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Allopurinol Reduced Oleic Acid Plasma on High-fat Diet-induced Hypertensive Rats

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Abstract

Hypertension is still a big challenge and is a key risk factor for cardiovascular disease which is the leading cause of death in the world. We evaluate the effects of Allopurinol in high-fat diet-induced hypertensiverats. To this end we induced hypertension of SD rats by high fat-diet for six weeks and divided into 6 groups (n = 5), for six weeks and one group was fed standard chow. Drugs were given for seven days orally; one group was only given a high-fat diet and CMC 0.5%, as Negative Control; one group was given a high-fat diet and Isosorbide Dinitrate (ISDN) 18 mg/kg as Positive Control, three groups were given a high-fat diet and Allopurinol with dose range of 45-180 mg/kg. Systolic, diastolic, mean arterial pressure and blood glucose were compared between diet groups and normal group; Free Fatty Acid Plasma and Amino Acid Plasma were determined by GC-MS. As a results, Allopurinol significantly reduced blood pressure in all three dose groups ($p<0.05$). The dose group of Allopurinol and ISDN gave similar results in lowering blood pressure and glucose levels which measured on the last day of drug administration. Allupurinol also reduced oleic acid plasma compared with high-fat diet group. In conclusion, Allopurinol has a potential as an antihypertensive agent by modulating linoleic acid.

Keywords: Allopurinol, Blood Pressure, High-fat Diet, Hypertension, Linoleic Acid

1. Introduction

Hypertension is a condition where there is an increase in systolic/diastolic blood pressure to 130/80 mmHg or more by measuring two times in the morning and evening in a state of rest or calm for 7 consecutive days[1]. High blood pressure or hypertension is a health problem that requires prompt treatment. However, controlling hypertension has not been adequate even though effective medicines are widely available[2].

Fructose, amonosaccharide found in many fruits and sweeteners such as corn syrup, stimulates the absorption of sodium and chloride, causing excess salt and other complications such as elevated levels of oxidative stress that affect the increase in blood pressure. Meanwhile, cholesterol contained in eggs, seafood, animal fat, butter, oil and other foods can contribute to increasing the blood pressure through oxidative stress and atherosclerosis, which in turn triggers hypertension[3,4]. Recent experimental studies have shown that the occurrence of oxidative stress in rats induced by fructose and fat has an important role in the development of hypertension. Oxidative stress caused by fructose or cholesterol promote endothelial dysfunction and decrease NO, causing hypertension[3]. In fructose, the absence of a feedback mechanism in fructose phosphorylation causes the formation of hypoxanthine which is converted to uric acid with Xanthine Oxidase (XO) with other results in the form of ROS[5].

Amino acid metabolism is essential for the conservation of nitrogen and for maintaining physiologic concentrations of amino acids, which cannot be stored up when in excess from the diet[6]. AA changes in the metabolic cycle may lead pathophysiologic condition[7]. Recent studies investigated the relation between amino acids and blood

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pressure[8]. Beside that fatty acids changes also showed significant pathophysiologic condition such as oleic acid caused cardiovascular diseases[9]

Allopurinol is a purine analog of hypoxanthine and is an inhibitor of the enzyme Xanthine Oxidase (XO). This drug is indicated for gout. In hypertension, there is a relationship with XO through the mechanism of the increase in oxidative stress caused by the reception of electrons of oxygen, so that XO generates superoxide and hydrogen peroxide. The superoxide anion can react with nitric oxide, leading to increased vasoconstriction of blood vessels and causing hypertension[6].

Based on this, the hypothesis emerged that animal hypertension models can be done through the provision of high-fat diets and Allopurinol provides a blood pressure reduction effect. The aim of this study was to create an animal model of hypertension using a high-fat diet, evaluate Allopurinol administration to hypertensive animal models, and evaluate the pathways of hypertension and the effect of Allopurinol on those pathways.

2. Material and Methods

2.1 Animals

Test animals used in this study were Sprague-Dawley (SD) male white rats aged approximately two months with a weight of 150-200 grams totaling 30 animals obtained fromFacultyof Animal Husbandry, Institut Pertanian Bogor (IPB), Bogor. Each cage contains one mouse with a 12-h cycle day and night.

