ACUTE TOXICITY TEST OF BENTONITE-PURIFIED FISH OIL IN WISTAR RATS (*Rattus Norvegicus*): HISTOLOGICAL, SGOT, AND SGPT STUDIES

Sri Widia Ningsih[1], Nita Andriani Lubis[2], Gabriella Septiani Nasution[3], Nadroh Br Sitepu[4], Nurul Hidayah[5]
[12345] Politeknik Kesehatan kementerian Kesehatan Medan
Email: [1]widianingsih29@gmail.com

ABSTRACT

Purifying fish oil boosts its nutritional and economic worth. This study examined the acute toxicity of bentonite-purified eel and mackerel tuna fish oil (2, 4, 6%) on female Wistar rats (Rattus norvegicus), using a 5000 mg/kg fatal dose limit test, along with histological alterations, SGOT, and SGPT studies. A quasi-experimental post-test-only control was designed using one-way ANOVA and post hoc Tukey HSD tests. All animal groups experienced a steady weight gain from 155 ± 4.966555 g to 194.25 ± 8.421203 g on day 15. EBIII treatment revealed the greatest SGPT value of 76.33 ± 21.733 u/L, while MTBIII showed the highest SGOT value of 110 ± 20.881 u/L. One-way ANOVA results for SGPT ($p > 0.05$) revealed no significant change between treatment groups, although SGOT ($p < 0.05$) did differ significantly. A Tukey HSD post-hoc showed that each group's SGPT levels were not statistically different ($p > 0.05$). Compared to the normal group, the MTB 6% group exhibited a substantially different SGOT level ($p < 0.05$). The liver organ index increased in all treatment groups. Fatty degeneration and necrosis of glomeruli and tubules were visible in all treatment groups. The study found that pure fish oil bentonite did not harm Rattus Norvegicus.

Keywords : *Acute-toxicity; bentonite; fish-oil-purification; liver-function.*

INTRODUCTION

As of 2022, Indonesia is the second-largest fish producer in the world after China, with a total production of 24.85 million tonnes of fish (Kementerian Kelautan dan Perikanan, 2022). Both saltwater and freshwater fish are rich in nutrients such as protein, omega-3 and omega-6, saturated fatty acids, vitamin D, and minerals (Wibawa *et al*., 2006; Nasution *et al*., 2025). Until now, fish have only been used as a food source with low economic value. By converting it into fish oil, the economic and nutritional value of the raw material can be maximized for use in medicines, cosmetics, and other health products (Robertson et al., 2014). To improve the performance and selling value of fish oil, a purification process is required. One of the materials used is bentonite (Ayu *et al*., 2019; Andhiarto & Wijaya, 2018; Suseno *et al*., 2014).

Eels and mackerel tuna are types of fish that are highly nutritious. Fish oil purified from bentonite from these two types of fish is known to contain 0.6% and 0.1% omega-3 fatty acids and 0.87% and 5.7% vitamin D in eel and mackerel tuna fish oil, respectively (Ningsih *et al*., 2024). In addition to nutritional content, the safety level of fish oil when used as a supplement or pharmaceutical product must be considered. Therefore, toxicity testing is required to identify the potential side effects or dangers of fish oil consumption, as well as to determine the safe dosage for use (Wibawa *et al*., 2006). Acute toxicity testing is an in vivo test conducted as a preliminary pre-clinical test to measure the degree of toxic effects after a single dose of a compound is administered within 24 hours (Wibawa *et al*., 2006; Berniawan, 2022). The purpose of this study was to determine the acute toxicity of purified mackerel tuna and eel fish oil using bentonite (2, 4, 6%) through a lethal dose 50 (LD_{50}) limit test on female Wistar rats (*Rattus norvegicus*) and to examine histological and SGOT SGPT values.

MATERIALS AND METHODS

Reagents

Mackerel tuna fish, eel fish obtained from Deli Tua Fish Market, Diethyl ether, Neutral Buffered Formalin (NBF), 96% Alcohol, Xylol, Liquid paraffin, Hematoxylin, Eosin.

