Hepatoprotective activity of mangrove snail (*Telescopium* sp.) extract in *sprague dawley* rats induced by paracetamol

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**Abstract.** The preliminary study showed that mangrove snails contain bioactive compounds and possess antioxidant activity, whereas these snails are only considered as pond pests. This study aimed to determine the activity of mangrove snails (*Telescopium* sp.) as hepatoprotective in Sprague Dawley rats induced by paracetamol. This study used a completely randomized design (CRD) with Duncan’s follow-up test, where mice were divided into 6 treatment groups (with n = 3). The observations included the levels of enzyme serum Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and malondialdehyde (MDA), and histopathological tissue. The results of histopathological analysis showed that dose of 75 mg/kg body weight were protective and repair on rat liver tissue.

1. Introduction
Mangrove snail (*Telescopium* sp.) is a water gastropod, generally inhabiting muddy areas that were rich in organic matter, nearly tidal areas and able to withstand high salinity. Mangrove snail often found in abundant quantities in aquaculture areas close to mangrove forests [7]. The empirical experience in coastal communities from Mentawai Island that mangrove snails (*Telescopium* sp.) were used as food, and believed to increase stamina.

Research by Hafiludin [8] showed that mangrove snails have a high protein content of 12.16% and has strong antioxidant activity with IC₅₀ value 22.08 ppm. Mangrove snail have active component that were alkaloids, steroids, and flavonoids.

The results of the research by Nugraha et al. [20] showed that the presence on liver cell repair from CCl₄-induced rats and then treated with red mangrove extract, which had antioxidant activity with value IC₅₀ = 0.76 ppm.

The utilization of mangrove snails were not optimal, even though that was believed to increase stamina, so mangrove snails were expected to be developed into traditional medicinal ingredients for liver protection. Lots of studies indicate that natural substances from edible and medicinal plants exhibited strong antioxidant activity that could act against CCl₄-induced liver damage, because they contain lots of free radical scavenger such as phenolic acids and flavonoid compounds [17].

Ponmari et al. [24] that carbon tetrachloride (CCl₄) is a highly toxic chemical and a well known hepatotoxin used extensively to investigate the hepatotoxicity in animal models. Makni et al. [18] told that CCl₄ by itself does not have cytotoxic effects on the liver but its metabolic products such as generated trichloromethyl free radicals are responsible for the toxicity and the production of lipid peroxidation.
The purpose of the study is to determine bioactive components, antioxidant activity and ability of *Telescopium* sp. as hepatoprotector with treatment on dose of extract. This studied using paracetamol which has a lower effect than CCl₄. The results of this study were expected to be able to provide information about the ability of mangrove snails as hepatoprotectors, so as to increased economic value of snail.

2. Materials and Method

2.1. Materials research
The samples used in this study were Mangrove snail (*Telescopium* sp.), collected from Sungai Pedada, Palembang, South of Sumatra. The samples were prepared and stored in the freezer until ready to be analyzed.

2.2. Research treatments
Indentification and proximate analysis, prepared material that mangrove snail separated between shells and meat. The meat of mangrove snails were extracted in water and then analyzed on active component and activities antioxidants.

The stage was to determine the best concentration in the treatments for *in vivo* test (50, 75, 100 mg/kg BW). The analysis on the parameters of in vivo test were Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and, malondialdehyde (MDA), histopathological tissue.

2.3. Chemical Analysis
The chemical analysis which comprised proximate analysis [1] on fresh meat, active component and activities antioxidants (Bloise 1958) on extract, SGOT [2], SGPT [2], and MDA [31] on serum of mice, also analysis on the rats liver histopathology [14].

2.4. Statistics Analysis
The experimental design for the determination of the best concentration method used a completely randomized design (CRD). If the ANOVA F test on different effects, then followed by Duncan’s test.

