The preventive effect of activated charcoal on HDL levels and aorta histopathological profiles in hypercholesterol rat models

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Abstract. Hypercholesterolemia is a condition which the cholesterol levels in the blood exceed the normal value. The use of hypercholesterolemia drugs or synthetic drugs in a long term will cause severe side effects. Therefore, it is important to explore an alternative preventive agent of hypercholesterolemia derived from natural materials, such as activated charcoal. The activated charcoal can absorb substances thousands times of its own weight and might be an effective and non-toxic material. The research aimed to determine the preventive effect of activated charcoal towards HDL level and aorta’s histopathology. This experiment used 24 male rats in randomized design that were divided into six groups: negative control (1); positive control (2), and four groups of treatments with activated charcoal doses of 2.250 mg/kg BW (3), 4.950 mg/kg BW (4), 6.750 mg/kg BW (5) 4.950 mg/kg BW (6), respectively, for 14 days. Simultaneously, all groups were received high cholesterol diet except group 1 and group 6. High Density Lipoprotein (HDL) level was measured by spectrophotometry method and the data of HDL were analyzed using One-Way ANOVA and followed by the Tukey test (p<0.05), while aorta’s histopathology were analyzed descriptively. Therapeutic dose of 4.950 mg/kg BW (group 4) showed the best dose in increasing HDL levels by 42% compared to positive control. Activated charcoal reduced the inflammation cells and the defect of aorta’s histopathology. Based on the results, activated charcoal prevents the decreasing of HDL level and a defect of aorta’s histopathology.

1. Introduction
Hypercholesterolemia is a metabolic disease, which is caused by an elevated total cholesterol level in blood circulation. The adverse effects of an increase in total cholesterol have associated with several life threatening diseases, such as hypertension, atherosclerosis, cardiovascular diseases, metabolic syndrome and obesity. About 40-70% of all human being suffer from hypercholesterolemia world wide [1]. This disease can also occured in pet animal such as dog, 32.8% from 192 American dogs [2] and cats 13% [3].

Hypercholesterolemia can decline of HDL level, because of continuous feeding of high cholesterol diet increase the free radicals in the body. The free radicals inhibit Apo-A1 activity as an apolipoprotein which is required in the formation of HDL. Consequently, the inhibition of apoA-1 activity can decrease the formation of HDL [4]. Another effect of hypercholesterolemia is a damage of
aorta. The damage of aortic endothelial cells can be indicated by the formation of random and rough tunica intima, and the appearance of inflammation cells. The occurrence of endothelial cells damage as an inflammation respond to oxidized-low density lipoprotein (Ox-LDL). The Ox-LDL can damage endothelial cell layers and the Ox-LDL infiltration in endothelia are phagocytes by monocytes and macrophages [5].

Currently available treatments for hypercholesterolemia is to regulate a diet that maintains normal body weight and reduce plasma lipid levels. However, in many cases, diet alone will not reduce blood lipid levels. In addition, 75-85% of serum cholesterol is endogenous and the change of diet will only reduce 10-30% of total cholesterol [6]. Another treatment is using a synthetic drug such as statin, the use of this drug in long-term will cause side effects including joint pain and liver damage. In order to overcome this problem, it is necessary to explore alternative treatment based on preventive hypercholesterolemia medication derived from natural product such as activated charcoal.

Activated charcoal is an adsorbent and commonly used as oral medication to treat poisoning and reduce intestinal gas in human. The activated charcoal is not toxic and safe to be used in oral administration because it cannot be digested in gastrointestinal tract [7]. Activated charcoal is expected to be able to absorb the excess of cholesterol in the intestine before entering the bloodstream. To date, there have been no reports of the treatment effect of activated charcoal to aorta condition of hypercholesterolemia animal. Therefore, the present study designed to determine the precautionary influence of activated charcoal towards HDL level and aorta’s histopathology.

2. Materials and Methods

2.1. Chemicals and Instrumentations

The activated charcoal used in the present study was purchased from PT. Haycarb. Eight to ten weeks old, male Wistar rats at weight range of 100-150 g were acclimatized for 7 days prior to the assay. The rats were housed in fully controlled conditions (room temperature of 25± 2 °C and 12 h of light and dark cycle) The research ethics committee of Brawijaya University approved the study protocol for animals (718-KEP-UB). Twenty-four rats were divided into six groups, each group consisted of 4 rats. Group 1 was the negative control received normal feed, while group 2 was the positive control given high cholesterol diet. Group 3, 4, 5, and 6 were chosen as treatments groups which received activated charcoal at doses of 2,250 mg/kgBW, 4,950 mg/kgBW, 6,750 mg/kgBW, and 4,950 mg/kgBW, respectively, for 14 days. Simultaneously, all treatment groups were received high cholesterol diet except in group 6. At the end of the experiment, all rats were sacrificed, aortic organ and serum were saved for assay examination.

2.1.1. Measurement of High Density Lipoprotein (HDL).

Measurement of HDL were carried out by spectrophotometry method. Serum HDL was determined by precipitation methods using various reagents [8].

2.1.2. Aortic histopathological observation.

Histopathologic analysis of the rats’ liver aortic were prepared using the HE staining method. Aortic histopathological images were captured using the Olympus BX51 microscope with 400× magnification to observe the damage of endothelia and the occurrence of inflammation cells. A digital camera was used to capture aorta histopathology pictures.

2.1.3. Statistical Analysis.

Quantitative HDL data levels was analyzed using One-way Analysis of variance (ANOVA), continued to Tukey test multiple comparison tests. The level for statistical
significance was set at a p value<0.05. The analysis used SPSS 23.0 for windows; and the aortic histopathologic profiles were analyzed descriptively.