Fish Oil Extraction

See Ningsih, *et al*. (2024)

Degree of Toxicity

Animals Test and Experimental Design

The animals used were female Wistar rats, weighing 150-200 grams, aged 8-12 weeks, healthy, and with no apparent anatomical abnormalities. This study used a quasi-experimental post-test-only control. There were 4 test animals in each treatment group (normal, 2% bentonite-purified eel oil (EBI), 4% bentonite-purified eel oil (EBII), 6% bentonite-purified eel oil (EBIII), 2% bentonite-purified mackerel tuna oil (MTBI), 4% bentonite-purified mackerel tuna oil (MTBII), 6% bentonite-purified mackerel tuna oil (MTBIII).

Research Ethics Approval

All treatment aspects of research involving animal studies comply with international animal welfare regulations and the ethics committee of the Medan Ministry of Health Polytechnic, Number 01.1711/KEPK/Poltekkes Kemenkes Medan 2023.

Acute Oral Toxicity Limit Test 5000 (LD_{50})

The limit test 5000 aims to determine whether the weight of the test animals is within the range of 2000–5000 mg/kg body weight (bw). The testing procedure is as follows: if three test animals show no mortality, the administration of the dose is stopped. If three test animals show mortality, the main test is conducted with the highest dose of 5000 mg/kg bw.

Histological Observation, SGPT, and SGOT of Animals Test

The observation was conducted for fifteen days by dissecting the test animals to examine the organ indices of rats that had been given oral preparations. The dissection was performed to observe macroscopic changes in the heart, lungs, spleen, liver, and kidneys due to the administration of mackerel tuna oil and eel oil. The mice that had been given the fish oil extract were then examined for liver function using the Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamate Oxyacetyltransferase (SGOT) tests. Microscopic histopathological examination was performed on cross-sectioned kidneys of female rats after 14 days of treatment in each treatment group using Hematoxylin and Eosin (HE) staining at 400x magnification.