2.2 Ethical Clearance

The study has received ethical approval from the Health Research Ethics Committee at the Faculty of Medicine, Universitas Indonesia following the certificate passed the ethical review with no. KET-227/UN2.F1/ETIK/ PPM.002/ 2019.

2.3 Induction of Hypertension

The method was based on the results of the previous team's research[10]. In subsequent studies, the test animals were divided into 2 groups, namely the normal control group and the induction group. The normal group was given the standard feed while the inductiongroup was given a mixture of standard chow (50%), quail egg yolk (30%), pure butter (Anchor, New Zealand) (10%), Fructose Corn Syrup (HFCS) (PT. Brataco, Indonesia) (10%)[10]. Subsequently divided into 6 treatment groups consisting of the normal control group, negative control, positive control, and three groups of variations in the dose of Allopurinol (Table 1).

Table 1. Rats treatment group

No.	Group	Total rats	Treatment
1.	Normal Control	5	Standard chowfeed and 0.5% CMC orally
2.	Negative Control	5	High Fat Diet (HFD) + CMC 0.5% orally
3.	Positive Control	5	HFD + Isosorbiddinitrate 18 mg/kg BWorally
3.	Allopurinol Dose 1	5	HFD + Allopurinol at a dose of 45 mg/kg BWorally
4.	Allopurinol Dose 2	5	HFD + Allopurinol at a dose of 90 mg/kg BW orally
5.	Allopurinol Dose 3	5	HFD + Allopurinol at a dose of 180 mg/kg BWorally

2.4 Blood Pressure and Glucose Level Recording

Blood pressure measurements were carried out with a non-invasive CODA blood pressure gauge (Kentscientific/USA). The sensor technology used was VPR (Volume Pressure Recording). At the beginning of the study and everyweek systolic blood pressure, diastolic blood pressure and mean arterial pressure would be measured. The procedure used to measure blood pressure refers to the White paper published by Kent Scientific Corporation[8]. Measurement of blood pressure in each rat was done every week from day 0 to 42. Blood glucose levels were measured at the end of the study on day 42. Blood samples were taken through the orbital sinus vein, then placed on the microtube. The blood-filled microtube is then centrifuged at 7000 rpm for 15 minutes. Plasma was measured using a GOD-POD glucose reagent kit. Measurement of blood glucose levels was carried out

by an enzymatic colorimetric method using Glucose Oxidase-Peroxidase (GOD-POD).

2.5 Administration of Drugs

Allopurinol as a test drug and ISDN as a positive control comparison drug were administered after measuring blood pressure every day for 7 days. The drug wasgiven in the form of suspension with 0.5% CMC. Doses for rats were obtained by multiplying human doses by conversion factors from humans to rats (0.018) and pharmacokinetic factors[12]. Human dose used in this study was 100 mg/human/day for Allopurinol and 20 mg/human/day for ISDN. The dose obtained was 90 mg/kg BW rats for the drug Allopurinol which was used as the second dose and 18 mg/kg BW rats for ISDN. The variation of Allopurinol dosage is ½ the second dose is 45 mg/kg which is determined as dose 1 and 2 times the second dose is 180 mg/g which is determined as dose 3.

2.6 Drugs and Chemicals

The following drugs and chemicals were used: Tablet Allopurinol 100 mg (Hexpharm Jaya, Indonesia), isosorbidedinitrate 10 mg (Novell, Indonesia), aquadest (PT. Brataco, Indonesia), dan CMC (PT. Brataco, Indonesia), ether (Merck, USA), glucose kit GOD-POD (HUMAN, Germany).

2.7 Statistical Analysis

Data obtained from this study were processed statistically using SPSS (SPSS Inc,USA). Blood pressure data obtained were performed data normality test (Saphiro-Wilk test) andhomogeneity test (Levene test). If the data is normally distributed and homogeneous the analysis continued using the parametric test, the one-way Analysis of Variance (ANOVA) variant method to see the relationship of all treatment groups. Significant Difference test (BNT) is used to see differences between treatment groups. Testing to see a significant difference between the two treatment groups that are not interconnected is done by independent T-test. If the data is not normally distributed or homogeneous, transformation is performed first. If the data is still not normally distributed and homogeneous, then the analysis uses a non-parametric test followed by a Kruskal-Wallis test to see the relationship of all treatment groups. Furthermore, the analysis is continued with the Mann-Whitney test to see differences between treatment groups.

