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Activities of *Cayratia trifolia* Fruit on Oxidative Stress and Histological Change in Physical Stress-Induced Mice

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ABSTRACT

Lakum fruit (*Cayratia trifolia* L. Domin) contains secondary metabolite compounds that have antioxidant properties. *C trifolia* belongs to the Vitaceae family, known as tropical grapes that have a sweet and itchy taste. This study aims to determine the potential of *C. trifolia* fruit methanol fraction as an antioxidant based on the parameters of malondialdehyde levels, superoxide dismutase enzymes, SGOT enzymes, SGPT enzymes, kidney and liver histology. The study used 24 male Swiss mice which were divided into 6 treatments, namely normal control, negative control, positive control (vitamin E), and *C. trifolia* methanol fraction 115; 230; 460 mg/kgBW. Physical stress induction was given in the form of fasting and swimming for 5 days. The data were analyzed by one-way ANOVA and descriptive analysis for histological parameters. The results showed that the methanol fraction of 115 mg/kg BW decreased the best levels of malondialdehyde serum (38 μM), SGOT (34.7 U/L) and SGPT (34.6 U/L) compared to other doses. Doses of 115 mg/kgBW also provided the best levels of the superoxide dismutase enzymes (1.73 U/L) almost equal to vitamin E as positive control. Observations of kidney and liver histology showed a decrease in damage to hepatocytes and renal glomerulus in the 115 mg/kgBW *C. trifolia* methanol fraction. This research contributes to the development of *C. trifolia* as a natural antioxidant.
Keywords: Antioxidant; *Cayratia trifolia*; Enzyme; Kidney; Liver

INTRODUCTION

Stress is a normal physiological reaction of the body aimed at mobilizing available resources and limiting the impact on the body of negative factors (Doreddula *et al*., 2014). However, stress that goes beyond the normal condition disrupts the homeostatic system, neuroendocrine, behavioural, and emotional reactions. Excessive physical activity is known to be one of the stress factors and causes an increase in free radical production. The increasing number of free Reactive Oxygen Species (ROS) that are not accompanied by the production of natural antioxidant compounds will cause oxidative stress conditions.

One of the critical parameters of oxidative stress is increased levels of malondialdehyde (MDA). Malondialdehyde compounds are toxic compounds resulting from fatty acid peroxidation in cell membranes (Sandhiutami *et al*., 2016). One of the endogenous antioxidant enzymes is superoxide dismutase (SOD). The superoxide dismutase enzyme converts superoxide anions into hydrogen peroxide which is a substrate for the enzyme catalase (Zheng *et al*., 2023). Exogenous antioxidants obtained from food such as vitamin A, vitamin C, vitamin E, enzyme cofactors (Q10), minerals (zinc and selenium), peptides (glutathione) and other secondary metabolites such as flavonoids and alkaloids from plants.

Exogenous antioxidant intake is important for maintaining the balance between ROS and the antioxidant system in the body. One of the natural resources that have the potential as an antioxidant is a lakum fruit (*Cayratia trifolia* L.). Lakum fruit (*C. trifolia*) which belongs to the family Vitaceae is a tropical grape. Raw lakum fruit is green colour and turns purple-black when it is ripe. The taste of this fruit is sweet but if eaten directly can cause itching in the lips and throat. The itching that occurs when eating the lakum is caused by the tannin compounds (Sowmya *et al*., 2015). *C. trifolia* fruit is found abundantly at the riverside in the West Kalimantan region. In traditional medicine, lakum has important properties as a medicine for women after childbirth and for the treatment of ulcers. Local people in West Kalimantan used lakum fruits as spices for local cuisine.

Traditionally, infusions *C. trifolia* seeds are used for the treatment of diabetes. Pharmacological evidence of stem extract has shown anti-inflammatory, antihiperlipidemia, and larvacides (Yusuf *et al*., 2017; Yusuf *et al*., 2021; Rousdy *et al*., 2021). Mohammed *et al*. (2017) reported that administration of the root ethanolic extract of *C. trifolia* showed recovery of glutathione and catalase enzymes in diabetic rats.

