mg/kg BW; **d** – combination at dose 1,000 mg/kg BW.

1 – normal; 2 – pyknotic nuclei;
3 – karyorrhexis; 4 – karyolysis

Рисунок 1. Результаты гистопатологического исследования печени крысы, подвергшейся воздействию комбинации чайной омелы и омелы манго в течение 28 дней. Патологический эффект отсутствует (окрашивание гематоксилином и эозином, ×100):
a – контроль (без лечения);
b – комбинация в дозе 250 мг/кг массы тела (МТ); **c** – комбинация в дозе 500 мг/кг МТ; **d** – комбинация в дозе 1,000 мг/кг МТ.

1 – норма; 2 – пикнотические ядра;
3 – кариорексис; 4 – кариолиз

Table 2. Normal, pyknotic, karyorrhectic, and karyolytic cells count in rat liver, n

Таблица 2. Количество нормальных, пикнотических, кариоректических и кариолитических клеток в печени крыс, n

Cells / Клетки	Group 1 (control) / 1-я группа (контроль)	Group 2 (250 mg/kg BW) // 2-я группа (250 мг/кг МТ)	Group 3 (500 mg/kg BW) // 3-я группа (500 мг/кг МТ)	Group 4 (1,000 mg/kg BW) // 4-я группа (1000 мг/кг МТ)
Normal / Нормальные	40	20	30	29
Pyknotic / Пикнотические	59	64	130	114
Karyorrhectic / Кариоректические	34	36	84	93
Karyolytic / Кариолитические	23	39	53	93

Note. *BW – body weight.*

Примечание. *МТ – масса тела.*

liver and a chemical structure change will occur which is catalyzed by an enzyme produced by the microsome of hepatocyte cell and called biotransformation. The medicinal or herbal compounds will be transformed into metabolites that are usually less active than the original medicines. The process of medicine metabolism is not always a process of medical detoxification or elimination of the compounds; sometimes the transformation of medicine results in the formation of intermediate compounds that are reactive and toxic to the liver. Acute liver damage will cause metabolic changes which, in turn, will result in structural and functional changes.

The test of SGOT and SGPT levels is one of the indicators which can be used to determine the presence or absence of liver function damage which is caused by increases in SGOT and SGPT levels found mainly in liver cells. Both enzymes are active in serum, which is used to measure indications of liver diseases. In this study, MESA-DP leaf extract showed no toxicity in experimental doses in animals indicating the safety of the bioactive phytochemicals present in the extract. The MESA-DP extract treatment group that was given had no significant effect on the levels of total bilirubin, SGOT, SGPT, albumin, cholesterol, and triglycerides compared to the control group. Otherwise, the biochemical parameters of globulin, serum total protein, HDL, and LDL experienced a significant decrease when compared to control (see Table 1). The previous study reported that male rats which have been exposed to a methanolic extract of tea mistletoe orally for 28 days (sub-chronic) did not show any abnormality on the histopathology examination and no effect compared to the control group rats on the levels of serum SGOT, SGPT, albumin, globulin and total protein [7]. Similarly, the previous study reported that female rats exposed to a methanolic extract of tea mistletoe orally for 28 days (sub-chronic) did not show any toxicity effect on the SGOT and SGPT levels caused by MESA, hence MESA was considered safe [19].

The liver is one of the organs of the body that functions as the center of metabolism for various substances such as bilirubin, cholesterol, and bile acids. Cell can release chemical substances such as enzymes, bilirubin, and coagulation factors and function as the synthesis of globulins, albumins, and immune bodies. Liver function will decrease if there is interference and an increase in the level of enzymes secreted by the liver. These enzymes are aminotransferase enzymes (SGOT and SGPT). In addition to these two enzymes, other liver function tests such as total bilirubin are used to establish the diagnosis. The total bilirubin level test is one of the indicators that can be used to determine the presence or absence of liver function