3. Results

3.1 Effect of Treatment of HFD on Body Weight

In this method, there was a statistically significant difference from the increase in body weight of test animals during the period of treatment between groups (P<0.05). The increase that occurred in the induction group was 28.73% of the initial body weight with an average weight gain per week was 9.75 ± 1.51 grams (P<0.05) compared to the normal group of 13.77% of initial body weight with an average weekly weight gain was 4.82 ± 1.42 grams (Figure 1).

3.2 Effect of HFD on Blood Pressure

Significant changes seen from the significance value (P<0.05) in the induction group for systolic blood pressure had occurred at the 3rd week (day 21). Treatment was continued until reaching hypertension for systolic blood pressure, that was in the 4th week (day 28) and the 5th week (day 35). By the 4th week, the mean systolic blood pressure had risen to 141.51 ± 7.01 mmHg and the 5th week (day 35) had reached 144.31 ± 5.86 mm Hg (Figure 1). For diastolic blood pressure, significant changes in the induction group were seen starting from the 3rd week or day 21. In the first week and the second week, there had been no significant increase in blood pressure (P = 0.154 and P = 0.152). In the 3rd week, a significant increase in the diastolic blood pressure had occurred (P = 0.002) so that its hould have been able to administer the drug. However, the increase was not evenly distributed, so the induction was continued. At 4th week (day 28), the diastolic blood pressure of induction group was 105.51 ± 7.89 mmHg and at week 5 (day 35), the diastolic blood pressure of the induction group was 114.63 ± 6.47 mmHg (Figure 1).

Similar result was shown in Mean Arterial Pressure (MAP), which showed a significant change on day 21 with P<0.05 when compared to data on day 0 with MAP values 104.89 ± 13.77 mmHg. MAP increased to 35 days and 42 days respectively 117.71 ± 7.29 and 124.52 ± 5.89 mmHg (Figure 1).

3.3 Effect of Allopurinol on Blood Pressure

A week after drug administration, there had been a significant decrease in the ISDN and Allopurinol group. The decrease was seen from the difference in blood pressure of both the systolic and diastolic of ISDN groups, dose 1 Allopurinol, dose 2 Allopurinol, and dose 3 Allopurinol to normal (P>0.05), except for systolic blood pressure in the dose 3 Allopurinol group which was decreased but not until it returns to normal (P = 0.031). Decreased blood pressure in the ISDN group was seen from the 5th week (day 35) with an average systolic/diastolic blood pressure of 145.55 ± 7.46/113.03 ± 4.81 mmHg to 118.3 ± 4.28 / 85.2 ± 5.14 mmHg at 6th week (D-42).

Decreased blood pressure in the dose 1 Allopurinol group was seen from week 5 (day 35) with mean systolic/diastolic blood pressure143.07 ± 2.89/111.43 ± 5.62 mmHg to116.98 ± 4.42/81.30 ± 9.09 mmHg at 6th week (day 42). Decreased blood pressure in the dose 2 Allopurinol group was seen from week 5 (day 35) with an average systolic/diastolic blood pressure of 140.73 ± 3.23/112.4 ± 5.42 mmHg to 117.73 ± 5, 43/85.63 ± 1.46 mmHg at week 6 (day 42). Decreased blood pressure in the dose 3 Allopurinol group was seen from week 5 (day 35) with mean systolic/diastolic blood pressure142.46 ± 2.90 /113.26 ± 4.17 mmHg to 119.47 ± 1 48/83.28 ± 1.60 mmHg at day 42 (Figure 2).

By comparing with the normal group, it was seen that Allopurinol had a significant effect in reducing systolic blood pressure (P = 0.000), with systolic blood pressure returning to normal for Allopurinol dose 1 (P = 0.364) and Allopurinol dose 2 (P = 0.459). Whereas for dosage 3, Allopurinol decreased even though it did not return to normal (P = 0.031) (Figure 2).