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The ethanol extracts from all parts of *C. trifolia* are known to contain secondary metabolites of alkaloids, flavonoids, steroids, and terpenoids, saponins and tannins. Flavonoid is the highest content in Cayratia etanol extract (Hikmawanti *et al*., 2021). The leaves contain stilbenoid compounds, kaemferol, myricetin, quercetin, and triterpenes. Secondary metabolite groups such as phenolic, alkaloids, flavonoids and terpenoids, are known as antioxidant compounds. Rabeta and Lin (2015) showed that the methanol extract of *C. trifolia* fruit had an antioxidant activity which was demonstrated through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test. The IC_{50} value of the DPPH test of the methanol extract of *C. trifolia* fruit was 318,621 µg/mL. Although *C. trifolia* fruit hold significant biomedical importance, there is still a lack of reports regarding its antioxidant activity to reduce the stress effect. Therefore, our study aims to determine the antioxidant activity of the methanolic fraction of *C. trifolia* fruits in mice with physical stress (in vivo).

MATERIALS AND METHODS

Materials

The materials used in this research were distilled water, methanol (Merck), ethanol (Merck), ethyl acetate (Merck), n-hexane (Merck), EDTA (Merck), chloroform (Merck), trichloroacetic acid (Merck), thiobarbiturate acid (Merck), epinephrine solution (Sigma), SGOT SGPT reagent kit (Glory Diagnostics), xylol (Merck), paraffin (Indopath), hematoxylin (Merck), eosin (Merck), buffered neutral formalin (Indopath) and canada balsam (Merck). All animal subjects were obtained from a mouse farm in Pontianak. The equipment used in this study included an analytical balance (Ohaus), a microtome, staining jars, syringes, micropipettes (Dragonlab), a spectrophotometer (Shimadzu), an incubator (Memmert), glasswares (Pyrex).

Methods

Plant collection and extract preparation

The mature fruits of *C. trifolia* were collected from Sungai Kakap District, West Kalimantan. Then the fruits were washed and blended. The fruit seeds were separated using a sieve. The fruits were freshly macerated in methanol for 3x24 hours. Every 24 hours, the extract is taken and filtered, then the solvent was replaced with a new one. The extract was concentrated under vacuum pressure using a rotary evaporator. The methanol extract was partitioned with ethyl acetate and n-hexane. The fraction used in the antioxidant test was the methanol fraction.

Animal Handling

Albino mice of 8-12 weeks old, weighing 20-35 g were used for the antioxidant test. Mice were acclimated under controlled conditions (temperature 25-28 C with a natural change in the daily cycle (12h a day, 12h a night). During acclimation, animals were not restricted in their intake of food and water (ad libitum). Ethical clearance letter was obtained from the Research Ethics Committee of the Faculty of Medicine, Tanjungpura University number 14592/UN22.9/PT.01.04.

Experimental design

The study used a randomized complete block design (RCBD) consisting of 6 treatments and 4 replications. The treatment given was as follows: normal control (mice were not given physical stress treatment), negative control (mice were given physical stress in the form of swimming and fasting), positive control (mice were given physical stress and vitamin E 26 mg/kg BW), treatment group (mice were given physical stress and given *C. trifolia* methanol fraction at a dose of 115, 230, 460 mg/kg BW).

Experimental protocol

The stress treatment was carried out for five days by fasting (not given food), but by given drinking water and swimming for ± 5 minutes every day (Wresdiyati, 2002). Swimming was done by placing a container filled with enough water so that it forces the mice to swim. After five days of stress treatment, the test animals were given *C. trifolia* methanol fraction orally, once every day for 7 days.

Blood sampling and preparation of red blood cell hemolysate

On the last day of treatment, the mice were anaesthetized with chloroform. The blood of the mice was taken through the heart and placed in a microtube which was added to the anticoagulant EDTA. Then the blood was centrifuged at 3000 rpm for 10 minutes. The yellow plasma layer was then taken with a micropipette and placed in a separate microtube for measuring MDA levels.

The pellet settled contained red blood cells, then 0.9% NaCl (0.25 mL) was added, shaken, and centrifuged at 3000 rpm for 10 minutes. The supernatant was removed and another 0.9% NaCl (0.25 mL) was added to a red blood cell pellet, shaken and re-centrifuged at 3,000 rpm for 10 minutes. The supernatant was removed. Subsequently, red blood cells (0.25 mL) were mixed with 1 mL of cold distilled water, shaken and centrifuged at 3,000 rpm fo 10 minutes.

After separating, the pellet (sediment) was removed while the supernatant was taken which is a red blood cell hemolysate (Sandhiutami *et al*., 2016).

