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Hepatoprotective activity of *Mitragyna speciosa* Korth. on liver damage caused by *Tuak*

R G P Panjaitan* & M W Mery

Biology Education Department, Faculty of Teacher Training and Education, Tanjungpura University, Pontianak 78124, Indonesia

*E-mail: ruqiah.gpp@fkip.untan.ac.id

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This study aims to evaluate the protection and restoration capacity of *kratom* leaves extracts on liver damage caused by *tuak*. Group A consist of 12 mice and divided into A1 administered 0.5 mL/20 g body weight aquadest, A2 administered *kratom* leaves ethanol extract at dose 0.29 mg/20 g body weight and A3 administered silymarin at dose 0.70 mg/20 g body weight on days 1 to 7, then continued given *tuak* at dose 0.26 mL/20 g body weight on days 8 to 14. Group B consist of 12 mice and divided into B1, B2, and B3, on days 1 to 7 they were administered *tuak* at dose 0.26 mL/20 g body weight then continued with aquadest at dose 0.5 mL/20 g body weight (B1), *kratom* leaves ethanol extract at dose 0.29 mg/20 g body weight (B2), and silymarin at dose 0.70 mg/20 g body weight on days 8 to 14 (B3). The average levels of ALT enzyme in the treatment groups respectively showed 29.13±0.40 U/L (A1); 25.25±0.69 U/L (A2); 20.51±1.00 U/L (A3) and 31.20±0.47 U/L (B1); 26.95±0.62 U/L (B2); 23.31±0.89 (B3) ($p<0.05$). Whereas, the average levels of AST respectively at 34.23±0.63 U/L (A1); 28.64±0.40 U/L (A2); 25.13±0.83 U/L (A3) and 35.69±0.63 U/L (B1); 30,83±0.63 (B2); 27.31±0.83 (B3) ($p<0.05$). The average scoring for the treatment groups resulted in 2.67; 2.33; 1.33 respectively A1, A2, and A3 ($p<0.01$) and 2.00; 0.67; 0.33 respectively B1, B2 and B3 ($p<0.01$). It is concluded that *kratom* leaves are not effective to protect and to restore the liver.

Keywords: *Kratom* leaves, Liver histopathological, Serum enzymes, *Tuak*

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The body is closely related to the process of metabolizing nutrients and xenobiotics in the liver. In connection with this function, the liver is often exposed to compounds that enter the body, even these compounds can cause liver damage. Some researchers report that liver damage can be caused by consuming drugs such as paracetamol,[1] and by viral hepatitis[2,3], aflatoxin fungi[4,5], as well as an unhealthy lifestyle due to consuming alcoholic beverages[6]. Liver damage is characterized by an increase in serum levels of the aspartate transaminase (AST) and alanine transaminase (ALT) enzymes[7-13], levels of alkaline phosphatase (ALP), total bilirubin, and total protein[9], as well as histopathological features which are characterized by steatosis in liver cells, necrosis, fatty degeneration, and hydropic degeneration[9-12].

Overall, it is stated that the hepatoprotector potency cannot be separated from the active compound contained in a preparation. Several types of plants that have been reported to have properties to protect the liver include *Eurycoma longifolia* Jack.,[7,10] *Pithecellobium lobatum* Benth.,[11] *Curcuma longa* L.,[12] *Silybum marianum* L. Gaertn.,[13] *Nigella sativa* L.,[14] *Piper betle* Linn.,[15] *Cayratia trifolia* L. Domin,[8] and *Cassia siamea* LAMK[16]. Besides, liver damage can be overcomed through the consumption of foods containing antioxidants[17].

Mitragyna speciosa Korth. is a type of plant belonging to the *Mitragyna* genus of the Rubiaceae family. *Mitragyna speciosa* Korth. is distributed in Africa[18,19], Asia[18] and Southeast Asia[19,20]. Gong (2012)[18] reported that several species in the genus *Mitragyna* have medicinal properties. *Mitragyna spesiosa* Korth. is also known as *biak-biak* in Malaysia and as *kratom* in Thailand[21]. The *kratom* plant is reported to contain various compounds including 7α-hydroxy-7H-mitragynine, paynantheine, speciogynine, and speciociliatine[22], ajmalicine, corynantheidine, isomitraphylline, mitraphylline, and isocorynanticatechin, as well as the flavonoids