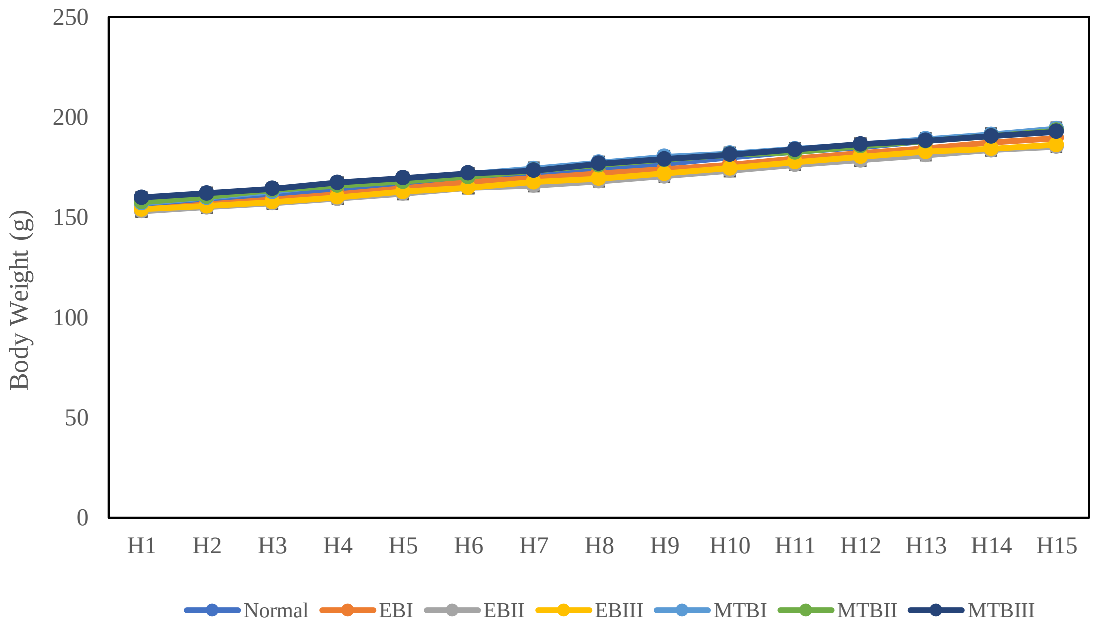
Statistical Analysis

All data are presented as mean ± standard deviation and analysed using IBM SPSS, USA, Statistical Package for Social Sciences (SPSS) version 22.0 software. The SGOT and SGPT values of the rats were tested using One-way ANOVA and further Tukey HSD (honestly significant difference) analysis to find differences between groups, with a significance threshold of $*p < 0.05$.

RESULTS AND DISCUSSIONS

Limit Lethal Dose 50 (LD_{50}) Test

Based on observations of animals test over fifteen days, all groups showed stable weight gain from 155 ± 4.966555 g to 194.25 ± 8.421203 g. The control group experienced the highest weight gain, while the EB and MTB groups showed slightly slower weight gain, especially at higher concentrations of purified bentonite (4% and 6%). This may indicate a mild physiological effect, such as a decrease in appetite, which could lead to a reduction in metabolism. However, overall, it shows that bentonite purification treatment of fish oil does not cause acute toxic effects on rat



growth. The results of this study align with Berniawan's study (Berniawan, 2022), which administered 3% bentonite-purified Sidat fish oil extract to white rats, demonstrating that there were no deaths among the test animals (LD_{50}) at a dose of 2400 mg/kgbw.

Figure 1. The changes in body weight at a LD_{50}

SGPT and SGOT Levels

The results of SGPT and SGOT liver function tests in rats showed varying values in each treatment group. The lowest SGPT value was found in the EBI treatment group, with a value of 63±12.166 units per liter of serum (u/L), and the highest SGPT level was found in the EBIII treatment group, with a value of 76.33±21.733 u/L. Meanwhile, the lowest SGOT level was found in the EBI % treatment group at 79.33±16.166 u/L, and the highest SGOT was found in the MTBIII treatment group at 110±20.881 u/L.

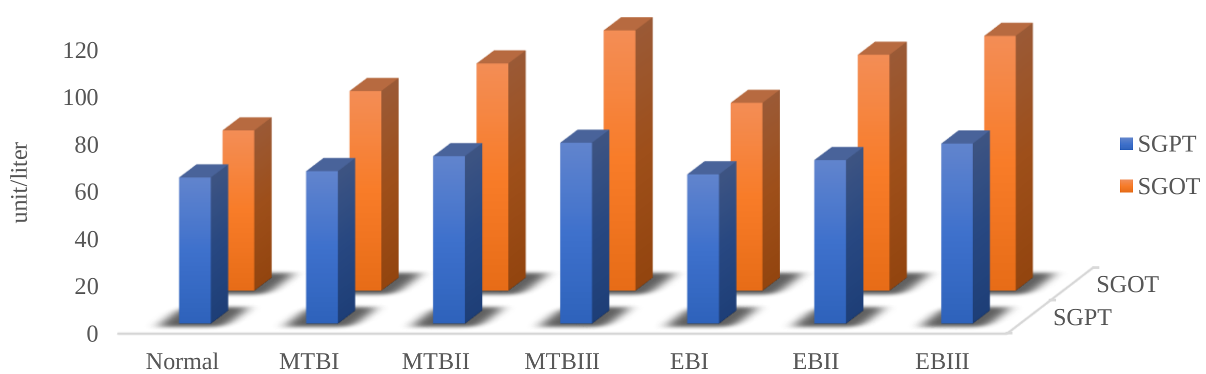


Figure 2. SGPT and SGOT Levels

The increase in SGOT was higher than the increase in SGPT in all treatment groups. This indicates that the higher the concentration of bentonite used in the fish oil purification process, the higher the SGOT and SGPT levels in rats. The pattern of increase in SGOT and SGPT levels was similar to increasing bentonite concentration in the fish oil purification process for both EB and MTB. Increased SGOT and SGPT levels can generally trigger hepatocellular stress (Wijaya, 2022).