Measurement of Superoxide Dismutase (SOD) enzyme

SOD levels were examined in red blood cells according to Misra & Fridovich (1972). Red blood cell hemolysate (250 µL) was mixed with a 3:5 chloroform-ethanol 96% mixture (400 µL) to remove haemoglobin. Mixtures were centrifuged at 3,000 rpm for 10 minutes. Clear yellow filtrate (10 µL) was taken and then added with 2960 µL carbonate buffer, 20 µL EDTA solution and 10 µL epinephrine solution. The solution was mixed homogeneously and incubated for 60 seconds at room temperature. The absorbance was measured at λ 480 nm after minutes 1, 2, and 3. The blank solution used 2970 µL of carbonate buffer, 20 µL of EDTA solution and 10 µL of epinephrine solution.

Measurement of malondialdehyde (MDA) levels

Malondialdehyde serums were examined according to the thiobarbituric acid reactive substance (TBARS) assay. The serum sample (100 µl) was mixed with 20% trichloroacetic acid (0.5 mL) and 0.67% thiobarbiturate acid (1 mL). The homogeneous solutions were heated in a waterbath at 95 °C for 15 minutes and then cooled to room temperature. After that, the solutions were centrifuged at a speed of 3000 rpm for 10 minutes. The pink supernatant was absorbed using a spectrophotometer at λ 532 nm. The absorbance was matched with the MDA standard curve (Draper & Hadley, 1990).

The standard curve for MDA levels was carried out using a 1,1,3,3-tetramethoxypropane (TMP) solution. The TMP solution was diluted to make five standard serial solutions. The TMP standard solutions were reacted with TCA and TBA as in serum treatment. The pink supernatant was absorbed using a spectrophotometer at 532 nm. Each concentration of a standard serial solution and its absorbance measurement results were plotted as a standard curve. The standard curve was analyzed using the regression equation y=a+bx, with the correlation coefficient (r) to determine the relationship between concentration and absorbance of standard solutions.

Measurement of SGPT and SGOT enzymes

The alanine aminotransferase (ALT/GPT) enzyme catalyzes the transfer of amino groups from alanine to oxoglutarate to form glutamate and pyruvate. Pyruvate is reduced by lactate dehydrogenase (LDH) with the coenzyme NADH. The reaction was seen from a decrease in the concentration of NADH to NAD^+. The aspartate aminotransferase (AST / GOT) enzyme catalyzes the transfer of amino groups from aspartate to oxoglutarate to form glutamate and oxaloacetate. Oxaloacetate is reduced by malic dehydrogenase (MDH) with the NADH cofactor. The reaction is seen by decreasing the concentration of NADH to NAD+. Reagent 1 (4 mL) was mixed with reagent 2 (1 mL) and plasma or serum sample (100 µL). The mixture was incubated for 1 minute at 30°C, and then the absorbance was read at 1, 2 and 3 minutes at 340 nm.

Histological observation

The mice were anaesthetized with chloroform and dissected. The kidneys and liver were washed in PBS solution and fixed in buffered neutral formalin (BNF). Liver and kidney histology is made by using the paraffin method and the hematoxylin-eosin (HE) staining method (Suvarna *et al*., 2019). Sections of the liver and kidney (5-6 µm) were prepared and then stained with hematoxylin and eosin dye, which were mounted in a Canada balsam medium for microscopic observations.

Data Analysis

All parameters were analyzed using a one-way Analysis of Variance (ANOVA) at a 95% confidence level. If there was a difference between treatments ($p<0.05$), it would be followed by Duncan's multiple range test (DMRT) using SPSS version 15. Data is displayed in mean ± standard deviation.

RESULTS

The physical stress treatment was given to mice by making them fast for 5 days without food and swimming every day for 5 minutes. The treatment of physical stress and fasting occurred in all treatment groups. On the 5th day, weight loss was found in the physical stress treatment group. After the stress treatment, the mice were given food again for 7 days. At that time, the recovery process occured so that body weight increased again (Figure 1). The normal controls which did not experience the stress treatment had relatively constant body weight.