By comparison with the normal group, it was seen that Allopurinol gave a significant effect in reducing diastolic

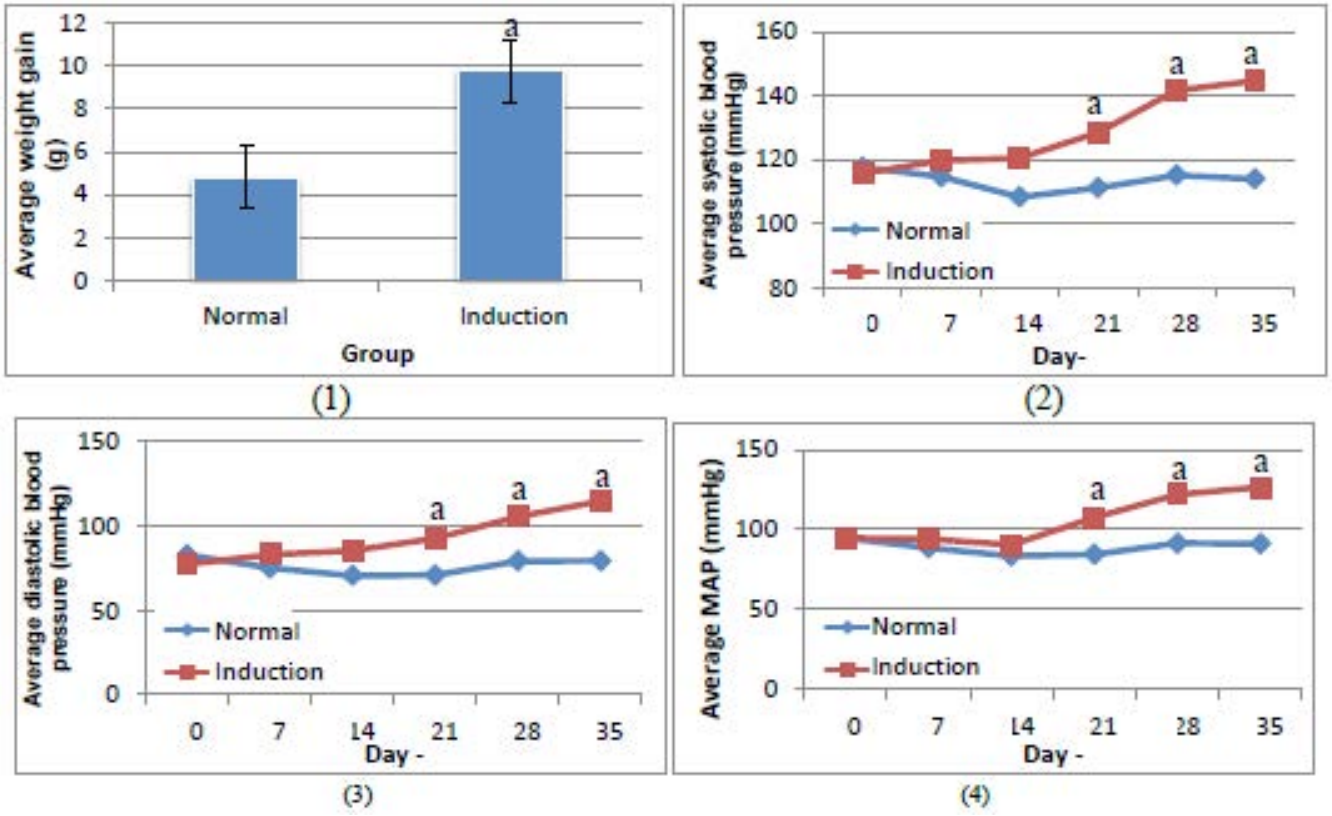


Figure 1. Average increase in body weight and blood pressure of rats after giving HFD induction treatment. Normal group = group that is given standard chow. Induction group = group given HFD, (a) P<0.01 compared to day 0. 1. Average profile bar diagram of rats' weight increase after treatment; 2. Graph of development of systolic blood pressure profile during treatment; 3. Diastolic blood pressure profile chart during treatment; 4. Graph of mean arterial pressure during treatment.

blood pressure (P = 0.000), with diastolic blood pressure returning to normal for Allopurinol dose 1 (P = 0.414), Allopurinol dose 2 (P = 0.067) and Allopurinol dose 3 (P = 0.053) (Figure 2).