Statistical Analysis of SGPT and SGOT

Based on the results of the one-way ANOVA statistical test, there was no significant difference between treatment groups for SGPT ($p > 0.05$), whereas SGOT values ($p < 0.05$) showed a significant difference. The results of the Tukey HSD follow-up test on SGPT examination showed no significant differences between groups. Although there was an increase in the average SGPT with increasing treatment concentration, this increase was not statistically significant ($p > 0.05$). Meanwhile, the MTB 6% group had significantly higher SGOT levels ($p < 0.05$) than the normal group. This indicates that the treatment increased SGOT enzyme activity, which could suggest a hepatotoxic effect. The fish oil purification process can show hepatotoxic effects such as lipid accumulation and hydropic degeneration. However, these findings depend on the type of fish oil, the purification process, and the dose and duration of administration (Muslim *et al.*, 2025).

.

Index of Wistar rats (*Rattus norvegicus*) Organs

According to measurements of the rat organ index, it is evident that the liver index increases more in all treatment groups compared to the normal group. This percentage increase indicates an adaptive response to toxic substance metabolism or hepatocellular enlargement (hepatomegaly). However, the increase in the index is still within physiological limits (<4) (Basheer *et al.*, 2017).

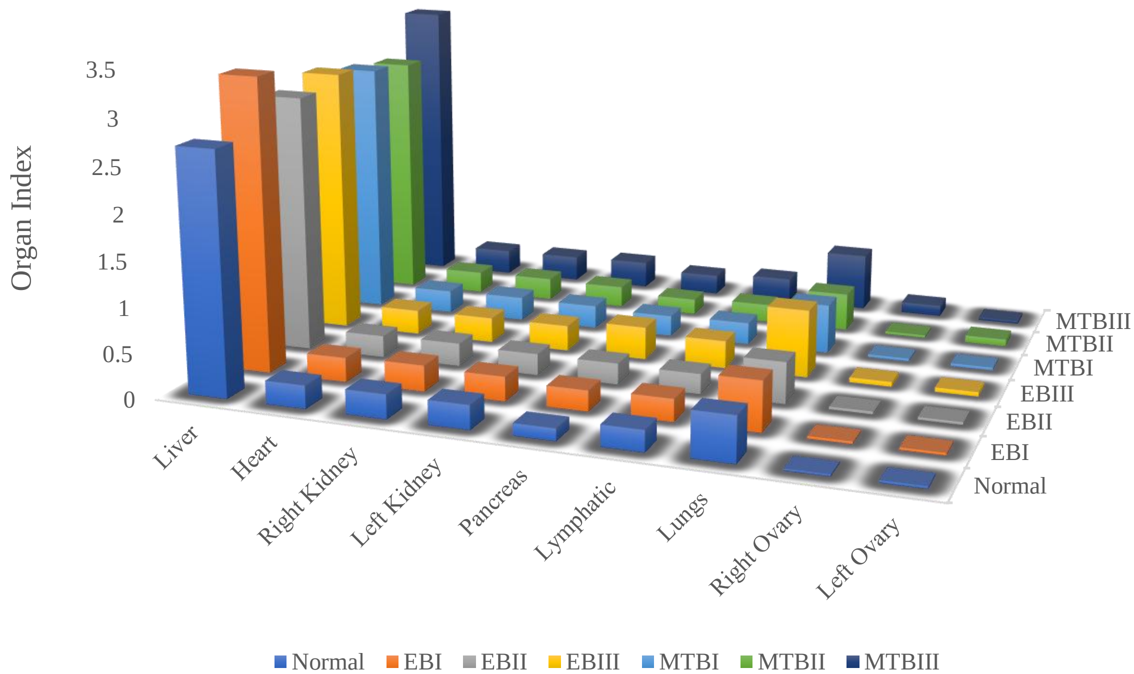
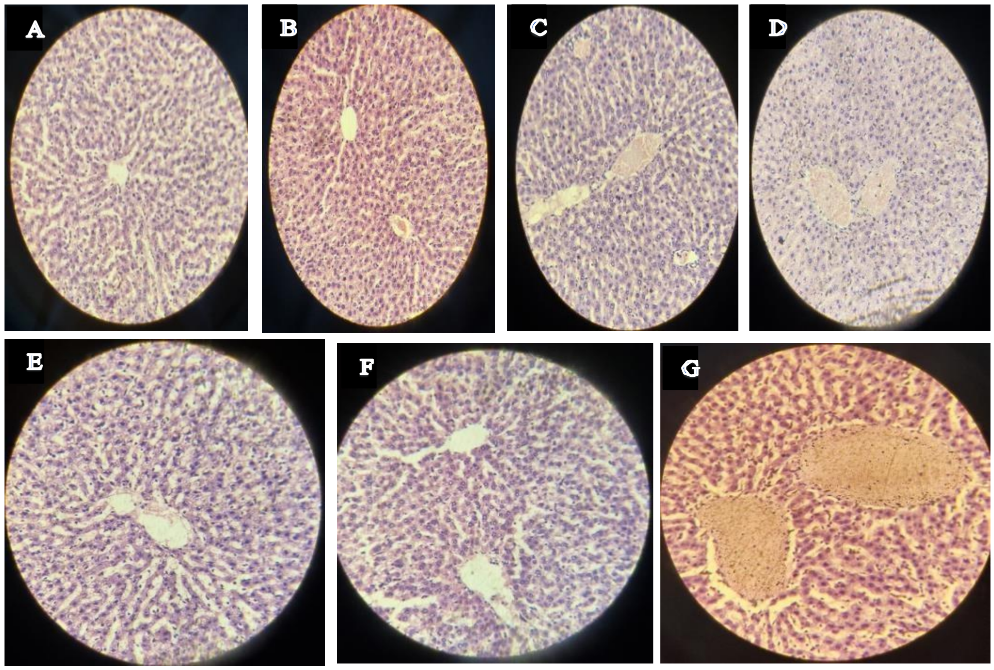