The liver MDA level in the negative control treatment which was only given stress was not as high as the kidney and serum levels (Figure 2). In this research, excessive oxidative caused by physical activity does not include toxic metabolites, therefore, it did not cause toxicity to the liver and kidney organs. Consequently, the liver dan kidney organ which plays a major role in the

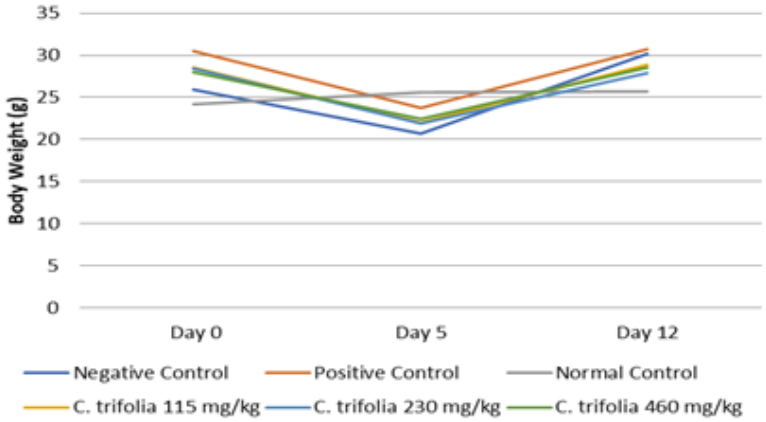

Figure 1. Graph of Animal Weight During Treatment

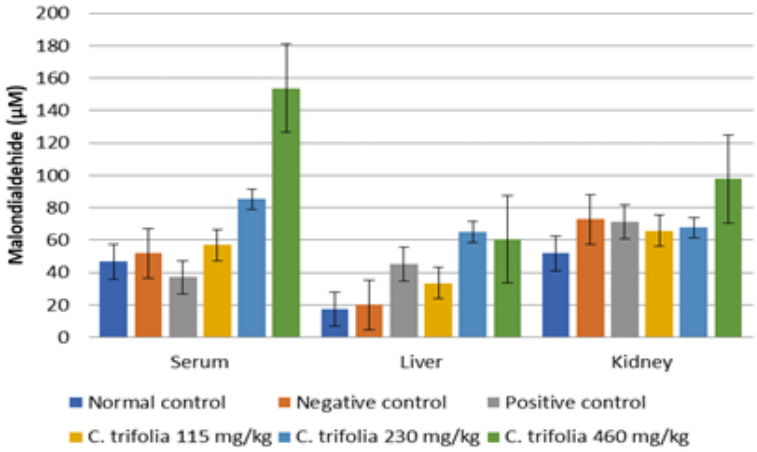

Figure 2. Effect of the methanolic fraction of *C. trifolia* on malondialdehyde (MDA) level

detoxification of toxic compounds were less affected by physical stress.

The fraction of *C. trifolia* 115 mg/kgBW can reduce MDA levels close to the positive control vitamin E (Figure 2). However, high doses of *C. trifolia* 230 and 460 mg/kgBW showed an increase in serum MDA levels, liver homogenate and kidney homogenate. It is suspected that the high antioxidant content could be turned into free radicals.

Table I shows SOD activity in the mice was affected by physical stress ($P<0.05$). Based on the result, normal mice that were not under stress showed the highest SOD levels but it was not significantly different from the negative control. This was probably because after the 5th days stress treatment, all mice had recovered after 7 days by given food intake. The treatment with *C. trifolia* at 115 mg/kgBW provided the most optimal SOD value (1.73 U/L). Whereas treatment with the highest dose of *C. trifolia* 460 mg/kgBW reduced SOD levels.

SGOT (Serum Glutamic Oxaloacetic Transaminase) is an enzyme that is usually found in the liver, heart, muscles, kidneys and brain. Meanwhile, SGPT (Serum Glutamic Pyruvic Transaminase) is the most abundant enzyme in the liver and a small amount in other organs. Both enzymes catalyze the transamination reaction or transfer of amine groups in amino acid metabolism. The fasting and physical stress treatment caused increased levels of SGPT and SGOT enzymes as in negative controls (Table I).

SGOT and SGPT activities in the mice were affected by physical stress ($P<0.05$). Treatment of *C. trifolia* 115 mg/kgBW decreased the SGOT and SGPT enzyme levels but was not significantly different from the negative control. *C. trifolia* dose of 115 mg/kgBW potentially repaired the damaged tissues of mice's liver and kidney during stress induction. The highest *C. trifolia* doses, 460 mg/kgBW increased in the SGOT SGPT enzyme. These results were consistent with the MDA level, SOD level and histological observations of the

Table I. Effect of methanolic fraction *C. trifolia* on Superoxide Dismutase (SOD), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT)

