Effect of mackerel (Rastrelliger) arsik soup on the LDL levels of hypercholesterolemic rats

A M S Mendrofa, E Julianti* and R J Nainggolan

Department of Food Science and Technology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: *elisa1@usu.ac.id

Abstract. Fish arsik soup is a popular traditional food in North Sumatera especially in Batak ethnic. This research aimed to evaluate the effects of different dose of mackerel fish arsik soup on body weight and LDL blood level in rats that were given a cholesterol rich feed namely quail egg yolk. Research was performed by using a Randomized Group Design. Forty male rats were grouped into 5 groups. The first group (K₁) was the negative control group which was given distilled water and basal diet. The second, third, and fourth groups were given fish arsik soup with the dose of 1 ml/200 g body weight (bw)/day (K₂), 2 ml/200 g bw/day (K₃), and 3 ml/200 g bw/day (K₄), and the last group was positive control group which was given gemfibrozil (K₅). The result shows that rats fed quail egg yolk had a state of body weight gain and hypercholesterolemic. Administration of mackerel fish arsik soup to hypercholesterolemic rats with various dose had significantly (p<0.05) lower LDL level of rat blood compared to negative and positive control groups. The administration of mackerel fish arsik soup 1 ml/200 g bw/day resulted in the highest reduction in LDL levels in the rat’s blood.

1. Introduction
Arsik soup is a traditional food from Batak. It is a fish soup that uses arsik seasoning with a short shelf life. Arsik seasoning is made from various spices such as andaliman, shallots, garlic, red chili, cayenne pepper, lemongrass, galangal, ginger, turmeric, lokio (Batak onion), hazelnut, salt, orange leaves, water, and cikala acid. The process of making Mackerel arsik soup takes a long time and great energy. People's habit of consuming food products nowadays is always wanting to be fast-paced, efficient, also practical in storage, preparation, and consumption, so traditional foods like Arsik Soup tend to be replaced with instant foods. Besides, the high cost of groceries also makes people switch to fast foods or instant foods [1].

Mackerel fish (Rastrelliger) is a fish that is rich in unsaturated fats, which has shown the result in lowering cholesterol levels in rats. Mackerel fish is known to have a lot of omega-3 and omega-6 content, that are useful in lowering triglyceride levels and preventing blood clotting [2]. The use of mackerel fish in arsik soup is expected to increase the nutritional value of arsik soup because it is an antioxidant-rich arsik seasoning such as those found in andaliman (Zanthoxylum acanthopodium DC), and cikala acid (Etlingera elator Jack) [3] and garlic which inhibits the activity of enzyme 3-hydroxy-3-methylglutaryl coenzyme A [4].

Cholesterol is a fatty substance produced by the liver and circulates inside the body. Excessive cholesterol in the blood will cause atherosclerosis (narrowing of blood vessels), coronary heart disease,
stroke, and high blood pressure. Blood contains cholesterol, of which the body itself produced 80% of blood cholesterol, and only 20% comes from food. Two types of cholesterol, namely High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL). If LDL is present in excessive amounts in the blood it will consolidate in the walls of the blood vessels and form a plaque which can cause blockage of the blood vessels [5]. Increased levels of LDL cholesterol in the blood will increase the risk of coronary heart attack. High LDL levels are also associated with cardiovascular disease, a blockage in the arteries (veins). The research was aimed to study the hypocholesterolemic effect of administration mackerel fish arsik soup in rats.

2. Materials and methods
The main ingredient used in this study was mackerel fish (Rastrelliger) obtained from the Percut fish auction site. The ingredients used in the manufacture of arsik seasoning were andaliman, onion, garlic, red chili, cayenne pepper, lemongrass, galangal, ginger, turmeric, lokio, pecans, salt, kaffir lime leaves, water, and torch ginger (Etlingera elatior (Jack) R.M.Smith) fruits those were obtained from the traditional market, Medan.

2.1. Arsik soup making
All seasonings except torch ginger fruits and kaffir lime leaves were blended using a blender until smooth and formed a flavor. The amount of mackerel fish used was 1 Kg. Mackerel fish was cut into 4 parts by removing the head and tail parts then washed until clean, up to 3 times washing. Then arsik seasoning as much as 30% of the weight of mackerel fish was added afterward. It was cooked using a skillet that had been added 1 L of water until the mackerel fish was cooked. For 1 Kg of mackerel fish, salt was added as much as 14.5 g to enhance the taste.

2.2. Experimental animals
Forty adult male Wistar rats (2-3 months old) with an average weight ± 150-200 g were divided into 5 groups. The adaptation of rats was done for 7 days by being fed and drinking ad libitum.

