Hypocholesterolaemic effects of fermented red onion \textit{(Allium cepa L. var aggregatum)} in rats \textit{(Rattus norvegicus)}

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Abstract. Red onion is known as one of spices, and it also benefits for human health evidently. Previous study that red onion has a beneficial for cardiovascular disease, including hypocholesterolaemic Red onion contains flavonoids. The extract of red onion decreased serum total cholesterol level of rats, which is mediated by quercetin. Quercetin could be increased by fermentation as one of the new methods in drug discovery, so the biological activity also increases through fermentation. This study aimed to anti-hypercholesterolaemic activity of red onion fermented extract. Twenty five rats were divided in 5 groups; 2 controls [negative and positive control (atorvastatin)] with treatment groups (100, 200 and 300 mg/kg bw of fermented red onion extract. Rats were given atherogenic feed until hypercholesterolaemic. All groups were given an atherogenic feed until hypercholesterolaemic. Total cholesterol of rats measured on the 7th, 14th and 21st day after they rats were treated by red onion fermented extract. Data analysed by ANOVA and continued by post hoc Duncan. All doses of red onion fermented extract decreased total cholesterol level of rats and dose of 200 mg/kg showed the strongest effect. Write the expectation for future fermented red onion has potential as a new agent cardiovascular health.

1. Introduction
Hypercholesterolaemic is one of the fat metabolism disorder, which is characterized by the increase in total cholesterol level. It is a risk factor of Coronary Heart Disease (CHD) [1]. CHD is the major cause of death and 	extit{disability-adjusted life years} (DALYs) in Europe, which is spend around €192 billion each year for treatment of CHD [2]. The Survey Sample Registration System (2014) in Indonesia showed that CHD was the highest cause of death [3]. In addition, World Health Organisation (WHO) reported that 17.5 million people died for cardiovascular disease or 31% of 56.6 million deaths worldwide in 2012. Heart disease may be initiated by atherosclerosis. Atherosclerosis is the accumulation of cholesterol in intima layer of the artery as a result of high blood cholesterol or hypercholesterolaemic. Decreasing blood cholesterol in hypercholesterolaemic can be treated by limiting cholesterol biosynthesis in liver, cholesterol absorption inhibitor, bile acid sequestrant, regulate the expression of lipoprotein metabolizing genes or others [4][5]. Cholesterol restriction can be done by inhibiting the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR).
HMGR is a key enzyme that has a role in cholesterol synthesis by overhauling HMG CoA into mevalonate, irreversible [6] [7].

Red onion, besides as a spices, it also influences the cholesterol levels. Red onion extract can reduce LDL cholesterol level in Sprague Dawley rat. Compounds that are thought has a role in this activity is quercetin. Red onion contains flavonoids, which mostly are quercetin [8]. One method for discovering new drug discovery is through fermentation, as the fermentation can increase the level of secondary metabolites in plants which also increase its biological activity [9][10]. Fermentation occurs naturally in foods, but others boarder view declare that fermentation takes place with addition of microorganisms. While fermentation process goes, it improves the preservation and organoleptic properties of foods by production a new biomass, enzymes, primary and secondary metabolites [11]. However, the effect of fermented onion in lowering LDL cholesterol is very rare reported. The current study was carried to evaluate the hypercholesterolaemic effect of red onion in rat.

2. Materials and methods

2.1 Fermentation and extraction of red onion
Red onion is processed by fermentation without additives using Automatic Fermentation Machine (Intelligent Fermenter). Fresh red onion (without peeling) was cleaned from impurities on skin and arranged into fermentation container, then the container was closed tightly, plugged in and left for 15 days at days at 50-80°C. Fermented Red Onion (FRO) was obtained after fermentation was completed. Then, FRO was extracted with 96% of ethanol refers to Indonesian of Herbs Pharmacopoeia 1st edition [12] and it was tested for antihypercholesterolaemic effect of red onion fermented extract in rats (rattusnovergicus).

2.2 In vivo test
The animal used in this study was wistar rats (rattus novergicus) (rattus novergicus) strains with 2-3 months of age and ± 200gram of body weight. Twenty five of rats were used in this study, which was divided into 5 groups. The first group was negative control (only be given CMC Na Carboxyl Methyl Cellulose (CMC) suspension as an extract vehicle), the second group was positive control (using atorvastatin calcium 20 mg from Hexparm Jaya), the third, fourth and fifth groups were given Extract of Fermented Red Onion (EFRO) at 100, 200 and 300 mg/kgbw, respectively. Rats were acclimatized for a week at the beginning of study to standardize the condition according to the research requirements.

2.3 Data analysis
Cholesterol levels reduction was analysed through ANOVA (Analysis of Varians) and continued with post hoc Duncan to find out which groups had the same or significantly different effect each other.

