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HYPOGLYCEMIC, ANTI-INFLAMMATORY EFFECT OF PORANG *(AMORPHOPHALLUS ONCHOPYLLUS)* ON ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Introduction: Iles-iles / Porang is a tuber-producing plant that is commonly found in Indonesia. One of the most sought after content from Porang is Glucomannan. One of Porang's health benefit is related to the effect of lowering blood glucose levels because it can prevent glucose absorption. These benefit is interesting to study because there has been no research linking the use of Porang to reduce inflammatory process in hyperglycmic conditions.

Purpose: This study aims to analyze the anti-inflammatory, anti-oxidant and hypoglycemis effects of Glucomannan from Porang (*Amorphophallus onchophyllus)* extract in experimental animals.

Method: The research design was a true experimental post-test only control group with random sampling to determine 5 white rats into the normal group, positive control group, negative control group and treatment 1, 2 and 3. The positive control group received Acarbose therapy thile the negative control group received carboxy-methyl-cellulose (CMC) therapy. This study used Porang extract *(Amorphophallus onchophyllus)* with concentrations of 200, 400 and 800 mg/Kg in hyperglycemic white rats that had been induced by Aloxan. The study was conducte for 50 days and then blood and serum samples were taken to assess the effect of hypoglycemia, anti-inflammatory and anti-oxidant using blood glucose, Malondialdehyde and C-reactiveprotein (CRP) measurement.

Result and Discussion: The results showed no significant difference between the groups of rats receiving Porang extract and the positive and negative control groups. However, the MDA levels after 50 days of intervention between the negative control group and the therapy with doses of 200, 400 and 800 mg/Kg showed significant differences. Similarly found for blood glucose levels after intervention between negative control group and the 200 and 400 mg/Kg therapy group. This results may be caused by the type of Porang used, the form of the Porang and the concentration level of the Porang extract.

Conclusion: Porang with the type of *Amorphophallus onchophyllus* can't be used directly, but requires further processing to obtain the active substance Glucomannan.

Keyword: *Porang, Diabetes Mellitus, Hypoglycemic, Anti-Inflammatory*

ABSTRAK

Latar belakang: Iles-iles / Porang adalah tanaman penghasil umbi yang banyak ditemukan di Indonesia. Salah satu kandungan Porang yang banyak dicari karena manfaatnya adalah *Glucomannan*. Penggunaan Porang di bidang kesehatan berkaitan dengan efek penurunan kadar glukosa darah karena dapat mencegah absorpsi glukosa. Fenomena ini merupakan hal yang menarik untuk diteliti sebab belum ada penelitian yang mengaitkan penggunaan Porang untuk menurunkan proses inflamasi pada kondisi hiperglikemia.

Tujuan: Penelitian ini bertujuan untuk menganalisa efek anti-inflamasi, anti-oksidan serta hipoglikemia yang dimiliki *glucomannan* ekstrak Porang *(Amorphophllus onchopyllus)* pada hewan coba.

Metode: Desain penelitian adalah *true experimental post-test only control group* dengan *random sampling* untuk menentukan 5 ekor tikus putih ke dalam kelompok normal, kontrol positif, kontrol negatif, intervensi 1, 2 dan 3. Kelompok kontrol positif mendapatkan terapi Acarbose sedangkan kelompok kontrol negatif mendapatkan terapi *carboxy-methyl-cellulose* (CMC). Penelitian ini menggunakan ekstrak Porang *(Amorphophallus onchophyllus)* dengan konsentrasi 200, 400 dan 800 mg/Kg pada tikus putih hiperglikemia yang telah diinduksi Aloxan. Penelitian dilakukan selama 50 hari kemudian dilakukan pengambilan sampel darah dan serum untuk menilai efek hipoglikemia, anti-inflamasi dan anti-oksidan menggunakan pengukuran gula darah, *Malondialdehyde* serta *C-reactive protein* (CRP).

Hasil: Hasil penelitian tidak menunjukkan perbedaan signifikan antara kelompok tikus yang mendapatkan ekstrak Porang dengan kelompok kontrol positif maupun kontrol negatif, namun tingkat MDA setelah intervensi selama 50 hari antara kelompok kontrol negatif dan intervensi dengan dosis 200, 400 dan 800 mg/Kg menunjukkan perbedaan yang signifikan. Hal serupa juga ditemukan pada tingkat gula darah setelah intervensi antara kelompok kontrol negatif dengan kelompok intervensi 200 dan 400 mg/Kg. Hasil ini mungkin disebabkan oleh karena jenis porang yang digunakan, bentuk sediaan porang serta tingkat konsentrasi ekstrak porang.

Kesimpulan: Porang dengan jenis *Amorphophallus onchopyllus* tidak dapat digunakan secara langsung, namun memerlukan pemrosesan lebih lanjut untuk mendapatkan zat aktif *Glucomannan.*

Keyword: *Porang, Diabetes Mellitus, Hipoglikemia, Anti-Inflamasi*

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INTRODUCTION

Diabetes Mellitus is estimated to have suffered by 463 million people in 2019 and predicted to increase to 700 million by 2045 based on data from the International Diabetes Federation (IDF) that Diabetes Mellitus becomes one of the main threats to global health in the 21st century. Indonesia itself ranks seventh as a country with 10,7 million people with Diabetes Mellitus in 2019(1,2).

Diabetes Mellitus is one of the top 10 causes of death in the world. It is associated with oxidative stress and chronic inflammation induced by chronic hyperglycemic conditions. Chronic hyperglycemic conditions in Diabetes Mellitus can cause reversible and irreversible molecular damage(3). Metabolic change due to hyperglycemic conditions are sorbitol metabolism which can increase de-novo diacylglycerol synthesis, protein kinase C (PKC) synthesis and activation, decrease Na^+ K^+ ATPase and increase reactive oxygen species (ROS) (4). The increasing tissue sorbitol also cause a decrease in oxidative stress dismutase which cause an oxidative stress and osmotic pressure leads to swelling and damage to cells, especially endothelium(5).