2.3. Treatment of experimental animals
Rats were fasted for 16 hours before testing and given water ad libitum. A blood sample of 40 rats was then tested for total blood cholesterol, this as the initial total blood cholesterol. Afterward, all the rats were given a suspension of quail yolks 1ml/day for 7 days. Furthermore, on the 7th day, the rat’s blood total cholesterol level was measured to determine whether the experimental rats were hypercholesterolemic. The rats used should be healthy and exhibit normal behaviour and hypercholesterolemia i.e., > 27.2 mg/dl [6]. After the rats were expressed positive hypercholesterolemia then they were grouped into 5 groups, namely:

Group 1: Negative control group was given distilled water and was fed ad libitum for the next 7 days.
Group 2: Treatment group was given mackerel fish arsik soup as much as 1 ml / 200 g BW / day per oral and was fed on an ad- libitum for the next 7 days.
Group 3: Treatment group was given mackerel fish arsik soup as much as 2 ml / 200 g BW / day peroral and was fed ad libitum for the next 7 days.
Group 4: Treatment group was given mackerel fish arsik soup as much as 3 ml / 200 g BW / day peroral and was fed ad libitum for the next 7 days.
Group 5: Positive control group was given the suspension of comparative provisions (gemfibrozil) 21.6 mg/200 g BW/day peroral and was fed ad libitum for the next 7 days.
2.4. Dosage planning test

2.4.1. The dose of fish arsik soup. The planned test dose is 1 ml, 2 ml, 3 ml/200 g BW/day. The dose was used based on the best formula adjusted for the maximum volume of the stomach in rats was 5 ml [7].

2.4.2. Quail egg yolk suspension dose. The cholesterol feed used in this study was made from quail egg yolks. Quail eggs were separated between the yolk and the egg white. Then the yolks were mixed homogeneously [8]. Then given as much as 2 ml/ head/ day for 7 days by oral [9].

2.5. Gemfibrozil suspension test preparation

Gemfibrozil for anti-cholesterol test comparison was given in the form of suspension with CMC according to the effective dose to humans. Gemfibrozil weighed as much as 80.36 mg then grinded into powder, then made suspension in 10 ml of distilled water and added CMC 1% sufficiently to prevent gemfibrozil precipitate. The calculation of the dose to obtain CMC 1% is 1 mg CMC in 100 ml of distilled water so that it gets 1% CMC [10]. Gemfibrozil effective dose to humans is 2 x 600 mg/day or 1200 mg and then converted to 200 gr wb rats (0.018), so the dose used is 21.6 mg/200 gr wb rats. Administration of the suspension dose of gemfibrozil in rats through the oral route with a maximum dose volume of 5 ml. Since the volume of the rat's stomach is ± 5 ml then we made the solution less than ≤ 5 ml, so that the volume squatting in rats is 3 ml/day.

2.6. Data analysis

The observation data was processed using Statistical Product and Service Solution (SPSS) ver.22 for Windows. The analysis of variance (ANOVA) was used to determine the effect of research treatment whether it had a significant effect or not. If the treatment had a p value <0.05, it indicates a significantly different effect and is continued with Least Significant Ranges (LSR) test.

3. Results and discussion

Evaluation of the hypocholesterolemic effects of mackerel fish arsik soup was conducted in vivo using hypercholesterolemic experimental rats. The trial rats were given High Cholesterol Diet Foods (HCFD) in the form of a 2 ml/day suspension of quail yolks for 7 days aimed to increase blood LDL levels in rats characterized by increased blood LDL levels to 27.2 mg/dl. After that rats which had experienced hypercholesterolemic were divided into 5 groups and given mackerel fish arsik soup at a dose of 1 ml/200 g bb/day, 2 ml/200 g bb/day, and 3 ml/200 g bb/day for 7 days. Initial and final weight observation after 7 days of HCFD administration is presented in Table 1.

| Group | Body Weight (g) |
|-------|----------------|
|       | Initial         | End             |
| K1    | 184.23±23.86    | 195.55±27.12    |
| K2    | 235.58±37.96    | 246.03±45.01    |
| K3    | 202.65±42.27    | 211.62±47.61    |
| K4    | 213.95±19.86    | 223.89±28.81    |
| K5    | 232.51±23.69    | 239.04±26.27    |

Table 1. The weight gain of rats after HCFD-induced for 7 days.

Description: The test repeated 5 times, the sign (±) indicates the standard deviation value

Table 1 shows that rats HCFD-induced suspension of quail yolks as much as 2 ml/day for 7 days experienced weight gain. Quail egg yolks contain protein (15.7-16.6%), fat (31.8-35.5%), carbohydrate (0.2-1.0%), and ash (1.1%) [11]. Quail eggs are categorized as foods with very high cholesterol levels,
namely 844 mg/dl [12], so that it can cause weight gain in rats after being induced by quail egg yolks. Rats that have experienced hypercholesterolemia were then grouped into 5 (five) groups. Each group of rats was given several treatments such as fed ad-libitum by distilled water, mackerel arsik soup products, and gemfibrozil for 7 days.