*Corresponding author

saponins, triterpenoid saponins quinovic acid 3-O-β-D-quinovopyranoside and quinovic acid 3-O-β-D-glucopyranoside, including some derivatives from a group of glycosides such as 1-O-feruloyl-β-D-glucopyranoside, 3-oxo-α-ionyl-O-β-D-glucopyranoside, roseoside, benzyl-β-D-glucopyranoside, epivogeloside, and vogeloside[23]. From generation to generation, people in the Southern Thailand region have used *kratom* leaves in various ceremonies and other informal activities. Usually, people in the Southern Thailand region consume *kratom* leaves by chewing or swallowing fresh *kratom* leaves[24]. More than that, it has been a decade since people in Malaysia, Thailand, and other parts of Southeast Asia have used *kratom* leaves in traditional medicine. In Indonesia, especially in the Kedamin Hulu area, Putussibau Selatan Subdistrict, Kapuas Hulu Regency, people traditionally use *kratom* leaves to treat diarrhoea, muscle pain and hypertension as well as a stamina booster.

The Dayak community in Kapuas Hulu Regency also has local wisdom to make *tuak. Tuak* is an alcoholic drink made from fermented *siwalan* sap[25], fermented black rice and black sticky rice[26], and fermented white sticky rice[27]. *Tuak* is popular for consumption by people in Indonesia, especially in the areas of North Sumatra, Pekanbaru[28], North Toraja[25], and West Kalimantan[27]. Toraja people consume *tuak* because it is a tradition and it is considered as an honorary drink. Therefore *tuak* is consumed not only at traditional ceremonies or parties but also in daily activities[25]. In contrast to the case in West Kalimantan, especially the Dayak people, the consumption of *tuak* is something that has become a habit during traditional ceremonies or parties.

Ethanol is the alcohol contained in alcoholic beverages. Ethanol is metabolized through oxidative and non-oxidative pathways in the liver. Through the oxidation process, ethanol that enters the body is converted by the alcohol dehydrogenase enzyme into acetaldehyde, then re-oxidized by the aldehyde dehydrogenase enzyme to form acetic acid, and ethanol metabolic products are reported to have contributed to various health problems[29-32]. When it is related to the habit of consuming *tuak* by the community with the benefits of *kratom* leaves as a stamina enhancer, this study aims to evaluate the protection and restoration capacity of *kratom* leaves ethanol extract on liver damage caused by *tuak*.

Material and Methods

Collection of plant material

Fresh *kratom* leaves were taken from a garden in Nanga Nyabau Village, North Putussibau District, Kapuas Hulu Regency, West Kalimantan Province, Indonesia. Furthermore, the identification of the plant species was carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Indonesia with a letter numbered 045/A/LB/FMIPA/UNTAN/2018.

Experimental animals

The experimental animals used were male Balb/c mice aged 2-3 months with body weight ranging from 24-36 g. The experimental animals were obtained from the Central Food and Nutrition Laboratory at Universitas Gadjah Mada, Yogyakarta, Indonesia. Before the experiment started, the experimental animals were acclimatized for 14 days. During the acclimatization period, the experimental animals were fed with standard feed and drinking ad libitum, and their health was observed by weighing their bodies. All procedures related to the care and treatment of animals in this study have met the ethical requirements stated in letter number 218.2/ UNRIYO/PL/IX/2018 from the Animal Ethics Commission of Universitas Respati Yogyakarta, Indonesia.

Plant extraction

The *kratom* leaves were separated from the stalks and stem then cleaned and weighed wet weight, then dried. Dried leaves as much as 285 g were extracted by maceration using distilled 96% ethanol solvent. Samples were immersed for 24 h at room temperature. Immersing was done 3 times. The filtrate was concentrated using a vacuum rotary evaporator. The extract obtained was 19.01 g with a yield of 6.67%. The whole extraction process was carried out according to Harborne[33].

Making yeast Tuak

The making of *tuak* yeast refers to the local wisdom of the Dayak tribe in Sungai Ayak Hamlet, Belitang Hilir District, Sekadau Regency, West Kalimantan Province, Indonesia. The steps for making yeast, namely pepper, coriander, galangal, cinnamon, and cloves are ground until smooth and then rice flour are added. After all the ingredients are evenly mixed, the dough is formed into a flat round with a diameter of ± 5 cm then dried in sun to dry and the yeast is ready to use.