Significant decreases were also seen from data before and after drug administration in the parameters of average arterial pressure (MAP). When compared with normal, it could be said that ISDN wasable to significantly reduce average arterial pressure in HFD rats to return to normal with a decrease from an average MAP of 123.87 ± 5.38 mmHg to 97.54 ± 4.47 mmHg (P = 0.089). The mean arterial pressure decreased from 121.98 ± 4.53 mmHg to 93.20 ± 6.12 mmHg (P = 0.332) for Allopurinol dose 1; 121.70 ± 4.44 mmHg to 95.54 ± 1.84 mmHg (P = 0.038) for Allopurinol dose 2; and 123.00 ± 2.81 mmHg to 95.34 ± 1.21 mmHg (P = 0.029) for Allopurinol dose 3 compared to the normal group (Figure 2).

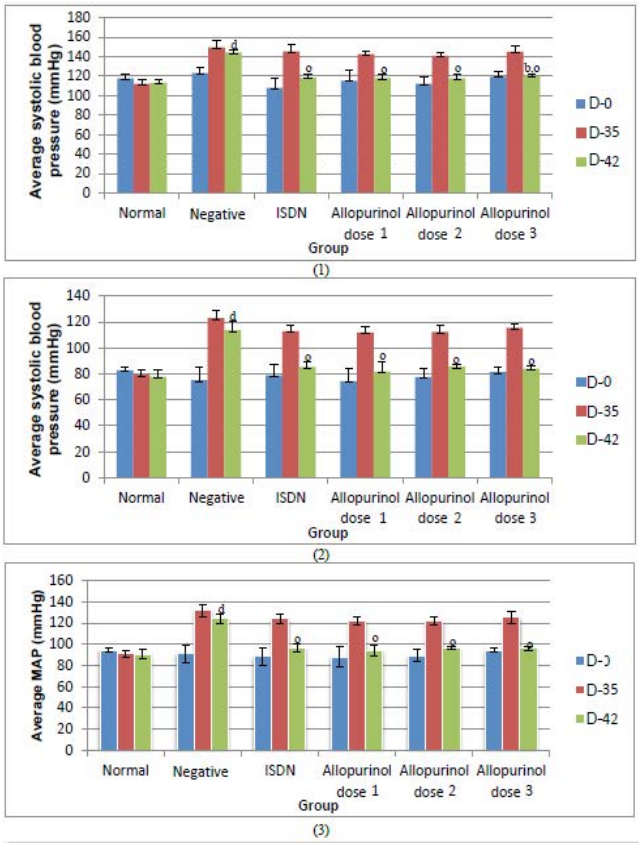


Figure 2. Average blood pressure changes in mice following treatment provision induction HFD. Normal, not given treatment; Negative, HFD and CMC 0.5%; ISDN, HFD and 18 mg/kg; Allopurinol dose 1, HFD and 45 mg/kg; Allopurinol dose 2, HFD and 90 mg/kg Allopurinol dose 3, HFD and 180 mg/kg; (b) P<0.05 compared to the normal group; (d) P<0.001 compared to the normal group; (o) P<0.01 compared to the negative group. 1. Graph of average systolic blood pressure during treatment; 2. Graph of average diastolic blood pressure during treatment; 3, Graph of average arterial pressure during treatment.

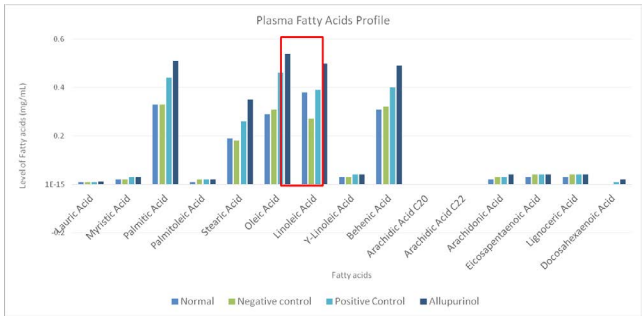


Figure 3. Plasma fatty acid profile following HFD and Allopurinol treatment. HFD treatment rat showed low concentration of Linoleic acid in plasma, administration of ISDN as positive control and Allopurinol showed increase the concentration of Linoleic acid.