damage caused by an increase in bilirubin level (hyperbilirubinemia). This is because bilirubin which should be secreted by the liver to the bile cannot be implemented and, as a result, bilirubin will accumulate in the blood. Hyperbilirubinemia is caused by damaged liver parenchyma cells. Damage to cell membranes can result in the entry of free radicals into cells so that cells will experience intracellular damage such as organelles, cytoskeleton, enzymes, non-membrane proteins, and DNA. Free radicals will attack enzyme components, especially ATPase which is composed of a series of amino acids containing a sulfhydryl group, eventually, ATPase becomes inactive and some of the cytosolic Ca^{2+} control functions are disrupted. Disruption of Ca^{2+} causes an increase in Ca^{2+} in the cytosol and attacks the mitochondria and endoplasmic reticulum. Because Ca^{2+} in the mitochondria and endoplasmic reticulum will be higher and exacerbated by not functioning properly ATPase and 1,4,5 inositol triphosphate (IP3) Ca^{2+} efflux will occur into the cytosol. As a result, the conformational change of the IP3 receptor triggers the opening of ion channels on the plasma membrane and ends up with the influx of extracellular Ca^{2+} into the cytosol which further increases from cytosolic C^{2+}. This increased the activity of phospholipases, endonucleases, and proteases. Increased phospholipase will damage lipid membranes, increased endonuclease activity will damage DNA, and protease activity will damage protein components. This causes complex biochemical changes and can eventually result in liver cell damage [20].

In the present study, it was assumed that the combined extract did not affect liver function. The administration of a combined extract of tea and mango mistletoe has not shown a significant effect on bilirubin, levels of SGPT, SGOT, albumin, cholesterol, and triglycerides, however, tends to reduce levels of globulins, serum total protein, HDL, and LDL due to scavenging activities in the combination of these extracts (see Table 1). This is because the extract contains flavonoids, which can lower LDL and prevent free radicals through inhibition of lipid peroxidation of unsaturated fatty acids in cell membranes and help restore cells damaged by oxidative stress such as liver cells in non-alcoholic fatty liver disease. Flavonoids reduce triglyceride levels by inhibiting the action of the HMG-CoA reductase enzyme, resulting in the inhibition of acetyl-CoA to mevalonate which is a precursor of LDL, VLDL, and triglycerides. Flavonoids have an anti-inflammatory effect by inhibiting the activity of cyclooxygenase and lipoxygenase which play a role in the conversion of arachidonic acid into cytokines such as prostaglandins and leukotrienes that cause inflammation in tissues [21].

Quercetin is an antioxidant flavonoid and, thus, able to inhibit free radicals, thereby unable to damage the liver cells. The word antioxidant is originated from the word “anti” (fight) and “oxidant” and known as free radicals. Thus, the antioxidant is a compound that can delay, inhibit or prevent lipid or other molecule oxidations by inhibiting the initiation or propagation of the oxidative chain reaction [22]. Oxidation is a chemical reaction that can produce free radicals that trigger a chain reaction that can damage the liver. Cell life depends on the supply of nutrition and oxygen. Nevertheless, oxygen also potential to cause cell damage through an oxidation process. Oxidation which is initiated by an oxidant will release free radicals, which will continuously attract stability by taking electrons from other atoms. This will result in the production of other free radicals. Every time an electron is released or caught by a free radical, then a new free radical will form. The newly formed free radical will do the same thing. Through this mechanism, the free radical chain is created. If this condition continues for a long time, the body’s cells will be damaged. Toxic substances, as well as free radicals, can damage the liver cells and tissues. Under normal circumstances, free radicals will not cause liver damage because the liver has a better defense system than other organs. However, if there is a part of the liver which has been damaged very extensively, the liver will lose its function immediately.

The liver is an organ that is important for detoxifying useless or harmful chemical substances. The liver has a high ability to bind chemical substances [17]. Most toxicants enter the body through the gastrointestinal tract. After being absorbed, toxicants are carried through the portal vein to the liver. The liver has many binding sites. The levels of enzymes that metabolize xenobiotic compounds in the liver are also high (especially cytochrome P450). This makes some toxins less toxic and more soluble in water and, thus, easier to excrete. But, in some cases, toxicants are activated and able to induce lesions. The liver lesion is centrilobular, which is associated with a higher level of cytochrome P450. In addition, the relatively low glutathione level when compared to the glutathione level in other parts of the liver has a role in activating toxicants [23]. Toxicants can cause various types of toxic effects on various organelles in the liver cells, such as fatty liver (steatosis), necrosis, cholestasis, and cirrhosis [17].