3. Result and discussion
Fermentation of red onion was carried out continuously for 15 days at temperature range of 50-80°C. Fermentation takes place automatically without using additive. Fresh red onions were arranged on fermentation machine shelf and it closed tightly. Fermented red onion has a soft texture, dark brown to black of colour, distinctive flavour like soy sauce-fried onions and has a slightly sour sweet taste. The appearance of fresh red onion and fermented red onion is shown in Figure 1. This fermentation process belongs to spontaneous short-term fermentation without additive, but using local ambient microbes and natural substrate from the fermented material itself.
After fermentation, the red colour in onions is change to dark brown because of browning process, which called as Maillard reaction. Maillard reaction is the reaction of carbonyl groups in reduction sugar with free amino groups in amino acid leads to the formation of Amadori compounds. This reaction occurs in red onions undergo thermal processing and create the tasty flavour compounds [13]. Sharma et al. in their research said that sugar content in onion decreases with increasing temperature. This is because of sugars in onions have undergone the Mallard reaction [14]. If temperature is increased or prolonged, caramelization will then occur. In contrast to the Maillard reaction, caramelization occurs between sugars which is degraded by amino acids [15].

In in vivo test, EFRO was given to rats orally at doses 100 mg/kg bw, 200 mg/kg bw and 300 mg/kg bw. After acclimatized, rats were given atherogenic feed until hypercholesterolaemic was obtained. Total cholesterol levels during study is shown on Table 1.

Table 1. Total cholesterol levels of rats (rattusnovergicus) (mg/dl)

| Groups          | Normal    | Hypercholesterolaemic | 7th day | 14th day | 21st day |
|-----------------|-----------|------------------------|---------|----------|----------|
| Negative control| 57.8±3.49 | 146.6±20.84            | 141.4±20.04 | 134.2±19.34 | 118±17   |
| Positive control| 61.6±6.22 | 148.6±17.03            | 125.4±14.45 | 110.2±16.68 | 94.4±10.78 |
| EFRO100         | 65.6±5.17 | 160±15.93              | 143.2±14.17* | 123±12.41* | 107.2±10.64* |
| EFRO200         | 69.4±7.16 | 174.2±16.85            | 138.6±13.67* | 109.2±11.43* | 87.8±8.04* |
| EFRO300         | 68.6±5.17 | 176.4±12.72            | 147.2±10.82* | 126.2±9.04* | 107±7.74* |

EFRO = Extract of fermentation red onions. Data are presented as mean ± SD (5 animals per group; three independent experiments were performed). Statistical significance between control and treated values was determined by post hoc Duncan and were given as a p value; *p value < 0.05.

The increase in total cholesterol levels of rats in hypercholesterololaemic states was 149.38%. Bukiya and Dantsker (2015) described that rat model of high cholesterol diet in hypercholesterolaemic is 70% elevation of total cholesterol levels to get rats as an experimental animal with hypercholesterolemia condition, total cholesterol levels of rats increased by at least 70% [16]. It This elevation indicated that rats were induced in this study have reached hypercholesterololaemic states. All groups have shown the decrease in total cholesterol levels on 7th day as in Table 1 that EFRO’s onset of action occurs in less than 7 days. Percentage of decrease in total cholesterol levels of rats significantly different in all treatment groups compared with negative control group as shown on Figure 2.

![Figure 1](image-url) Figure 1. (a) Fresh red onion and (b) fermented red onion.
The ability of EFRO to reduce total cholesterol levels increases with length of the treatment day. At 7th day, EFRO dose 200 mg/kg bw decreases 20.44% of total cholesterol levels, which is higher than EFRO 100 (10.49%), EFRO 200 (16.56%) even positive control (15.625%). At 14th day, EFRO dose 200 mg/kg bw decreased 37% of total cholesterol levels and it still be higher than others. The greatest reduction of total cholesterol levels was found in EFRO dose 200 mg/kg BW (49.58%), which was followed by EFRO dose 300 mg/kg bw (39.34%), positive control (36.47%) and EFRO dose 100 mg/kg bw (32.99%).

Decreasing of total cholesterol levels on the seventh, 14th and 21st day are significantly different. The decrease in cholesterol indicates that extract of fermentation red onions has the ability that was not less great compared to Atorvastatin as commercial antihypercholesterolaemic. Atorvastatin can reduce total cholesterol levels up to 42.9% [17]. In this study, atorvastatin reduces total cholesterol levels of rats at 36.47%.

4. Conclusions
Fermentation is one of methods for discovering or developing a moiety as drug discovery marker. Red onion can be fermented without additive at controlled temperature. Extract of fermented red onion has the strongest hypocholesterolaemic effect at dose 200 mg/kg bw which can reduce 49.58% of total cholesterol levels in rats.

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