Making Tuak

The making of *tuak* refers to the local wisdom of the Dayak tribe in Sungai Ayak Hamlet, Belitang Hilir District, Sekadau Regency, West Kalimantan Province, Indonesia. The steps for making *tuak* are 4 kg of white sticky rice, washed, then cooked for ± 50 min by adding 4 L of water. Furthermore, the cooked sticky rice is cooled and then sprinkled with ± 100 g of fine yeast. The sticky rice that has been mixed with yeast is put in a plastic jar and let stand for 21 days. After 21 days of fermentation, the water produced from the fermentation process is filtered. The water that is produced is called *tuak*. From the test results conducted by the Center for Drug and Food Control Pontianak City which is reported in the letter number LP -18.097.99.13.06.0031.K, and the alcohol content in the *tuak* produced from the fermentation process of white sticky rice is 14.00% and is stated to be included in the alcoholic beverage class B. In the Presidential Regulation No. 74 of 2013 concerning Control and Supervision of Alcoholic Drinks, alcoholic drinks of class B are alcoholic drinks with an ethanol content of more than 5% to 20%. Alcohol at this level is high enough and can make one drunk, especially if consumed in large quantities.

Protection and restoration capacity of kratom leaves extract

As many as 24 mice were grouped into 6 groups, each consisting of 4 mice. Group A (protection), divided into A1 was given distilled water at a dose of 0.50 mL/20 g body weight for 7 consecutive days followed by giving *tuak* at a dose of 0.26 mL/20 g body weight for 7 consecutive days. Group A2 was given *kratom* leaf extract at a dose of 0.29 mg/20 g body weight for 7 consecutive days followed by giving *tuak* at a dose of 0.26 mL/20 g body weight for 7 consecutive days. Group A3 was given silymarin at a dose of 0.70 mg/20 g body weight for 7 consecutive days followed by giving *tuak* at a dose of 0.26 mL/20 g body weight for 7 consecutive days. Then, group B (restoration) was divided into group B1 was given *tuak* with a dose of 0.26 mL/20 g body weight for 7 consecutive days followed by giving distilled water with a dose of 0.50 mL/20 g body weight for 7 consecutive days. Group B2 was given *tuak* at a dose of 0.26 mL/20 g body weight for 7 consecutive days followed by giving *kratom* leaf extract at a dose of 0.29 mg/20 g body weight for 7 consecutive days. Group B3 was given *tuak* at a dose of 0.26 mL/20 g body weight for 7 consecutive days followed by giving silymarin a dose of 0.70 mg/20 g body weight for 7 consecutive days. After being given the treatment for 14 days, on the days 15, blood samples and liver were taken.

Dosage of tuak and dose of kratom leaf extract

Traditionally, for one drink, people consume a certain amount of *tuak* and this amount is converted from the human dose to the dose of mice to obtain 0.26 mL/20 g body weight. Likewise, for *kratom* leaf doses, for one-time consumption, people usually use 5 fresh *kratom* leaves weighing 6.20 g and after extraction, it is obtained 0.113 mg. This dose was converted from the human dose to mice to obtain 0.29 mg/20 g body weight.

Biochemical evaluation

The blood sample is taken from the orbital sinus. The blood samples obtained were centrifuged at 4000 rpm for 15 min. Measurement of levels of alanine transaminase and aspartate transaminase enzymes using a kit from DiaSys (Diagnosis System) with the UV-Test method.

Histopathological examination

Animals were sacrificed through cervical dislocation. The collected liver was routinely processed and then stained with hematoxylin-eosin (HE). The results of histopathological staining were observed under a light microscope. The procedure for making histopathology refers to Kiernan.[34] The changes that appeared on the histopathological features of the liver were scored (Table 1).

Data analysis

This experiment was carried out using a completely randomized design. Data on levels of alanine transaminase and aspartate transaminase enzymes were statistically analyzed using the SPSS 20 for Windows program and followed by Duncan's test at the 5% level if significantly different. The scoring

Table 1 — Criteria for liver histopathological assessment

Score	Description
0	No specific changes were found
1	Liver cells undergo hydropic degeneration and mild degeneration of fat, evenly
2	Liver cells undergo fatty degeneration and moderate steatosis, are multifocal
3	Liver cells undergo fatty degeneration, steatosis, and a severe degree of dystrophy, which is multifocal

data for the degree of liver damage were statistically analyzed using the SPSS 20 for Windows program and continued with the Duncan test at the level of 1% if significantly different.