The administration of distilled water, canned mackerel arsik soup products, and gemfibrozil for 7 days also showed an increase in mouse weight from the beginning to the end of the study. The increase in weight in rats was not separated from the amount of feed intake of each group of animals tried. The increasing of feed intake will increase the weight gain. The weight of rats after a feed-induced intervention (mackerel fish arsik soup and gemfibrozil) and standard feed for 7 days was shown in Table 2.

### Table 2. The weight gain of rats after induced feed intervention + standard feed for 7 days.

| Group | Body Weight (g) | Initial | End     |
|-------|-----------------|---------|---------|
| K₁    |                 | 195.55±27.12 | 198.91±26.42 |
| K₂    |                 | 246.03±45.01 | 249.68±45.53 |
| K₃    |                 | 211.62±47.61 | 214.88±47.31 |
| K₄    |                 | 223.89±28.81 | 227.24±28.76 |
| K₅    |                 | 239.04±26.27 | 242.22±25.99 |

Description: The test repeated 5 times, the sign (±) indicates the standard deviation value.

From Table 2, it was obtained that the weight gain of rats from the highest to the lowest was directly proportional to their feed intake of the K₂, K₅, K₄, K₃, and K₁ groups. Rats that were given hypercholesterolemic feed and continued with interventional feeding and standard feed can trigger weight gain when compared to acclimatization (standard feed only) because it can experience increased energy intake. The feed intake is directly proportional to the energy intake. The greater the feed intake, the energy intake that is stored as fat is also greater and finally has implications for the weight gain of the try animals [6].

### 3.1. Cholesterol levels in rats (mg/dl)

Rats cholesterol levels after being treated for 7 days and the relevance of the effect of administering distilled water, canned mackerel arsik soup, and gemfibrozil to the percentage of decreased rats cholesterol levels present in Table 3.

### Table 3. LDL blood levels of rats after being treated for 7 days.

| Group | LDL levels (mg/dl) | LDL Levels reduction (%) |
|-------|--------------------|--------------------------|
| Initial | End       |                          |
| K₁    | 41±2.96          | 9±1.94                   | 76.75±5.34<sup>b</sup> |
| K₂    | 32.6±2.51        | 3.6±1.34                 | 88.94±4.21<sup>a</sup> |
| K₃    | 38.4±2.70        | 6.8±2.86                 | 82.17±7.24<sup>ab</sup> |
| K₄    | 50.0±13.58       | 10.4±1.14                | 77.80±7.37<sup>b</sup> |
| K₅    | 39.0±2.73        | 8.0±1.67                 | 80.54±3.88<sup>b</sup> |

The test repeated 5 times, the sign (±) indicates the standard deviation value. Different superscript letters in LDL level reduction indicate significantly different at p<0.05.
Based on Table 3 it can be noted that LDL cholesterol after intervention feeding in all treatment groups decreased. The decrease in LDL cholesterol from high to lowest is K2, K3, K5, K4, and K1. The results of the LDL cholesterol measurement of rats showed that after hypercholesterolemia feeding, all groups experienced hypercholesterolemia with LDL cholesterol > 27.2 mg/dl in all groups and after intervention feeding, all treatment groups experienced a significant decrease in LDL cholesterol. Hypercholesterolemic condition in this group is caused by feeding of high cholesterol in the form of quail egg yolks resulting in increased cholesterol absorptions in the intestines. Increased cholesterol absorption in the intestines can lead to increased synthesis of LDL cholesterol in hepar so that LDL cholesterol in the blood of rats exceeds normal limits [6].

The decrease in LDL cholesterol in group K2 was greater compared to the K3 and K4 groups due to the food intake of the K3 and K4 groups greater than the K2 group which allowed the total calories of the K3 and K4 groups to be greater than the K2 group. Total calories were the number of calories gained from a day's intake of food in rats. [6] said that the body's metabolism requires calories from food to be processed in the body so that metabolic results can be used or stored in the body. Lower total calories in the K2 group compared to the K3 and K4 groups likely had an impact on fat metabolism. In addition, K2 has an average weight that is greater than K3 and K4 which causes the tendency to consume feed is also greater.

Rats given gemfibrozil were also effective in lowering the blood LDL levels of rats. However, canned mackerel fish arsik soup at a dose of 1 ml/day (K2) was more effective in lowering LDL levels in rats when compared to gemfibrozil. This is because gemfibrozil provides full benefits in lowering newly obtained cholesterol levels during 3 months of drug use. The usual dose of gemfibrozil consumed by adults is 600 mg orally 2 times a day. But for children, there is no dosage provision yet, because this drug can be dangerous for children. Side effects after consuming gemfibrozil such as hives and difficulty breathing.

4. Conclusions
Provision of mackerel arsik soup with 30% seasoning for 7 days to hypercholesterolemic rats at a dose of 2 ml/200 g body weight per day can reduce the blood cholesterol levels by 88.94%. Based on this results, mackerel arsik soup can be developed as a functional food, especially for hypercholesterolemic people.

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