Treatment	SOD level (U/L)	SGOT level (U/L)	SGPT level (U/L)
Normal control	1.78±0.13[a]	38,25±1,70 [a]	32,30±13,87[ab]
Negative control	1.72±0.52[ab]	31,52±1,89[ab]	35,79± 9,79[a]
Positive control	1.56±0.52[ab]	26,33±10,11[b]	20,95± 2,01[b]
C. trifolia 115 mg/kg	1.73±0.23[ab]	34,75±10,04[ab]	34,62±7,17[ab]
C. trifolia 230 mg/kg	1.54±0.28[ab]	36,00±13,08[ab]	38,70±9,88[a]
C. trifolia 460 mg/kg	1.30±0.50[b]	43,00±4,60[a]	46,27±17,11[a]

Data showed an average ± standard deviation. Different values in the same column showed a significant difference between treatments (P<0,05)

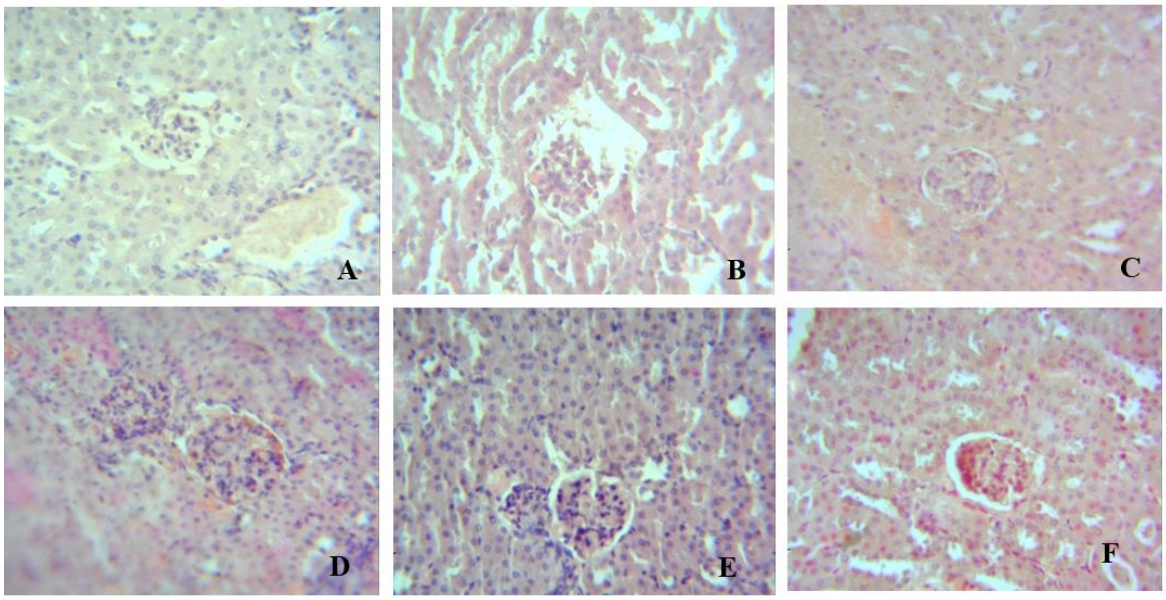

Figure 3. Light micrograph of renal section. Normal control (A), negative control (B), positive control (C), *C. trifolia* 115 mg/kgBW (D), *C. trifolia* 230 mg/kgBW (E); *C. trifolia* 460 mg/kgBW (F)

kidneys and liver which showed tissue damage at dose 460 mg/kgBW.

Kidney histology observations showed abnormal tissue structure in the negative control treatment and *C. trifolia* dose 460 mg/kg BW. The glomerulus is part of the renal nephron that filters the blood from the renal artery so that the glomerulus is vulnerable to damage. The glomerulus appeared to shrink or atrophy (Figure 3B). Shrinking glomerulus will cause Bowmann's space to become bigger. In the negative control (Figure 3B), a dilated proximal tubule was seen as indicated by the disappearance of the brush border from the tubular epithelium.

The treatment of the highest dose of *C. trifolia* 460 mg/kgBW also caused glomerular atrophy (Figure 3F), but not as damaging as the negative control. This result was consistent with kidney malondialdehyde levels showing an increase in the highest dose of *C. trifolia* 460 mg/kg. Treatment of methanol fraction of *C. trifolia* at a dose of 115 mg/kgBW, m/kgBW and vitamin E showed normal glomerular histology (Figure 3C, 3D, 3E). The normal glomerulus is characterized by the absence of atrophy and a proportional Bowman space.

