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Hypotensive Effect of Tuber Based Artificial Rice on Hypertension Rats

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Abstract : Hypertension is one of public health problems in developed and underdeveloped countris. Food for hypertension prevention or treatement is still limited. One of food crops that contains bioactive compounds for hypertension prevention or treatment is tuber. Most of tubers contain phenolic compounds that have a role in hypertension management. *Dioscorea* sp or yam tubers contain tuber storage protein, namely dioscorin, that has an ability to inhibit angiotensin converting enzyme (ACE). ACE converts angiotensin I to angiotensinII that constricts blood vascular and increasesblood pressure. Artificial rice based on tubers was expected to provide food for hypertension treatment. This study aimed to examine the effect of tuber based artificial rice on blood pressure of salt induced hypertension rats. Artificial rice made from arrowroot, wild yam, lesser yam, greater yam, and cocoyan was separately fed to 5 groups of hypertensive rats *ad libitum*. As controls, a group of hypertensive rats was fed by rice IR 36 and a group of normotensive rats was fed by rice IR 36. Experiment was conducted for 4 weeks, and every week the systolic blood pressure of rats was examined. Body weight and feed intake were measured daily. Phenols content and dioscorin of tuber based artificial rice were also analyzed. The results showed that all types of tuber based artificial contained phenolic compounds and only yam based artificial rice had dioscorin. Tuber based artifical rice was able to reduce systolic blood pressure in hypertension condition. The greatest effect was found in wild yam artificial rice. The magnitude of reduction was wild yam > cocoyam > arrowroot > greter yam > lesser yam based artificial rice. Meanwhile, rice IR 36 did not exhibit blood pressure decline. This finding showed that not only yam based artificial rice was able to reduce blood pressure but also non yam tuber such as arrowroot and cocoyam. Phenolic compounds also influenced the reduction of blood pressure after tuber based artificial rice feeding.

Keywords : angiotensin converting enzyme, artificial rice, dioscorin, phenols, systolic blood pressure, tuber.

Introduction

Hypertension, also known as high blood pressure, is a long term medical condition in which the blood pressure in the arteries is persistently elevated[1].Long term high blood pressure; however, is a major risk 1factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease[2,3].

Many medicines are available for hypertension treatment. Most of hypertension drugs have an action as ACEi (Angiotensin Converting Enzyme inhibitor). ACE converts angiotensin I to angiotensin II that constricts blood vascular and increases blood pressure.Side effects of blood pressure medications are hypokalemia, hyperuricemia, hypercalcemia, impaired glucose tolerance, and erectile dysfunction[4]. Thus, non-pharmacological approaches for lowering blood pressure have been widely studied[5].

The trend to view many foods not only as substance but also as medicine, so-called functional foods, is increasing[6,7]. However, foods for hypertension prevention or teratment are still limited. Products for hypertension treatment might use food materials that contain antihypertensive bioactive compounds. Among them, tubers are one of antihypertensive compound containing food crops, beside fruits, legumes, nuts, and vegetables.

Numerous studies have shown that yams are sources of diverse nutrients and non-nutrient molecules, many of that display bioactive properties. In the recent years, several beneficial properties of yam tubers were demonstrated in many papers[8]. Yam tubers contains dioscorin, a storage tuber protein, that has capability in inhibition of ACE activity[9]. The research of Liu *et al.*[9] showed that feeding trial of yam powder containing instant meal to hypertensive subject improved blood pressure. Beside dioscorin in yam tubers, tubers also contain phenolic compounds. Study of Ciociuo *et al.*[10] showed that polyphenolic extract from *Aronia melanocarpa* reduce systolic and diastolic arterial tension values in rats with drug-induced hypertension.

Tuber powder is an intermediate product that is usually processed into variuos foods. Artificial rice is a product that has similarity to rice in appearance, texture, mouthfeel, preparation, and functions. This product is produced as an alternative of rice consumption. Due to lilmited acceptability, artificial rice was designed to have advantage over original rice, especially benefits to health. Tuber based artificial rice is expected to provide health benefits in hypertension management due to dioscorin content in yam tubers and phenolics content in most tubers. This study was aimed to examine the effect of tuber based artificial rice from wild yam (*Dioscorea hispida*), greater yam (*Dioscorea alata*), lesser yam (*Dioscorea esculenta*), cocoyam (*Xanthosoma sagittifolium*), and arrowroot (*Marantha arundinaceae*).It was supposed that tuber based artificial rice reduced blood pressure in hypertension condition. Among tuber based artificial rice, yam based artificial rice is allegedly to have better effect.