3. Results and Discussion

Hyper cholesterol rat models have been produced successfully by force feeding rats with high cholesterol nutrition. All hypercholesterolemia rats of groups 2 showed total cholesterol levels in blood above 54 mg/dL that were 151 mg/dL, while the healthy rats showed 43.25 mg/dL. It is known that at range of 10-54 mg/dL is categorized as normal value of total cholesterol level in rat blood [9]. The high total cholesterol level is linked to free radical such as reactive oxygen species (ROS). The decrease levels of HDL result from the imbalance of ROS and endogenous anti-oxidant. Groups 3, 4, 5, 6 were preventive treated with activated charcoal and the HDL levels of hypercholesterolemia rats are displayed at Table 1.

Table 1. Average HDL Levels in Groups 1, 2, 3, 4, 5, 6 after 14 days of therapy with activated charcoal (AC).

| Group | HDL levels (mg/dL)* |
|-------|---------------------|
| 1. Negative control (healthy group) | 40.74 ± 1.42c |
| 2. Positive control (hypercholesterolemia group) | 28.08 ± 0.75a |
| 3. Therapy AC 2.250 mg/kg bw | 37.20 ± 0.82b |
| 4. Therapy AC 4.950 mg/kg bw | 39.86 ± 0.98c |
| 5. Therapy AC 6.250 mg/kg bw | 36.06 ± 0.69b |
| 6. AC 4.950 mg/kg bw | 43.02 ±0.85d |

*different letters (a-d) show significant statistical different effect in each group p<0.05

As shown in Table 1, HDL level of hypercholesterolemia rats dropped significantly compared to healthy rat. The standard normal of rat HDL level is ≥35mg/dL. The decline of HDL level due to receiving hypercholesterol diet. The high cholesterol intake triggers an increase levels of total cholesterol and LDL due to inadequate HDL level to bring cholesterol ester back to liver. In hypercholesterolemia condition, the body tries to balance plasma cholesterol level by converting cholesterol to bile acids. The synthesis of bile acid involves 7 α-hydroxylase, O2, NADPH and cytochrome P450; ROS as by product is resulted from this process.

The oral administration of activated charcoal may decrease the high level of total cholesterol and increase HDL level. These were significantly different (p<0.05) between HDL level of hypercholesterolemia and therapeutic hypercholesterolemia rats. At the dose of activated charcoal 0.4950 mg/kg body weight is effective dose. This is because the HDL level in group 4 was not different significantly compared to group 1 (healthy rats).

Feeding rats with high cholesterol nutrition, and activated charcoal therapy changed the histopathologic image of rats’ aorta. Variations in the histopathologic image of aorta can be seen in Figure 1, particularly, the changes of tunica intima. The aorta layer comprised of Tunica intima, tunica media and tunica adventitia. The tunica intima condition of negative control group (Group 1) shows that normal layer of endothelial cells which are neatly arranged, flat shapes with the long axis of the cells parallel to the bloodstream. Tunica media consists of elastic fiber, fibroblast, collagen fiber and smooth muscle, while tunica adventitia is composed of fibro elastic connective tissue and smooth muscle cells [10]. That negative control groups shown as the healthy rats. The aorta of group 2 indicates hypercholesterolemia condition. In tunica intima shows that the damaged endothelial cells release to lumen, while in tunica adventitia occur the inflammation cells amongst fibroblast. Enhancement of histopathologic features of the aorta can be shown in the therapy groups. Group 3 shows a decline of endothelial cell erosion in tunica intima compared to Group 2. In addition, group 4
shows a more reduction of endothelial cells erosion and inflammation cells in tunica adventitia. Group 5 shows that aorta histopathology does not different with group 3. Group 6 received 4.950 mg/kg bw without hypercholesterol diet, that shows no damage in tunica intima and inflammation cells, this condition is similar to negative control groups. This group is carried out to investigate the effect of activated charcoal to aorta histopathology and HDL levels in healthy rat.

**Figure 1.** Comparison of histopathologic images of rats aorta from groups 1, 2, 3, 4, 5, 6 after 14 d treatments with activated charcoal with HE staining (400× magnification), TI: Tunica intima, TM: Tunica media, TA: Tunica Adventitia.

Decreases of HDL levels and repair in aorta histopathology of hypercholesterolemia rats treated with activated charcoal can be connected to physical properties of activated charcoal as an adsorbent. Activated charcoal function to inhibit intestine from fully absorbing of cholesterol diet, because The porous of activated charcoal can absorb large molecules such as cholesterol before being absorbed in intestine. The adsorption mechanism of cholesterol takes places through an internal diffusion of cholesterol from a boundary layer to the surface of adsorbent. Some of them are adsorbed on outer surface and most of its further diffuse internally inside adsorbent pore. The activated charcoal cannot be absorbed by intestine or digestive tract; the activated charcoal is only bound to molecules in gastrointestinal lumen. Therefore, activated charcoal are not metabolized in the body and excreted together with feces in the unchanged form [11]. The absorbed cholesterol in activated charcoal will be wasted with feces that can prevent the damage of aorta. The result of SEM shows that activated charcoal in this study has diameter pore of 4.23 μm, while diameter pore of chylomicron of 75-1200 nm or 0.075-1.2 μm [12], that shows activated charcoal has ability to adsorb cholesterol. Based on the results of aorta histopathology observation, activated charcoal can be used as a preventive medication of hypercholesterolemia at effective doses of 4.950 mg/kg body weight.

4. Conclusion
This paper confirmed that activated charcoal at dose of 4.950 mg/kg bw is effective to prevent the decrease of HDL level on hypercholesterolemia animal models using *Rattus norvegicus*. Moreover, the activated charcoal can improve histopathology of its aorta which shown by reducing endothelial cell erosion and inflammation cells. Further study should be conducted in order to investigate the highest and nontoxic dose of activated charcoal.
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