Liver histology observations revealed abnormal tissue structure. The physical stress treatment and starvation would lead to hypertrophy centrilobular hepatocytes, showed by the negative control (Figure 4B). The central vein in negative control was also damaged. The flattened epithelial cells that line the central vein, were separated from the vein. The damage of the hepatocytes and sinusoids was also found in the highest dose of *C. trifolia* 460 mg/kgBW (Figure 4F). Treatment of *C. trifolia* did not show centrilobular hepatocyte hypertrophy or sinusoidal dilatation.

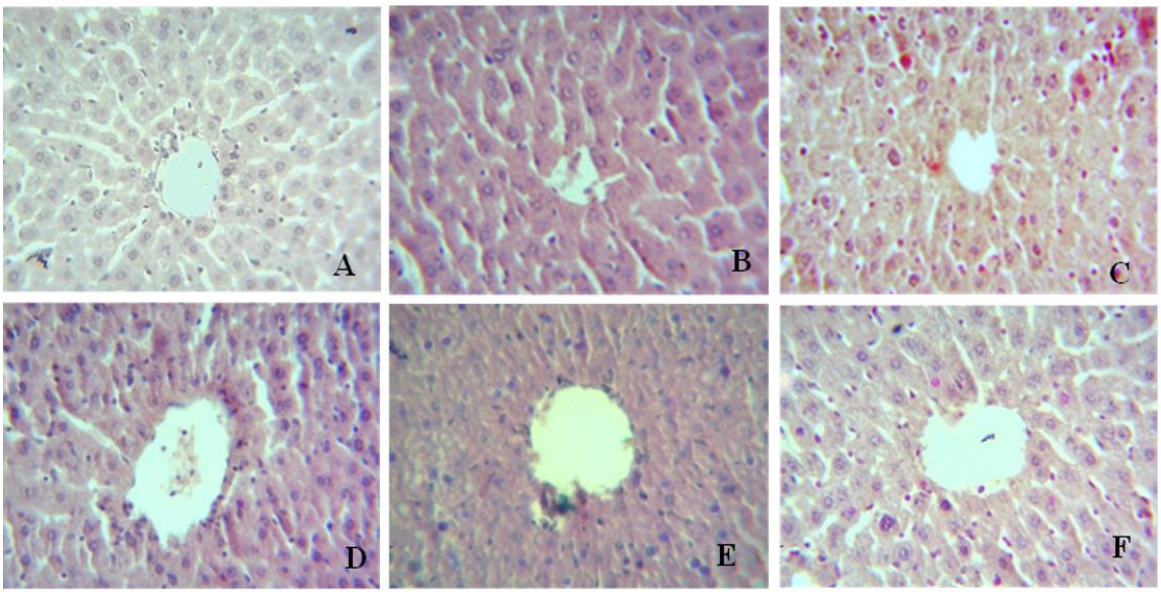

Figure 4. Light micrograph of liver section. Normal control (A), negative control (B), positive control (C), *C. trifolia* 115 mg/kgBW (D), *C. trifolia* 230 mg/kgBW (E); *C. trifolia* 460 mg/kgBW (F)

DISCUSSION

The observation of body weight indicated a decrease in weight after thephysical stress. Starvation conditions caused an increase in glucose catabolism so that glucose reserves in the form of glycogen stored in the muscles and liver will be converted to glucose. This process is called glycogenolysis. Increased glycogenolysis in muscle cells and the liver will cause weight loss. In addition, fasting conditions and physical stress also increase the lipolysis of fatty acids from adipose tissue. Fatty acids released from adipose tissue are then catabolized to form ATP energy. This mechanism is the body's response when glucose reserved in the cells are depleted. After the stress treatment, all mice were fed again therefore recovery occurred which was marked by an increase in body weight.

Malondialdehyde (MDA), an indicator of oxidative stress, is the result of lipid peroxidation due to free radicals. Lipids in the form of fatty acids are oxidized by free radicals. Oxidative stress in the form of fasting and swimming can increase MDA levels in body cells. In this research, MDA levels were measured from blood plasma or serum, liver homogenates and kidney homogenates.

MDA levels are different between serum, liver and kidney. Analysis of MDA levels in renal homogenates was higher than blood plasma MDA and liver homogenate MDA (Figure 2). During physical stress, there is an increase in mineralocorticoid (aldosterone) through the renin-angiotensin-aldosterone system (RAAS) (Correa *et al*., 2022) The renin-angiotensin-aldosterone system (RAAS) is one of the most important hormonal mechanisms in controlling hemodynamic stability by regulating blood pressure, fluid volume, and sodium-potassium balance. Over secretion of aldosterone will cause water and salt retention so the kidneys work harder (Munoz-Durango *et al*., 2016; Bruce *et al*., 2015).