3. Results and Discussions

3.1. Physical Characteristics
Molluscs that live in water with constant change of current will grow better than do in coral or in the flowing waters (Suwignyo *et al.* 2005). Mangrove snails have a bilateral symmetrical body that is protected by a strong shell, cone-shaped at the tip and circular. Physical characteristics of mangrove snails are presented in Table 1. Oemarjati and Wardhana [22] classifying mangrove snails (*Telescopium* sp.) as follows:

| Phylum  | Mollusca       |
|---------|----------------|
| Class   | Gastropoda     |
| Order   | Neotaenioglossa|
| Family  | Potamididae    |
| Genus   | Telescopium    |
| Species | *Telescopium telescopium*, Linnaeus 1758. |

Hamsiah *et al.* [7] stated that mangrove snails derived from the waters of Barru Regency, South Sulawesi had a length of 7.0-7.5 cm and a width of 3.2-3.85 cm with a body weight of 38.47-40.17 g. Factors that influence the growth of mangrove snails (*Telescopium* sp.) Namely environmental conditions, mangrove snails can grow well at pH 7-8, temperature 28-32°c, salinity ranges from 29-30.33 ppt, dissolved oxygen content greater than 5 mg / L, with organic C of 5.50%.
Table 1. Physical characteristics for *Telescopium* sp.

| No | Character     | Research       | *Telescopium* sp.* |
|----|---------------|----------------|--------------------|
| 1  | Length (cm)   | 7.80±0.62      | 8.94               |
| 2  | Width (cm)    | 3.07±0.11      | -                  |
| 3  | Thick (cm)    | 3.11±0.21      | 4.73               |
| 4  | Weight (g)    | 40.81±3.37     | -                  |
| 5  | Edible portion (%) | 8.01±1.24 | -                  |

Information: * Efendi et al. [5]

3.2. Proximate Composition of The Mollusc Muscle

Proximate analysis in this research was determined moisture content, ash content, protein content, and lipid content. Results of proximate analysis from mangrove snails (*Telescopium* sp.) can be showed in Table 2.

Table 2. Chemical composition of Mangrove snails (*Telescopium* sp.).

| Parameter          | Mangrove snails (*Telescopium* sp.) | Mangrove snails (*Telescopium* sp.)* | Mata merah snails (*Cerithidea obtuse)* |
|--------------------|-------------------------------------|--------------------------------------|----------------------------------------|
| Moisture content (%)| 72.42±0.43                          | 78.14                                | 72.10 ± 1.02d                          |
| Ash content (%)     | 3.41±0.55                           | 5.42                                 | 4.71±0.78                              |
| Fat content (%)     | 0.25±0.09                           | 0.38                                 | 7.80 ± 0.87                            |
| Protein content (%) | 13.87±0.28                          | 12.16                                | 14.39 ± 0.25                           |

Information: * Hafiludin, [8]

**Purwaningsih [25]

The result of proximate analysis showed that the ash content of mangrove snail is 3.41%. The amount of ash content can be affected by various of organisms, differences of habitat and environmental conditions. Padidela and Thummala [23] were explains that the ash content caused by habitat and environmental differences. Each aquatic environment can provide different mineral intakes for the living aquatic organisms. Animals will need more energy to multiply stored in the form of lipid. Lipid content of mangrove snails 0.25%, this is lower than [8] research on mangrove snails and Purwaningsih [25] on matah merah snails. The proximat analysis showed mangrove snails has 13.87 % protein content. Purwaningsih *et al.* [25] explains that the various of moisture content, ash content, lipid content, and protein content of matah merah snail was affected of many factors. The factors are species, size, gonado somatic index, temperature, various of feed and sampling location.

3.3. The Active Compounds of Mangrove Snails (*Telescopium* sp.) Extract

Marine organisms have ability to synthesize chemical for self defense. The chemical help them to survive from changes of temperature, extreme salinity and pressure, prevent from predators, paralyze prey, and prevent poisoning and infection [30]. The active compounds of mangrove snails showed on Table 3.