Results

The results of this study showed that the average levels of ALT, AST, and liver histopathological scoring in the aquadest group and in the group with *kratom* leaf ethanol extract which preceded the *tuak* administration were higher than silymarin. Likewise, the average ALT, AST enzyme levels, and liver histopathological scoring in the *tuak* group which preceded the administration of distilled water and the group with the ethanol extract of *kratom* leaves were higher than silymarin ($p<0.05$) (Table 2,3,4, and 5). For the histopathological analysis of the liver, the group given distilled water and ethanol extract of *kratom* leaves showed inflammation which was marked by infiltration of lymphocytes and Kupffer

Table 2 — Average levels of ALT and AST enzymes in mice by administering distilled water at a dose of 0.5 mL/20 g body weight on days 1 to 7 and followed by administration of *tuak* at a dose of 0.26 mL/20 g body weight on days 8 to 14 (A1) (A2), with the administration of ethanol extract of *kratom* leaves at a dose of 0.29 mg/20 g body weight the on days 1 to 7 and followed by administration of *tuak* at a dose of 0.26 mL/20 g body weight on days 8 to 14, and A3 by giving silymarin a dose of 0.70 mg/20 g body weight on days 1 to 7 and followed by the administration of *tuak* at a dose of 0.29 mL/20 g body weight on days 8 to 14. (n = 4)

Group A	Average Enzymes Level	
	ALT (U/L)	AST (U/L)
Aquadest (A1)	29.13^{c}±0.40	34.23^{c} ±0.63
Kratom Leaves Extract (A2)	25.25^{b}±0.69	28.64^{b}±0.40
Silymarin (A3)	20.51^{a}±1.00	25.13^{a}±0.83

^{a,b,c}the same letter is not significantly different in the Duncan test with a level of 5% The number after the ± indicates the standard deviation (SD)

Table 3 — Average levels of ALT and AST enzymes in mice by giving *tuak* at a dose of 0.26 mL/20 g body weight on days 1 to 7 and followed by giving distilled water a dose of 0.5 mL/20 g body weight on days 8to 14 (B1), B2 with giving *tuak* at a dose of 0.26 mL/20 g body weight on days 1 to7 and followed by giving *kratom* leaf ethanol extract at a dose of 0.29 mg/20 g body weight on days 8 to 14, and B3 by giving *tuak* at a dose of 0.29 mL / 20 g body weight on days 1 to 7 and followed by giving silymarin a dose of 0.70 mg/20 g body weight on days 8 to 14. (n = 4)

Group B	Average Enzymes Level	
	ALT (U/L)	AST (U/L)
Distilled water (B1)	31.20^{c}±0.47	35.69^{c}±0.63
Kratom Leaves Extract (B2)	26.95^{b}±0.62	30.83^{b}±0.63
Silymarin (B3)	23.31^{a}±0.89	27.31^{a}±0.83

^{a,b,c}the same letter is not significantly different in the Duncan test with a level of 5% The number after the ± indicates the Standard Deviation (SD)

Table 4 — Average liver scoring of mice by giving distilled water at a dose of 0.5 mg/20 g body weight on days 1 to 7 and followed by giving *tuak* at a dose of 0.26 mL/20 g body weight on days 8 to 14 (A1), A2 with the administration of leaf ethanol extract *kratom* dose of 0.29 mg/20 g body weight on days 1 to 7 and followed by administration of *tuak* at a dose of 0.26 mL/20 g body weight on days 8 to 14, and by giving silymarin at dose 0.70 mg/20 g body weight on days 1 to 7 and followed by the administration of *tuak* at a dose 0.29 mL/20 g body weight on days 8 to 14 (A3)

Group A	Average Score
Aquadest (A1)	2.67^{a}
Kratom Leaves Extract (A2)	1.33^{a}
Silymarin (A3)	0.67^{a}

^{a,b,c}indicates the same letter is not significantly different in the Duncan test with a level of 1%