The fraction of *C. trifolia* at high concentrations of 230 mg/kgBW and 460 mg/kgBW was not able to reduce levels of malondialdehyde. These results were in accordance with Onoja *et al*. (2018) that antioxidants are dependent on the dose. High concentrations of antioxidants will cause an increase in malondialdehyde levels. This was due to the content of secondary metabolites specifically phenolic compounds, if given in high quantities it will act as a prooxidant that triggers the formation of free radicals (Sotler *et al*., 2019).

The measurement of SOD enzyme activity reaction used epinephrine method. The production of oxidative radicals (ROS) will cause autoxidation of epinephrine, which was originally colourless, to a pink adenochrome compound. The presence of SOD enzymes produced by erythrocyte cells will inhibit the oxidation of epinephrine. The superoxide dismutase enzyme is a natural antioxidant enzyme that acts as a catalyst for the dismutase reaction from superoxide anions to hydrogen peroxide (H_2O_2) and oxygen (O_2). Hydrogen peroxide which is toxic to cells is then converted by the catalase enzyme into water.

Starvation and physical stress conditions cause heavy loss of fat body storage. Triglycerides are catabolized to fatty acids and glycerol. Under normal conditions, catabolism of fatty acids occurs in the mitochondria through a process known as β-oxidation. However, in hunger conditions, there is an increase in the β-oxidation process in peroxisomes which under normal conditions is a minor pathway in the β-oxidation process. Wresdiyati and Makita (1995) report that stress conditions such as fasting can increase the number of peroxisomes which has an impact on the increase in oxidation in the peroxisomes. With the increasing activity of β-oxidation in peroxisomes, the number of free radicals also increases as a by-product of an increase in metabolism. In this study, the high levels of free radicals under stress conditions were detected by increasing levels of MDA and decreasing SOD enzyme in the stress group compared to the control group in this study.

Fasting and physical stress also increase protein catabolism into ATP energy. The protein catabolism reaction requires SGOT and SGPT enzymes to transfer the amine group from the amino acid glutamate to acceptors in the form of oxaloacetic and pyruvic acid compounds. The amine group is then released through a deamination reaction. The SGOT and SGPT are indicators that can be used to assess liver and kidney damage. In this research, the SGOT and SGPT of *C. trifolia* 115 mg/kgBW decreased the SGOT and SGPT enzyme levels. However, *C. trifolia* at high concentrations of 230 mg/kgBW and 460 mg/kgBW was not able to reduce levels of SGOT and SGPT.

The positive control vitamin E, yielded the best results in reducing SGOT and SGPT levels. Vitamin E works as an antioxidant that prevents oxidation by donating one hydrogen ion from the 6-hydroxyl group which can convert peroxyl radicals into a less reactive tocopherol radical so that it is unable to damage the fatty acid chain (Niki, 2015). Vitamin E is a powerful antioxidant that acts as an electron donor to free radicals.

The methanol fraction of *C. trifolia* based on phytochemical screening contained antioxidant compounds in the alkaloids, phenolic, flavonoid and terpenoid groups (Sowmya *et al*., 2015). The leaves contain stilbenoid compounds, kaemferol, myricetin, quercetin, triterpenes, and epifriedelanol. *Cayratia trifolia* stem extracts have alkaloid compounds, tannins, flavonoids and amino acids (Kumar & Goel, 2019; Hikmawanti *et al*., 2021). All of these compounds are antioxidants.

Based on Kumar & Goel (2019) phenolic component is a terminator of free radicals and chelating active redox metal ions. These phenolic antioxidants block the oxidation of lipids and other molecules by donating hydrogen atoms to radical compounds to form phenoxyl radical intermediates. Alkaloid compounds also act as natural antioxidants. Alkaloid extract of *Cyclea peltata* in CCl_4-induced rats caused a significant reduction of liver malondialdehyde and liver enzymes (Shine *et al*., 2014).

Flavonoid compounds also act as antioxidants through the following four mechanisms: donating a hydrogen atom to free radicals, chelating metal ions, suppressing enzymes involved in the formation of free radicals and stimulating antioxidant enzymes. The antioxidant ability of flavonoids is closely related to the shape of the flavonoid structure that contains hydroxyl groups in rings C and B, and has a double bond in the carbonyl group (Banjarnahor & Artanti, 2014).