The testing of alkaloid compounds showed positive on mangrove snails extracted by water. Alkaloids found in marine invertebrates can be developed as anticancer. Imperatore *et al.* [11] explains that alkaloids found in marine invertebrates include *indole, quinoline, pyrrole, pyrazine, and pyridoacridine*.

Saponins test showed positive on mangrove snails extracted by water. That were according with Putri *et al.* [26] that saponins test was positive on mangrove snails and Azizah [3] that saponins tests was positive on matah merah snails. Francis *et al.* [6] explains that saponins are present in natural materials that content high protein and some saponins have antioxidant activity. Navarro *et al.* [19] explains that saponins in plants and medicines also have several kinds of bioactivity, such as anti-inflammatory, antiviral, and antiparasitic.
3.4. Antioxidant Activity of Mangrove Snails (Telescopium sp.) Extract

The analysis of antioxidant activity of mangrove snails (Telescopium sp.) was carried out using the DPPH method. Antioxidant analyses were carried out by comparing the percentage of inhibition between aquadest extract of mangrove snails with vitamin C as a standard. The results of the analyses of antioxidant activity from aquadest extract of mangrove snails can be seen in Table 4.

| Type of Mollusk | Concentration (µg/ml) | Activities (%) | Regression equation | IC₅₀ (ppm) |
|----------------|-----------------------|----------------|---------------------|-----------|
| Mangrove snail (Telescopium sp.) | 50 | 7.33 | | |
| | 100 | 7.57 | | |
| | 200 | 13.93 | y = 0.0188 + 6.9176X | 2291.61 |
| | 400 | 19.07 | | |
| | 800 | 27.38 | R² = 0.9887 | |
| | 1600 | 44.25 | | |
| | 3200 | 56.41 | | |
| Matah merah snail (Cerithidea obtusa)* | 12.5 | 18.50 | | |
| | 25.0 | 32.65 | Y = 29.544 + 0.3516X | 58.19 |
| | 50.0 | 68.80 | | |
| | 100.0 | 80.20 | R = 0.836 | |
| | 200.0 | 88.80 | | |
| Vitamin C | 1.25 | 8.83 | | |
| | 2.50 | 24.35 | y = 7.5283 + | |
| | 5.00 | 45.68 | 3.2417X | 6.21 |
| | 10.00 | 76.07 | R² = 0.9852 | |
| | 20.00 | 92.02 | | |

Information: * Purwaningsih [25]

The results of antioxidant activity from aquadest extract of mangrove snail mangrove snail were classified as very low, because the value IC₅₀ = 2291.61 ppm (IC₅₀ > 1000 ppm). This was different with...
research by Purwaningsih [25] that antioxidant activity from ethanole extract of matah merah snail was strong with IC$_{50}$ = 58.19 ppm. In this study using vitamin C as a standard, where the value of IC$_{50}$ from vitamins was 6.21ppm (very strong).

This was different from the results study which showed that the best antioxidant activity was in the treatment of evaporation temperature on 70 °C with IC$_{50}$ = 0.72 ppm. The evaporation temperature of 70 °C is the optimal temperature in the process of separating mangrove antioxidant compounds.

3.5. Alanine Aminotransferase and Aspartate Aminotransferase Levels in Rat Serum

The reactivity of these free radicals alters the integrity or permeability of cell membrane due to oxidation of polyunsaturated fatty acid in cellular membranes. This causes leakage of liver enzymes such as ALT, AST and ALP into the blood circulation. There for CCl$_4$-induced or paracemal-induced hepatotoxicity, the normal liver functions are affected which include substantial increase in the levels of liver enzymes such as ALT, AST, ALP, and TP.

Hann et al. [9], that the levels of these liver enzymes and TP concentration are the main biochemical markers which indicate the status of liver function. Low levels of these biomarkers are normally present in the blood; however, in case of hepatotoxicity or injury to liver, levels of these biomarkers elevated in the blood. Therefore, the levels of ALT, AST, ALP and TP in the blood are directly related to the extent of the liver tissue damage. The results of the analysis of AST and ALT levels in rat liver can be seen in Figure 1 and 2.