Figure 3. Index of Wistar rats (*Rattus norvegicus*) Organs

The heart organ index values appear relatively stable across all groups, nearly matching the values in the normal group. This indicates that there is no significant toxic effect on heart tissue due to the addition of bentonite in the fish oil purification process. There was an increase in the kidney organ index in both the right and left sides, with insignificant values in the EBIII and MTBIII treatment groups. This indicates increased excretory activity or mild stimulation of kidney function, but does not yet indicate severe toxicity. Meanwhile, the pancreas, spleen, lungs, and right and left ovaries showed relatively similar index values between groups and were close to normal values. This indicates that there was no significant toxic effect on these organs (Akil, 2019; Heramnto *et al*., 2022).

Histopathological Examination of Rats after Administration of Fish Oil

Microscopic observation showed that the glomeruli and tubules had undergone fatty degeneration and necrosis. These conditions were found in all treatment groups. In fatty degeneration, the nuclei of the tubular epithelial cells were pushed to the periphery, and fat vacuoles were seen filling the cytoplasm. Necrosis occurring in the tubules included loss of epithelium, detachment of the



basement membrane, pyknotic nuclei, ruptured nuclei (karyorrhexis), disappeared nuclei karyolitic), and desquamation or loss of a group of epithelial cells due to the absence of surrounding tissue to hold them in place.

Figure 4. Histopathological of female rat kidneys in cross-section after 14 days of treatment for each treatment group, stained with hematoxylin and eosin (HE) at 400x magnification: A. Normal treatment group (16 ml/kg BW); B. MTBI treatment group; C. MTBII treatment group; D. MTBIII treatment group; E. EBI treatment group; F. EBII treatment group; G. EBIII treatment group.