4. Discussion

4.1 Effect of HFD on Body Weight

In subsequent studies, rats were divided into 2 groups, namely the normal control group and the induction group. There was a significan tincrease (P<0.05) from the increase in body weight of rats during the period of treatment between groups statistically. This is consistent with research conducted by El-Bassossy and Shaltout (2015) in which rats given HFD had a higher weight gain in the induction group compared to the normal group. Rats given a high-fat diet became more obese and had higher glucose levels than the normal group. Increased blood pressure does not seem to occur due to a high-fat diet itself but seems to be a consequence of hyperphagia and obesity that occurs[13]. Weight gain resulting in obesity can be associated with increased oxidative stress, lipid peroxidation and Xanthine Oxido Reductase (XOR) activity[14].

4.2 Effect of HFD on Blood Pressure

The difference in blood pressure in the normal group with the diet induction group illustrates the increase in blood pressure in rats that is thought to have metabolic syndrome. This is consistent with research conducted by El-Bassossy and Shaltout (2015) in which rats given a high-fat diet had higher mean arterial pressure than the normal group. High-fat dietsare associated with hypertension, hyperinsulinemia, hyperglycemia, and hypercholesterolemia. Dietary linoleic acid intake may contribute to prevention and control of adverse blood pressure levels in general populations[15]. In this research, HFD decreased the concentration of Linoleic acid plasma and lead high systolic blood pressure. BP-lowering effects of linoleic acid may be mediated through changes in Prostaglandin (PG) metabolism[16]. In animal studies of diets enriched with n-6 PFA, the vasodilator PGs (PGI2 and PGE2) increased in kidney, aorta, blood or urine. In humans, there is evidence that PG production in kidney and other tissues from dietary PFA is involved in BP regulation. Therefore, decreasing linoleic acid should be increase blood pressure.

4.3 Effect of Allopurinol on Blood Pressure

The reduction in systolic, diastolic and MAP of the three doses of Allopurinol was not significantly different when compared to the ISDN group, thus providing the possibility that the pathway to decrease blood pressure was the same. The decrease in blood pressure is in accordance with previous research which states that Allopurinol has a hypotensive effect; i.e, reducing systolic blood pressure, diastolic blood pressure and heart rate; suppress both systolic and diastolic blood pressure; and aorticvasodilation. These effects provide strong evidence that Allopurinol can relieve hypertension, possibly through cardiac hemodynamic suppression and decreased blood vessel resistance[17]. In addition, given Allopurinol can reduce uric acid levels, serum uric acid levels and significantly inhibit XO activity compared to the normal group in the study[12].

Xanthine oxidase activity also produces reactive oxygen species, namely superoxide, hydrogen peroxide and hydroxyl radicals. Reactive oxygen species cause tissue damage and deactivate nitric oxide, which leads to endothelial dysfunction, a precursor to atherosclerosis and blood vessel injury. Allopurinol inhibits xanthine oxidase activity, thereby decreasing reactive oxygen species and increasing the availability of biological nitric oxide. Treatment with Allopurinol has been shown to improve endothelial function, measured by forearm blood flow in patients with heart failure and type 2 diabetes mellitus with mild hypertension[18].

Allopurinol reduces oxidative stress in diabetic rats, an effect that can be attributed to its inhibitory effect on XO. The study shows that the possible effect of Allopurinol is not caused by decreased uric acid production and the evidence presented supports the hypothesis that the reduction in oxidative stress contributes to the beneficial effect of Allopurinol[19].

5. Conclusion

It can be concluded that the treatment of high-fat diet is able to form animal models of hypertension. In addition, Allopurinol has been shown to reduce systolic/diastolic blood pressure and MAP in Sprague-Dawley rats as a model animal with hypertension at 45 mg/kg bodyweight rats.

Further research needs to be done to evaluate mechanism of action of Allopurinol that decreased blood pressure through decreasingl evels of linoleic acid.

6. Acknowledgement

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