Experimental

Materials

Wild yam, greater yam, and lesser yam were ontained from loval farmer in Tuban, East Java, Indonesia. Meanwhile, cocoyam and arrowroot were obtained from ILETRI, Malang, Indonesia. Male Wistar strain rats with age of 2.5-3 months and body weight of 200-300 g were used for *in vivo* study. All chemical reagents for analysis were from Merck, Germany.

Tuber flour preparation

All tubers were separately washed, peeled, and sliced with 2-3 mm in thickness. Sliced lesser yam tuber was soaked in Na-bisulfite (1 g/L) solution and salt (50 g/L) for 6 h. Peeled wild yam tuber was rubbed with salt for 24 h, and then soaked in water for 30 h. Every 3 h the soaking water was replaed by fresh water and subsequently sliced into chips. Sliced greater yam, arrowroot, and cocoyam ware separatelysoaked in Na-bisulfite (1 g/L) solution with different time, i.e. 15 min for greater yam, and 12 h for arrowroot and cocoyam. Each sliced tubers after soaking were then dried in cabinet drier with temperature 55ºC in different time, i.e. 6 h (lesser yam, arrowroot), 12 h (wild yam, cocoyam), and 10 h (greater yam). The dried chips were ground to obtain 80 mesh tuber flour.

Tuber based artificial rice preparation

Separately, each tuber flour was mixed with rice flour in 70:30 (w/w). Water, palm oil, salt, STPP (sodium tripolyphosphate), sodium alginate, and $CaCl_2$ were added to flour mixture. The mixture was kneaded and then molded to have rice shape. The molded dough was then steamed at 100ºC for 5-7 min and dried at 60ºC for 1 h. The semi dried artificial rice was dipped into sodium alginate solution (3% w/v) and $CaCl_2$ 1000 ppm for 15 min for coating. The drying was continued for 3 h at 60ºC

Antihypertension *in vivo* test of tuber based artificial rice

As many as 35 male Wistar strain rats were divided into 7 groups. Each rat was caged individually at animal laboratory with temperature 20-25^{o}C, and 12 h lighting. One group of rats was normotensive rats that fed by rice IR36. This group was declared as control normal group. Other 6 groups were hypertensive rats that separately fed *ad libitum*by rice IR 36 and wild yam, lesser yam, greater yam, cocoyam, and arrowroot based artificial rice. To obtain hypertensive rats, rats were force fed by pure NaCl (1 g/kg bw) for 7 d to increase blood pressure to stadium 3 with systolic blood pressure 180-209 mmHg. During salt induction time, rats were fed by standard diet AIN-93 M. Only rats with blood pressure more than 180 mmHg were included in this experiment. First measurement of blood pressure was declared as systolic blood pressure at week 0. During subsequent 4 weeks, rats were fed according to the treatment. Fed intake was measured every day and body weight was measured every two days. Systolic blood pressure was measured by systolic blood pressure was measured every week by tail cuff method using sphygmomanometer. Data from this experiment was analyzed statistically for analysis of variance, and subsequently Least Significance Difference (LSD) analysis.

Tuber based artificial rice snalysis

Phenol content was analyzed by method of Ukom *et al*.[11]and dioscorin was analyzed by SDS PAGE[12]. Anaylisis of cyanide content for wild yam artificial rice was according to method of Andama and Lejju[13].

Ethical Clearance

The protocol of bioassay had been approved for ethical clearance No. 202-KEP-UB 2014 from Animal Care and Use Committee, Brawijaya University, Indonesia.

Results and Discussions

Phenol and dioscorin of artificial rice

All artificial rice contained phenols but their amount is measly (Table 1). Processing of tuber into artificial rice caused some phenols lost or oxidized. Phenols are antioxidant compounds that have some roles on health, including antihypertension[14]. Phenols impair endothelial functions by regulating eNOS (endotheilial Nitric Oxide Synthase) expression and increasing NO (Nitric Oxide) production. In oxidative stress condition, total amount O_2^- increases and O_2^- is a strong vasoconstrictor that constricts blood vessel an increases blood pressure. NO will interact with O_2^- immediately for O_2^- neutralization and reducing O_2^- amount. This condition causes a reduction of NO bioavailability. Phenols contribute to incrase NO bioavailability by activating eNOS. eNOS will produce NO that has a role as a strong vasodilator and relaxing blood vessel[15].