Table 5 — Average liver scoring of mice with *tuak* administration at a dose of 0.26 mL/20 g body weight on days 1 to 7 and followed by giving distilled water at dose 0.5 mL/20 g body weight on days 8 to 14 (B1), B2 with a dose of *tuak* 0.26 mL/20 g body weight on days 1 to 7 and followed by giving *kratom* leaf ethanol extract at dose 0.29 mg/20 g body weight on days 8 to 14, by giving *tuak* at dose 0.29 mL/20 g body weight on days 1 to 7 and followed by silymarin at dose 0.70 mg/20 g body weight on days 8 to 14 (B3)

Group B	Average Score
Distilled water (B1)	2.33^{b}
Kratom Leaves Extract (B2)	2.00^{b}
Silymarin (B3)	0.33^{a}

^{a,b,c}indicates the same letter is not significantly different in the Duncan test with a level of 1%

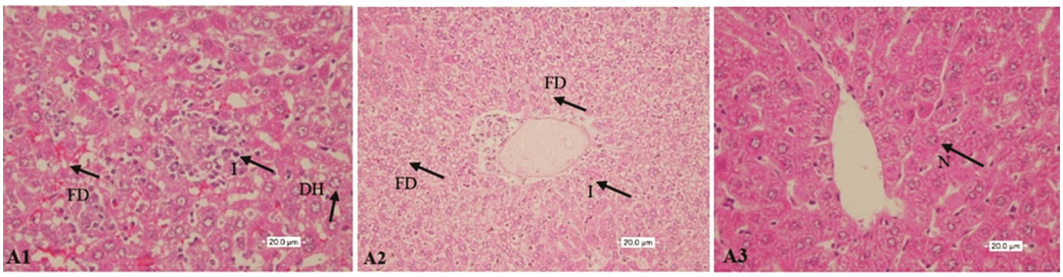

Fig. 1 — Histopathological picture of the liver of mice. A1 by administering distilled water at dose 0.5 mL/20 g body weight on days 1 to 7 and followed by administration of *tuak* at dose 0.26 mL/20 g body weight on days 8 to 14, A2 by administering *kratom* leaf ethanol extract at dose 0.29 mg/20 g body weight on days 1 to 7 and followed by administration of *tuak* at dose 0.26 mL/20 g body weight on days 8 to 14, and A3 with silymarin administration at dose 0.70 mg/20 g body weight on days 1 to 7 and followed by administration of *tuak* at dose 0.29 mL/20 g body weight on days 8 to 14. HE 40X. bar = 20 µm. I = inflammation, N = normal, HD = hydropic degeneration, FD = fatty degeneration

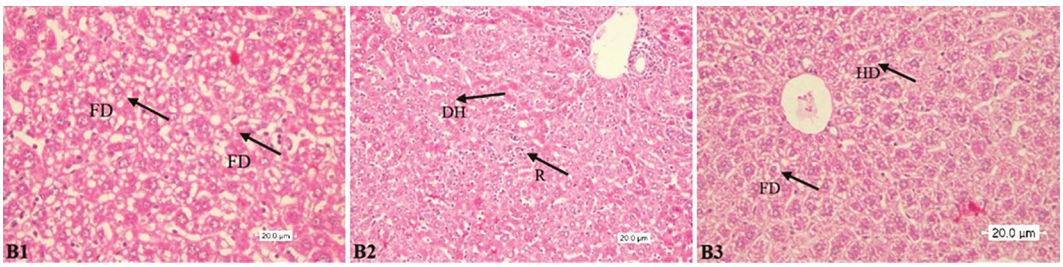

Fig. 2 — Histopathological picture of the liver of mice. B1 by administering *tuak* with at dose 0.26 mL/20 g body weight on days 1 to 7 and followed by giving distilled water at dose 0.5 mL/20 g body weight on days 8 to 14, B2 by giving *tuak* at dose 0.26 mL/20 g body weight on days 1 to 7 and followed by giving *kratom* leaf ethanol extract at dose 0.29 mg/20 g body weight on days 8 to 14, and B3 by giving *tuak* at dose 0.29 mL/20 g body weight on days 1 to 7 and followed by giving silymarin at dose 0.70 mg/20 g body weight on days 8 to 14. HE 40X. bar = 20 µm. I = inflammation, N = normal, HD = hydropic degeneration, FD = fatty degeneration