Based on histopathology examination of the female rats' liver on both pyknotic and karyolysis cells showed more increase between all treatment groups (Groups 2, 3, 4) than the control group (Group 1) (see Table 2). One of the causes of many karyorrhectic liver cells was too many toxic substances exposed to the liver cells. Liver cells play important roles in lipid metabolism. If the liver cells are exposed to the toxic substances continuously, then the metabolic process in the liver will be disrupted and cause damage to the liver cells’ tissue structure in the form of the necrotic liver [17]. Necrosis in the liver cells is usually indicated by shrinkage of liver cell nuclei, their boundaries are irregular, and the colors are dark, which are the characteristics of pyknotic cells. The pyknotic nuclei are shrunk nuclei as a result of cytoplasm homogenization and eosinophilic increase. After becoming pyknotic, the liver nuclei can be destroyed and leave chromatin fragments scattered in the cells; this process is called karyorrhexis. Then, if the nuclei are dead due to losing the ability to be colored, this process is called karyolysis [24]. Pyknotic nuclei are the initial stage of necrosis; necrosis is indicated by changes in the liver cells' nuclei [25].

MESA-DP which enters the body through the digestive tract will undergo the first metabolism in the liver because the liver is the main metabolic site that will detoxify and eliminate all toxic substances, both endogenous and exogenous. Therefore, the liver is a potential organ to be damaged by countless various types of pharmaceutical and environmental chemical compounds. Injury is the result of various things, such as direct toxins, liver convention against a xenobiotic becoming an active toxin, and immune mechanism [17].

One of the causes of liver necrosis is too many free radicals. Free radicals are molecules that have unpaired electrons and are reactive. The presence of these electrons in the body tends to attract other electrons belonging to other molecules, thus changing the molecules into free radicals. These molecules are called free radicals because there are reduced or added electrons. The increase of oxidative stress in the liver is caused by free radicals, which can damage the liver cells. In the present study, necrotic cells were observed in the control group. This might be due to no examination of the rats’ liver before sampling. Hence, it might be that the rats taken as samples already had liver damage. Other possible reasons might be because the cage condition was less than ideal, administration of the food and drink was less varied and following the standard, stress factor, the effects of other substances or diseases, immunities, and susceptibilities of the rats. Nevertheless, there was another factor caused by MESA-DP preparations, which was the drying process, that will affect the antioxidant level in the mistletoes because the duration of the drying and storage process also affects the antioxidant power [26]. There was a tendency for hepatocytes to

undergo increases in the pyknotic, karyorrhectic, and karyolysis cells count due to the administration of the mistletoe's combination.

The present study not only examined the clinical biochemistry but also observed the histopathology. The liver can have several changes such as irreversible and reversible damage. Degeneration is reversible damage in which the normal structure of cells changes. The cause of degeneration is a biochemical disorder caused by anemia, abnormal metabolism, and toxic chemical substances. The degeneration that occurs continuously will cause irreversible cell death. Cell death may happen through necrosis and apoptosis. Necrosis is cell death caused by acute cell damage, while apoptosis is a type of programmed cell death used by multicellular organisms to remove cells that are no longer needed by the body [27]. Necrosis can be focal (central, intermediate, peripheral) or massive. Usually, necrosis is acute [17]. The characteristics of necrosis are the appearance of necrotic heart muscle fragments or cells without nuclear outlay or no cells with an inflammatory reaction. Whether or not the remaining liver cells appear depends on the duration and type of necrosis [28].

CONCLUSION / ЗАКЛЮЧЕНИЕ

The outcomes of the current study demonstrated that serological studies for liver function tests proved the protective consequences of MESA-DP in mice and did not cause changes in the levels of total bilirubin, SGOT, SGPT, albumin, cholesterol, and triglycerides, indicating the safe effect of MESA-DP. However, administration of MESA-DP caused a significant increase in necrotic liver cells compared to the control group. The observed hepatoprotective effects may be due to the presence of phytochemicals as well as various biologically active secondary metabolites such as quercetin and flavonoids identified as the main compounds. The protective mechanism is through modulation of oxidative stress and augmentation of antioxidant enzymes and quercetin is known to protect against liver damage by inhibiting inflammatory biomarkers. In future, it is necessary to maintain the availability of natural materials wisely so that conventional and *in vitro* propagation and cultivation are needed. Because the tea and mango mistletoe are beneficial for the liver health of experimental animals, it is advisable to perform clinical testing on humans.

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In this study, the natural ingredients used have limitations in the availability of phytopharmacy	Используемые в исследовании натуральные ингредиенты имеют ограничения в плане доступности фитопрепаратов
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Data sharing	**Раскрытие данных**
Raw data could be provided upon reasonable request to the corresponding author	Первичные данные могут быть предоставлены по обоснованному запросу автору, отвечающему за корреспонденцию
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