Table1. Phenol and dioscorin content of tuber based artificial rice

Artificial Rice	Phenols Content (mg GAE/ 100g)	Dioscorin (%w/w based on water soluble protein)
Rice IR36	0.1	-*
Lesser yam	0.26	2.49
Wild yam	0.18	1.21
Greater yam	0.65	5.18
Arrowroot	0.48	-**
Cocoyam	0.40	-**

* not analyzed ** not detected

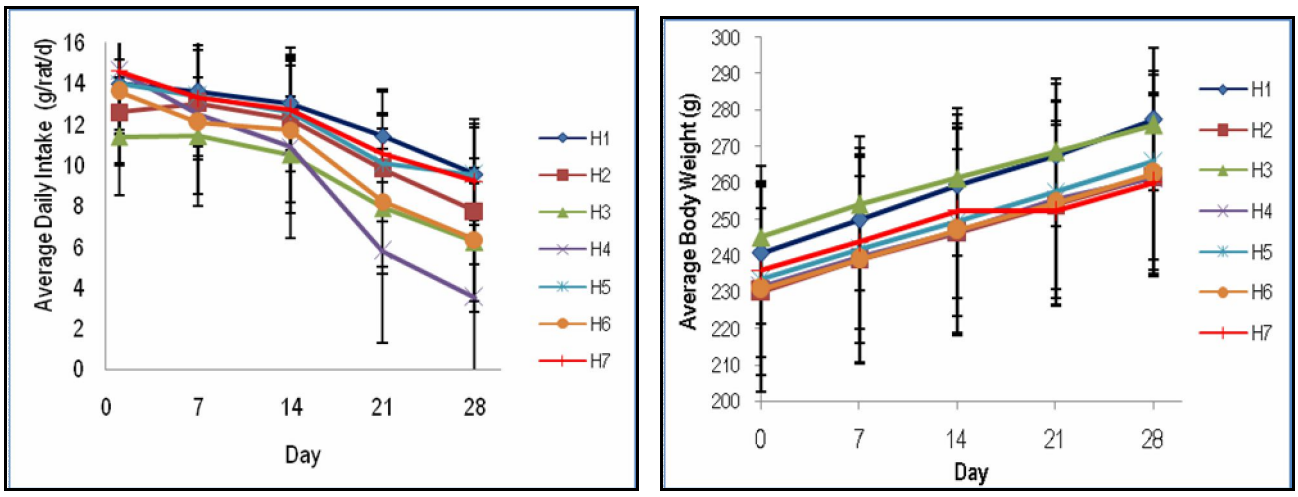
Dioscorin was found in yam based artificial rice such as lesser yam, wold yam, and greater yam. Meanwhile cocoyam and arrowroot are not yam family and dioscorin was not detected in both tubers. Greater yam had the highest amount of disocorin, and the least was found in wild yam. Dioscorin is water soluble protein[16] of yam family[17,18,19] that has several activities such as trypsin and carbonic anhydrase inhibitors[20], radical scavenging activity[21], and immunomodulator[22].

Several studies showed that dioscorin inhibits angiotensin converting enzyme (ACE), an enzyme that is responsible to increasing blood pressure[21,22,23]. Dioscorin has competitive inhibitory to ACE and is able to control blood pressure in hypertension[21]. It was expected that the existence of dioscorin in tuber based artificial rice would contribute to lower blood pressure in hypertension condition.

Feeding intake and body weight

Rats had preference to artificial rats tat indicated by average daily amount of intake (Figure 1a). The highest intake was found in group of normotensive rats treated by rice IR 36. The lowest intake was group of hypertensive ratsfed by wild yam based artificial rice. It was shown that feeding intake gradually decreased during experiment. The sharp decline was found in wild yam based artificial rice treated group. Cyanide content was usually found in wild yam. Wild yam artificial rice contained cyanide of 27.64 ppm. Bitter test of cyanide and its toxic effect might contribute to low intake of this artificial rice. Commonly, hypertensive groups consumed lower amount of feed than normotensive group.