Reduction of liver enzymes (SGOT and SGPT), alkaline phosphatase (ALP), and GST was also observed in rats with the administration of antioxidants, such as ellagitannins (Banjarnahor & Artanti, 2014), hesperidins (Pari *et al*., 2015), watercress extract (Azarmehr *et al*., 2019), curcumin (Mansour-Ghanaei *et al*. (2019). The stems of grape plants (*V. vinifera*) which are included in the Vitaceae family, the same family as *C. trifolia*, have hepatoprotective activity. It was proven by their ability to reduce SGPT levels in rats induced by CCl_4 (Ahmed *et al*., 2012). Grapes (*V. vinifera*) also show antioxidant and hepatoprotective potential because they contain resveratrol compounds (Bhaumik *et al*., 2015). Phytochemical constituents act as antioxidants which significantly reduce the oxidative threat leading to the reduction of pathological changes and restoration of normal physiological functions (Pari *et al*., 2015)

The kidneys are the main organs to maintain the balance of blood volume, blood chemical composition, excrete solutes, metabolic waste and other toxic compounds (Albert 2022). The nephron damage due to physical stress and starvation is the intraglomerular hemodynamic mechanism. This event is triggered by an imbalance in blood pressure in the glomerular filtration process due to vasoconstriction of afferent arterioles, thereby reducing the glomerular filtration rate (GFR). Therefore, abnormal blood flow due to starvation and stress over time causes damage to podocyte cells and results in shrinkage of the glomerulus (Dalal *et al*., 2023).

Starvation and physical stress treatment also cause the lumen of the tubular cells to dilate. Physical stress will cause cells to starve and lack ATP. Lack of ATP in proximal tubular epithelial cells will disrupt and change the position of the cytoskeleton actin on the brush border. Rapid disruption of cytoskeletal integrity caused the brush border released, promoting epithelial desquamation and the formation of cellular debris in the tubule (Chatauret *et al*., 2014).

Wresdiyati *et al*. (2002) also reported that the greatest damage to the nephron during physical stress conditions of hunger occurred in the glomerulus and proximal tubule. This can be seen from the decreased levels of the SOD enzyme and increased levels of malondialdehyde. A lack of cell ATP will increase the number of peroxisomes in kidney cells (Wresdiyati & Makita 1995). Peroxisomes play an important role in oxidation reactions. Langseth (1995) in Wresdiyati *et al*. (2002) reported that oxidation-reduction reactions in peroxisomes and mitochondria will trigger the formation of oxidative radicals.

The liver is the main organ in the metabolism of food and the biotransformation of other compounds. Catabolism and anabolism of carbohydrates, proteins and lipids occur in the liver. In hepatic hepatocyte cells, processes of glycogenolysis and gluconeogenesis occur in starvation conditions (Steinhauser *et al*., 2018).

According to Hayati *et al*. (2014), enlargement of the hepatocytes is a sign of hepatocyte damage. The hypertrophy of the hepatocytes showed among the central vein because this zone is specialized for detoxification (Gilgenkrantz and de I'Hortet, 2018). Hypertrophy hepatocytes have an irregular shape, larger size, and are often associated with elevated liver enzyme levels, but it also a sign of the first stage of liver regeneration (Morangiu *et al*., 2017)

The treatment of *C. trifolia* 115 mg/kgBW, 230 mg/kgBW and vitamin E showed the normal condition of hepatocytes, sinusoids and central veins. The flattened epithelium comprising the central veins is still arranged at the edges of the vessels. Putri *et al*. (2019) also reported the hepatocyte regeneration of *C. trifolia* methanol extract in paracetamol-induced rats. This result was also consistent with kidney histological analysis, malondialdehyde, SOD level and liver enzymes. Hepatocyte regeneration occurs because secondary metabolite compounds in *C. trifolia* fruit extract can reduce liver enzyme levels in the blood, increase SOD levels, reduce levels of malondialdehyde (MDA) and can improve the regeneration of hepatocyte damage due to lipid peroxidation chain reactions.

CONCLUSION

Bioactive compounds in *Cayratia trifolia* are potentially used as an antioxidant. Methanol fraction of *C. trifolia* fruit dose 115 mg/kg BW is able to reduce levels of malondialdehyde, SGOT, SGPT liver enzyme. It also repairs the damaged structure of hepatocytes and kidney glomerulus, induced by starvation and physical stress.

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CONFLICT OF INTEREST

There was no conflict of interest between the authors regarding the publication of this manuscript.

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