cells in the liver parenchyma, as well as hydropic degeneration and fatty degeneration (Fig. 1 and Fig. 2). According to Widyaningsih and Pertiwi[35], hydropical degeneration is caused by an increase in the amount of water in the cell so that the cytoplasm and cell organelles appear to be swollen and vacuole. This situation occurs because of the presence of a toxic substance that causes the mitochondria to be disrupted to produce the ATP energy needed to activate the sodium pump. In the absence of ATP energy, the sodium in the cell will not come out, while the sodium attracts water. Not only that, but the presence of toxic substances will also disrupt cell permeability so that the extracellular fluid will enter the intracellularly and form clear, small, and large vacuoles. These vacuoles unite to form larger vacuoles that occupy the cytoplasm and replace the cell nucleus and cause swelling which is called hydropic degeneration. Furthermore, Suhita *et al.*[36] stated that fatty degeneration can occur due to the accumulation of abnormal fat in the cytoplasm with the size of the vacuole varying in size, pushing the nucleus to the edge. Some of the causes of fatty degeneration are toxins and protein malnutrition.

Discussion

Hepatoprotectors are compounds or substances that can protect cells as well as repair damage to liver tissue due to the influence of toxic substances. Measuring levels of ALT and AST enzymes is one way to detect liver damage[7-11,15,37-43]. Alanine transaminase is an enzyme found in the cytosol of the liver and is specific for detecting changes in the liver, while AST is commonly used to detect changes in the liver, but the presence of this enzyme is not specific only to the liver, so the levels of this enzyme can be used to detect trauma. or necrosis in other organs[7]. In normal conditions, in serum, ALT enzyme levels are lower than AST[7,10,15,20]. An increase in the levels of AST and ALT enzymes is a marker of damage to cells in the liver[7-13], but in severe liver damage, the levels of this enzyme will even be very low[9].

Based on the results of this study it is known with certainty that giving *tuak* at a dose of 0.26 mL/20 g body weight for seven consecutive days can cause damage to liver cells. The ethanol content in *tuak* which used in this study was 14%, and this ethanol content was higher than the *tuak* used in the previous study. Previously, Panjaitan and Rahmawati[27]

reported that consuming *tuak* with an alcohol content of 9.95% at a dose of 0.26 mL/20 g body weight for seven consecutive days can result in changes to the histopathological picture of the pancreas and an increase in blood glucose levels. This means that the ethanol contained in *tuak* is indeed proven to be toxic like ethanol in other alcoholic beverages.

Several researchers have previously reported that consuming alcoholic beverages will increase the number of free radicals, in turn, reduce antioxidants in the body, resulting in oxidative stress. More than that, oxidative stress will cause lipid peroxidation which will result in damage to cell membranes so that the process of regulating passive and active transport processes will be disrupted. Furthermore, the loss of cell membrane integrity will lead to accumulation of fluid in the tissue which is called oedema and leads to necrosis[29-32].

Silymarin belongs to the class of flavonoids (silybin, isosilybin, silychristin, silydianin and taxifoline). Silymarin is contained in the dried fruit of the *Silybum marianum* plant. *Silybum marianum* is a plant in the Asteraceae family and has been used to treat liver and bile duct diseases, especially cirrhosis, jaundice, and hepatitis. Silymarin is reported to have antioxidant power as well as serve as a hepatoprotector[7,13]. The mechanism of action of silymarin as a hepatoprotector is related to its role as an antioxidant, lipid anti-oxidation, and increasing detoxification power. Silymarin also plays a role in increasing protein synthesis of liver cells, reducing the activity of substances that cause tumours, maintaining the presence of mast cells (a type of cell in connective tissue that contains basophils, possibly associated with the formation of histamine and heparin), modulating immunity, anti-inflammatory, and antifibrosis[7,13].

Overall, the results of this study indicate that although the group with *kratom* leaf extract also showed high results for measuring the levels of ALT and AST enzymes and scoring high degrees of liver damage, the administration of *kratom* leaf extract does not worsen damage to liver cells that have been damaged due to ethanol contained in *tuak*. However, the ethanol extract of *kratom* leaves cannot be said to be able to overcome liver cell damage as well as silymarin.

Conclusion

The ethanol extract of *kratom* leaves with a dose of 0.29 mg/20 g body weight did not have the ability of protection or restoration of the liver.

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Conflict of Interest

The authors declare no conflict of interest.

Author's Contributions

All authors fully contributed to all stages, including the stages of methodology, data analysis, and article writing-review. Further, all authors have read and approved the manuscript.

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