Feeding rats with artificial rice increased body weight but the magnitude of increment depended on type of tubers (Figure 1b). Normotensive group had the best body weight gain. Among tuber based artificial rice, the best body weight gain was found in group treated by lesser yam and the least was found in cocoyam artificial rice treated group. It seemed that there was no correlation between feeding intake and body weight because the average starting point of weight was vary among groups.



(a) (b)

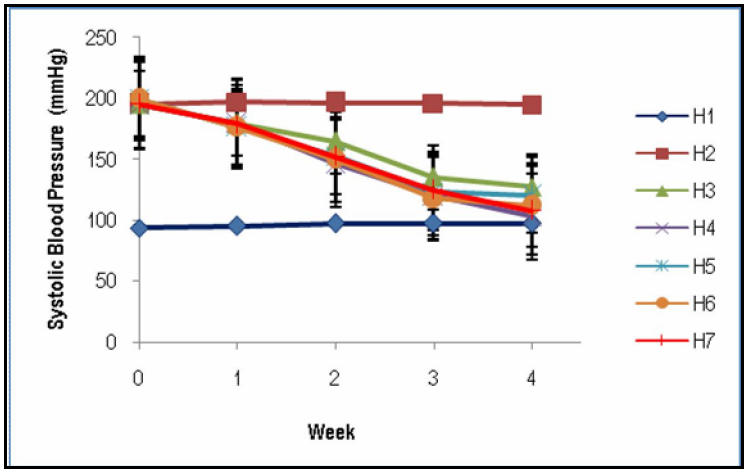
H1 = normotensive group fed by rice IR 36, H2 = hypertensive group fed by rice IR 36, H3 = hypertensive group fed by lesser yam artificial rice, H4 = hypertensive group fed by wild yam artificial rice. H5 = hypertensive group fed by greater yam artificial rice, H6 = hypertensive rice fed by arrowroot artificial rice, H7 = hypertensive group fed by cocoyam artificial rice

Figure 1. Average feeding intake (a) and body weight (b) of group of rats fed by artificial rice

Antihypertensive effect of tuber based artificial rice

In this experiment, systolic blood pressure was measured by tail cuff method. Normotensive group showed normal blood pressure during 4 week experiment. Hypertensive group fed by IR 36 exhibited high blood pressure, meanwhile blood pressure of hypertensive groups treated by tuber artificial rice gradually decreased (Figure 2). Degree of blood pressure reduction was affected by the type of tuber. The highest decline was found in wild yam artificial rice fed group and the lowest was in group treated by lesser yam

artificial rice. The magnitude of reduction was wild yam > cocoyam > arrowroot > greter yam > lesser yam based artificial rice.



H1 = normotensive group fed by rice IR 36, H2 = hypertensive group fed by rice IR 36, H3 = hypertensive group fed by lesser yam artificial rice, H4 = hypertensive group fed by wild yam artificial rice. H5 = hypertensive group fed by greater yam artificial rice, H6 = hypertensive rice fed by arrowroot artificial rice, H7 = hypertensive group fed by cocoyam artificial rice

Figure 2. Systolic blood pressure of groups of rats fed by tuber based artificial rice

In hypertensive group, high blood pressure was achieved by force fed salt for 7 d. Increased salt intake may aggravate the rise in blood pressure and the development of consequential damage of the heart, the vessels and other organs. Elevated sympathetic activity, angiotensin II (ANG II), endogenous Na/K-ATPase inhibitors and other influences exert their hypertensive effects mainly via vascular smooth muscles inducing vasoconstriction, and this reaction is more pronounced on high salt intake. An important mediator of increased blood pressure under high salt intake is endogenous ouabain (EO). EO is thought to induce this sensitization and to increase the activity of endothelial ACE[24].

In this experiment, salt induction successfully increased blood pressure in hypertensive groups to systolic blood pressure 195-200 mmHg. Blood pressure of hypertensive group fed by rice IR 36 was still high although the salt induction was terminated after 7 day. This is an indicator that salt induction almost permanently changed blood pressure.

All tuber based artificial rice decreased systolic blood pressure gradually (Table 2). Yam based artificial rice (wild yam, lesser yam, greater yam) contained disocorin in different amount. The sharp decline of systolic blood pressure was found in wild yam artificial rice (Figure 2), although this rice did not contain the highest amount of dioscorin and phenols (Table 1). Likewise, lesser yam artificial rice exhibited the lowest changes of blood pressure during 4 week feeding, although this rice contained dioscorin and phenol and the amount was higher than that found in wild yam artificial rice. The highest dioscorin and phenol content was found in greater yam artificial rice, but this rice did not show the best performance in reducing systolic blood pressure.