Treatm ... not show fa ... had a differ ... of purified ... ore fatty degeneration and necrosis were observed in the tubules and glomeruli. Similarly, with the administration of eel fish oil extract (EB) in each treatment group, the higher the concentration of bentonite in the eel fish oil extract, the more pronounced the fatty degeneration and necrosis in the tubules and glomeruli, as shown in E and F, especially in the microscopic images in Figure G.

The proximal tubule is the part of the nephron most susceptible to damage from toxic substances or ischemia because it is where absorption and secretion occur, resulting in higher concentrations of toxic substances (Mudiana *et a*l., 2023). Other causes of proximal tubule damage include high levels of cytochrome P-450, which can activate toxic substances. The process of renal tubule damage begins with the entry of toxic substances into the tubule epithelial cells, which then undergo degeneration in response. Fatty degeneration indicates the occurrence of toxic nephritis in the tubules. Further epithelial damage can take the form of necrosis and cell desquamation (Sijid *et al*., 2020)]. Tubule tissue that undergoes desquamation then transforms into connective tissue, causing a decline in kidney function. A decline in kidney function of more than 25% can lead to kidney failure (Mardiastuti, 2002).

CONCLUSIONS

A stable increase was observed in all groups' body weight, from 155 ± 4.966555 g to 194.25 ± 8.421203 g. There was an increase in SGOT and SGPT levels, following the same pattern, along with an increase in bentonite concentration during the fish oil purification process for both EB and MTB. The increase in liver organ index was higher in all treatment groups compared to the normal group, but the increase in index was still within physiological limits (<4). Microscopic observation showed that the glomeruli and tubules had undergone fatty degeneration and necrosis in all treatment groups. The results of the study showed that bentonite purification of fish oil did not have a toxic effect on the test animals (*Rattus Norvegicus*).

ACKNOWLEDGEMENT

The authors would like to extend their appreciation for the research grant Penelitian Pemula awarded by Politeknik Kesehatan Kementerian Kesehatan Medan.

REFERENCES

Andhiarto, Y., Wijaya, S., (2018) ‘Perbandingan Kualitas Suplemen Minyak Ikan Layang (Decapterus Ruselli) Menggunakan Bentonit dengan Berbagai Konsentrasi pada Tahap Bleaching’, *Pharmaceutical Journal of Indonesia*. 15(2), pp. 2019 – 2017.

Akil SNA. (2019) ‘The clinical and histopathological aspect of the liver, lung, and kidney in malaria’, *Qanun Medika Jurnal Kedokteran FK UM Surabaya*, 3(2), pp. 132-135.

Ayu D.F., Diharmi, A., Ali A. (2019) ‘Karakteristik Minyak Ikan dari Lemak Abdomen Hasil Samping Pengasapan Ikan Patin (*Pangasius hypopthalmus*)’, JPHPI. 22(1), pp. 187 - 197.

Basheer, A. S., Siddiqui, A., Paudel, Y. N., Hassan, Md. Q., Imran, Mohd., Najmi, A. K., & Akhtar, Mohd. (2017) ‘Hepatoprotective and Antioxidant Effects of Fish Oil on Isoniazid-Rifampin

Induced Hepatotoxicity in Rats' *Pharma Nutrition*, *5*(1), pp. 29–33. https://doi.org/10.1016/j.phanu.2017.01.002

Berniawan, W. (2022) *Uji Toksisitas Akut Fase Minyak Ekstrak Ikan Sidat (Anguilla Marmorata Q.) Fase Yellow Eel pada Tikus Putih Galur Wistar*. Universitas Tadulako.

Hermanto, F., Nur AI., Wahyuningsih, S., Alatas, F., Lucky Rachmawan, R., Ahsanul Haq, F., Adhary, F. (2022) 'Aktivitas Antiplasmodium dan Pengaruh Resveratrol terhadap Indeks Organ Mencit yang Terinfeksi Plasmodium berghei ANKA Antiplasmodium Activity and The Effect of Resveratrol on Index Organs of Mice Infected with Plasmodium berghei ANKA', *Pharmaceutical Journal of Indonesia*. 19 (1), pp. 28-39.