![Figure 1. The average of AST levels in rat liver tissue](image)

Where:  N=normal, N= paracetamol, KB1= P+50mg/kg BW extract, KB2=P+75mg/kg BW extract, KB3=P+100mg/kg BW extract, Syl=P+Sylimarin 25 mg/kg BW. Information: the numbers followed by different letters on the figur indicated differences [p <0.05].

Test results showed that the treatment has a significantly different effect ($\alpha = 0.05$) on AST value. The Duncan's follow-up test that the treatment in normal control rats was not significantly different from rats with treatment given paracetamol and then given 75 mg /kg BW extract. The highest AST value is rats with treatment given paracetamol without given extract, that was different with other treatment. That was present in the serum AST to protect against cellular injuries caused by oxidative stress. Our results have indicated that the intoxication of rats with paracetamol causes significant increase in the serum levels of these antioxidative enzymes.
Where: N=normal, N= paracetamol, KB1= P+50mg/kg BW extract, KB2=P+75mg/kg BW extract, KB3=P+100mg/kg BW extract, Syl=P+Sylimarin 25 mg/kg BW. Information: the numbers followed by different letters on the figur indicated differences [p <0.05].

Figure 2. The average of ALT levels in rat liver tissue.

Test results showed that the treatment has a significantly different effect ($\alpha = 0.05$) on ALT value. The Duncan's follow-up test that the treatment in normal control rats was not significantly different from rats with treatment given paracetamol and then given sylimarin, but different with others. There are present in the serum to protect against cellular injuries. The results indicated that the intoxication of rats with paracetamol caused significant down regulation in the serum levels on ALT (oxidative enzymes). In this studies the hepatoprotective efficacy of mangrove extract wws investigated by evaluating its potential to alleviate the elevation of liver enzymes (AST and ALT) and MDA levels.

3.6. MDA levels in rat liver

Regarding non-enzymic antioxidants, GSH is an intracellular reductant and protects cells against free radicals, peroxides and other toxic compounds. In addition, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx and GST. The CAT enzyme catalyzes the decomposition of hydrogen peroxide to water and oxygen and is a very important enzyme in protecting the cell from oxidative damage induced by ROS Niedernhofer [21], that the hepatic tissue destruction caused by free radicals generated by the exposure of chemical toxin (CCl₄) also cause substantial oxidative stress and produce ROS in the cellular systems. The mechanism of liver injury induced by ROS is to change the liver pathology. CCl₄are metabolized into their metabolite such as CCl₃ and CCl₃OO free radicals in the presence of cytochrome p450 in liver and kidney [28].

Han et al. [9], that these ROS bind to the macromolecules protein, carbohydrate, lipid, and DNA and cause severe oxidative stress that may lead to cell death or regeneration. Lipid peroxidation is the measure of thiobarbituric acid reactive substance, and MDA is a direct biochemical marker of oxidative stress induced by chemicals, drugs, or external noxious injuries and MDA is a direct biochemical marker of oxidative stress induced by chemicals, drugs, or external noxious injuries.

Knockaert et al. [15] showed that CCl₄ is a hepatotoxic compound causing severe liver injury. It undergoes metabolism by the action of cytochrome p450 that is present in endoplasmic reticulum of liver cells and leads to the production of unstable and complex metabolites of CCl₄, which may cause hepatotoxicity. CCl₄ is activated in the presence of cytochrome p450 (Cyp2E1), and (CYP2B, CYP3A) both are marginally involved in the transformation of CCl₄ to its metabolites such as trichloromethyl (CCl₃) free radicals that can also convert into trichloromethyl peroxy radical (CCl₃OO') in the presence of oxygen [13]. These metabolites of CCl₄ are very reactive. CCl₃ free radical covalently binds to the biomacromolecules and CCl₃OO' involves in lipid peroxidation to dissolve the polyunsaturated fatty acid
and change into small fragment called MDA or 4-hydroxynonenal. The results of the analysis of MDA levels in rat liver can be seen in Figure 3.