It seemed that bioavailability of dioscorin and phenols among tuber based artificial rice was different. According to Carbonell-Capella *et al.*[25] when studying the role of bioactive compounds in human health, their bioavailability is not always well known. Before becoming bioavailable, they must be released from the food matrix and modified in the gastrointestinal (GI) tract. Bioavailability includes also in its definition the utilization of a nutrient and therefore can be defined as the fraction of ingested nutrient or compound that reaches the systemic circulation and is utilized[26]. Bioavailability includes GI digestion, absorption, metabolism, tissue distribution, and bioactivity[25].

Table 2. Systolic blood pressure changes during 4 week feeding by tuber based artificial rice

Group	Week					Changes	
	0	1	2	3	4	mmHg	%
H1	93.8±2.3a	95.2±3.3a	97.2±1.3a	97.2±1.5a	97.2±1.9a	2	+2
H2	195.6±4.5a	196.8±2.3a	196.6±1.1a	195.8±2.3a	194.8±2.9a	-2	-1
H3	195.6±1.9e	179.4±6.2d	164.8±5.1c	135±4.1b	127.4±2.3a	-52	-29
H4	194.8±3.4e	179.6±6.5d	146.4±4.5c	120±2.5b	103±3.5a	-76.6	-43
H5	198.8±4.0d	176.6±3.3c	152.6±3.0b	123.2±1.9a	120.4±2.1a	-56.2	-32
H6	199.6±4.2e	176.6±5.9d	149.8±5.4c	117.8±4.7b	112.2±3.3a	-64.4	-36
H7	195.4±3.7e	179±4.1d	152.2±5.0c	124.6±2.1b	108±2.5a	-71	-40
LSD 5%	4.6						

H1 = normotensive group fed by rice IR 36, H2 = hypertensive group fed by rice IR 36, H3 = hypertensive group fed by lesser yam artificial rice, H4 = hypertensive group fed by wild yam artificial rice. H5 = hypertensive group fed by greater yam artificial rice, H6 = hypertensive rice fed by arrowroot artificial rice, H7 = hypertensive group fed by cocoyam artificial rice

(+) increased, (-) decreased

It is suggested that bioavailability of penolic compounds and dioscorin in tuber based artificial rice affected on their performance in reducing blood pressure. Different phenolic compounds among tubers might influence their role in reducing blood pressure. Study of Ozo *et al.*[27] showed that there were different phenolic compounds of *Dioscorea* species. Similarly, Champagne *et al.*[28] revealed that there were various antocyanin and phenolic compounds among tropical root crops .

Dioscorin is atuber storage protein of yams. Liao *et al.*[29]revealed that there was different secondary structure of three cultivars of greater yam. According to Liu *et al.*[30]different structure of dioscorin caused different biological activity. Therefore, the effect of dioscorin on reducing blood pressure was not only affected by its amount but also affected by its bioavailability.

Groups of rats fed by non yam based artificial rice from cocoyam and arrowroot also exhibited reduction in systolic blood pressure. Cocoyam artificial rice showed the best decline after wild yam artificial rice. Cocoyam and arrowroot did not contain dioscorin (Table 1), and the bioactive compound that responsible to reduce blood pressure was phenolic compounds through antioxidant activity. As previously explained, antioxidant play a role in reducing blood pressure through impairment of endothelial functions by regulating eNOS (endotheilial Nitric Oxide Synthase) expression and increasing NO (Nitric Oxide) production[15]. Different types of phenolic compounds had different antioxidant activity[31]. Therefore, the different ability of tuber based artificial rice on reducing blood pressure was supposed to be affected by type of phenolic compounds and structure of dioscorin.

Conclusion

Tuber based artificial rice was able to reduce systolic blood pressure in hypertension condition. The phenolic compounds and dioscorin in artificial rice was supposed to contribute in lowering blood pressure. The greatest effect was found in wild yam artificial rice. The magnitude of reduction was wild yam > cocoyam > arrowroot > greter yam > lesser yam based artificial rice. Meanwhile, rice IR 36 did not exhibit blood pressure decline when fed to hypertensive rats. This finding showed that not only yam based artificial rice was able to reduce blood pressure but also non yam tuber such as arrowroot and cocoyam.

Acknoeledgement

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