Kementerian Kelautan dan Perikanan. *Rilis Data Kelautan dan Perikanan Triwulan IV Tahun* (2022). Jakarta: Kementerian Kelautan dan Perikanan. Pusat Data, Statistik dan Informasi Sekretariat Kementerian Kelautan dan Perikanan.

Mardiastuti E. (2002) *Gambaran Histopatologi Organ Hati dan Ginjal Tikus Diabetes Mellitus yang Diberi Infus Batang Brotowali (Tinospora tuberculate L.) sebagai Bahan Antidiabetik*. Institut Pertanian Bogor.

Mudiana, W., Gusti, I., Sudisma, N., Luh, N., Setiasih, E., & Sudira, W. (2023) 'Gambaran Histologi Hati Tikus Putih *(Rattus norvegicus)* yang Diberikan Ekstrak Bunga Kecubung *(Datura metel L.)* sebagai Anestesi' *Acta Veterinaria Indonesiana*. 11(2), pp. 102–108. http://www.journal.ipb.ac.id/indeks.php/actavetindonesia.

Muslim, WR., Santoso, AH., Destra, E., Jap AN., Teguh SKMM. (2025) 'Peran Skrining SGOT dan SGPT sebagai Deteksi Dini Risiko Hepatotoksisitas pada Usia Produktif', *Pengabdian Kesehatan Masyarakat Health Community Service (HCS)*, 3 (1), pp. 28-32. https://doi.org/10.47709/hcs.v3i1.6286.

Nasution, G. S., Lubis, N. A., Ningsih, W., Hamsar, A. (2025) 'The Comparison Of Vitamin D Content Of Fish Oil Extracts Of Several Types Of Freshwater Fish' *in Proceeding The 3rd International Conference on Health Science*. Poltekkes Kemenkes Medan, pp. 1-15.

Ningsih, W., Lubis, N. A., & Nasution, G. S. (2024) 'The Quality of Purified Eel Fish (*Monopterus Albus Zuieuw*) Oil and Mackerel Tuna Fish (*Euthynnus Affinis*) Oil'. *Health Notions, 8*(1). pp. 1-7.

Robertson, B., Burri, L., & Berge, K. (2014) 'Genotoxicity Test and Subchronic Toxicity Study with Superba™ Krill Oil in Rats'. *Toxicology Reports, 1*, pp. 764–776. https://doi.org/10.1016/j.toxrep.2014.07.007.

Suseno, H. S., Tambunan, J. E., Ibrahin, B., Izaki A. F. (2014) 'Improving the Quality of Sardine Oil (Sardinella sp.) from Pekalongan-Indonesia Using Centrifugation and Adsorbents (Attapulgite, Bentonite and Zeolite)', *Advance Journal of Food Science and Technology*, 6(5), pp. 622-628. Tersedia di http://repository.ipb.ac.id/handle/123456789/77128. [diakses 18 September 2025].

Sijid SA, Muthiadin C, Zulkarnain, Hidayat AS, dan Amelia RR. (2020) 'Pengaruh Pemberian Tuak Terhadap Gambaran Histopatologi Hati Mencit (*Mus musculus*) ICR Jantan' *Jurnal Pendidikan Matematika dan IPA*. 11(2), pp.193-205.

Wibawa, P. J., Listiyorini, D., & Fachriyah, E. (2006) 'Penentuan Komposisi Asam Lemak Ekstrak Minyak Ikan Kembung (*Rastrelliger kanagurta*) dengan GC-MS dan Uji Toksisitasnya Menggunakan Metode BSLT', *Jurnal Sains & Matematika (JSM*). 14(4), pp.169-174.

Wijaya, R. C. (2020) 'Lethal Concentration 50% Of Pathchouli Oil (Pogostemon Cablin) Towards Zebrafish Embryo (*Danio Rerio*)', *Herb-Medicine Journal*, 3(2), pp. 1-6. https://doi.org/10.30595/hmj.v3i2.6360.