![Graph showing MDA levels in rat liver tissue.](image)

**Figure 3.** The average of MDA levels in rat liver tissue, N=normal, N= paracetamol, KB1= P+50mg/kg BW extract, KB2=P+75mg/kg BW extract, KB3=P+100mg/kg BW extract, Syl=P+Sylimarin 25 mg/kg BW.

The high level of MDA in the liver in animals showed the level of liver damage, given paracetamol on a dose 30 mg/kg BW for 5 days were significant increased on MDA level. This showed that paracetamol is a hepatotoxic compound causing severe liver injury.

The value of MDA levels in rat liver showed that the highest in paracetamol treatment at a dose 28 mg/kg BW that was 0.0183 nmol/mL and lowest is treatment paracetamol and then given mangrove snails extract at a dose 75 mg/kg BW (KB2) that was 0.0096 nmol/mL. The figure showed that treatment with a dose 75 mg/kg BW (KB2) more better than Sylimarin (25mg/kg BW).

3.7. The Hepatic Histopathological Analyses

The liver as a vital organ has a wide range of functions in the body, including detoxification, plasma protein synthesis, and glycogen storage. Oxidative stress is considered as the imbalance between reactive oxygen species (ROS) production and antioxidant protective mechanism. It is principal cause of the development of hepatic disorders.

Slater [27] that oxidative stress plays a major role in CCl4-induced liver injury; it is mediated by the production of free radical de- rivatives of CCl4 and is responsible for cell membrane damage and the consequent release of marker enzymes of hepatotoxicity.

Szymonik-Lesiuik *et al.* [29] that oxidative injury induced by CCl4 can be monitored in experimental animals by detecting oxidative stress parameters such as NO, MDA, SOD, GPx, and GRd. [4] that SOD is an effective antioxidant enzyme for catalyzing the dismutation of superoxide radicals into H2O2. GPx and GRd are glutathione-related enzymes that catalyze the reduction of H2O2 and hydroperoxides into nontoxic products and then end the chain reaction of lipid peroxidation.

Jaeschke *et al.* [12] that the free radical NO is a highly active nitrogen species produced by both parenchymal and nonparenchymal liver cells from L- arginine through NO synthase. NO may have a cytotoxic effect on neutrophils by forming peroxynitrite after it reacts with various ROS, especially. Lipid peroxides or ROS can easily block these antioxidant enzymes. The results of hepatic histological analyses can be seen in Figure 4.
Figure 4. The hepatic histological analyses: (a) control group, (b) animals pre-treated with paracetamol (28,80 mg/kg of bw), (c) animals pre-treated with paracetamol (28,80 mg/kg of bw) and then treated with extract (75 mg/kg of bw), (d) animals pre-treated with paracetamol (28,80 mg/kg of bw) and then treated with silymarin (25 mg/kg of bw), (d) kuffer cell (K), normal hepatocytes (HpN), sinusoid (Snd). Information: Liver tissues were stained with H & E (40 X 0.65).

The results showed that major change in histology induced by paracetamol resulted in increased kuffer cell, sinusoid, vacuolization, and inflammation (Fig. 5B) when compared with the control group (Fig. 5A). Liver injuries were reduced by pre-treatment with paracetamol (28,80 mg/kg of bw), and the injuries significantly decreased (Fig. C and D). The histological finding demonstrated that treatment with extract on 75 mg/kg of bw significant prevented paracetamol induced liver injury, and that was not different with treatment with silymarin.

4. Conclusion
Component active from mangrove snails extract were alkaloids, saponin, steroids, triterpenoid. Antioxidant activity from mangrove sanils was very low (IC$_{50}$ = 2291.61 ppm). In conclusion, we demonstrated that daily dose of 75 mg/kg BW showed greater protective potential against paracetamol induced hepatotoxicity and thus can be used as an alternative natural hepatoprotective agent.

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