Oxidative stress induced pro-inflammation protein production as well as infiltrating macrophages which secrete pro-inflammatory cytokines such as Tumor Necrosing Alpha (TNF-alpha). The secretion of inflammatory cytokines plays a role in causing insulin resistance and increasing the production of reactive oxygen species (ROS)(6–8). In patients with Diabetes Mellitus, ROS can be produced through several pathways such as increase in Polyol pathway, Advanced-Glycation End Products (AGE) and PKC activation(7).

Chronic hyperglycmic conditions in Diabetes Mellitus can inhibit antioxidant activity such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and decreasing glutathione production causing damage to DNA, fatty acid and protein. This causes reactive oxygen species and pro-inflammatory mediators to increase. This interaction between oxidative stress and inflammation in the long term will casue microvascular and macrovascular complications in Diabetes Mellitus patients. Those complications are Diabetic hepatopathy, retinopathy, nephropathy, neuropathy and cardiomyopathy(7).

Iles-iles / Porang is a tuber-producing plant that is commonly found in Indonesia. Ile-iles / Porang also called *Amorphophallus muelleri* in latin or more commonly known as Konjac are also found in sub-tropical areas of Asia, Southeast Asia, Africa and Australia. In China, Porang is known as Moyu, while in Japan it is known as Juruo and Elephant Foot Taro in Europe(9).

One of the most sought after content from Porang is Glucomannan. One of Porang's

health benefit is related to the effect of lowering blood glucose levels because it can prevent glucose absorption. Some animal studies have shown that Porang can decrease blood glucose through increased mRNA insulin gene expression(10). Another animal studies using processed polysaccharide Porang flour showed the effect of slowing gastric emptying time, lowering total cholesterol and triglyceride levels. Besides that the fiber content which is easily fermented by gastrointestinal flora is thought to stimulate Glucagon-Like Peptide (GLP) production(9,11–13) These benefit is interesting to study because there has been no research linking the use of Porang to reduce inflammatory process in hyperglycmic conditions. This study aims to analyze the anti-inflammatory, anti-oxidant and hypoglycemis effects of Glucomannan from Porang (*Amorphophallus onchophyllus)* extract in experimental animals. We hope that the results of this study can serve as a reference and provide information about the clinical effect of Porang in Indonesia.

METHOD

The design of this study is ture experimental with Post-test only control group design. The experimental animal model of hyperglycmia used was a male white rat. The sample of this study was divided into normal, positive control, negative control, treatment 1, 2 and 3. Each group consisted of 5 white rats with random sampling technique.

The 5 groups of rats treated with intraperitoneal injection of Aloxan at a dose of body weight divided by 100 times 0,2 to make the diabetes rats. The positive control groups rats were given acarbose peroral therapy at a dose of 0,2 mL/200 gram body weight. The negative control groups were given carboxy-methyl cellulose (CMC) peroral at a dose of 0,2 mL/100 gram body weight. The treatment groups were given whole extract porang (*Amorphophallus onchophyllus)* peroral at a dose of 0,2 mL/100 gram body weight with different concentration, Treatment group 1, 2, & 3 were given 200 mg/Kg, 400 mg/Kg & 800 mg/Kg of body weight concentration accordingly.

Whole extract Porang was administered for 56 days to determine the effect of chronic hyperglycmia induced by Aloxan. The study was conducted at the Biochemistry laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya. During the treatment, the rats were given food and water to drink regularly. Malondialdehyde level was measured to determine the oxidative stress caused by hyperglycemic condition, C-reactive protein (CRP) as a marker of inflammation and blood sugar levels were measured to determine the effect of hypoglycemia.

Measurement of blood sugar levels was carried out 3 times, after the adaptation period, after Aloxan induction and after 50 days of treatment with porang through blood specimen, while MDA and CRP measurements were carried out on day 51 through serum specimen.

The methodology for determinating the levels of Malondialdehyde based on the derivatization of MDA using thiobarbituric acid to form "MDA-TBA" compound which is highly fluorescent at acidic pH. The formed "MDA-TBA" compound was preconcentrated using vortex assisted liquid-liquid microextraction and measured spectro-fluorimetrically(14).

C-reactive protein measurement used two reagent, immunoturbidimetric system. First of all, the specimen is combined with a Tris buffer then incubated. After that, latex particles coated with mouse anti-human CRP antibodies added to the specimen. In the presence of circulating CRP, the latex particles aggregate forming immune complex. These complex cause an increase in light scattering that is propotional to the CRP concentration. The light absobance resulting from this light scatter is read against a stored CRP standard curve to determine the concentration of CRP. Turbidity is measured at a primary wavelength of 546 nm or 800 nm(15).

This study has received a certificate of ethics from the Komite Etik Penelitian dan Klinis (KEPK) Faculty of Medicine Widya Mandala Catholic University Surabaya with the number 0222/WM12/KEPK/DOSEN/T/2021.

RESULTS

Results of Examination of Body Weight, Blood Glucose, Malondialdehyde and C-Reactive Protein.

Table 1 shows the mean value of Body Weight Post Aloxan and 8-weeks after intervention from each groups. Normality test shows p value > 0,05 which means the body weight data has normal distribution.

Data analyze using One Way Anova shows p value 0,654 and 0,869 which means there are no significant differences.

Table 1. Mean Value and Standard Deviation of Body Weight (gram) (Mean ± SD)

Group	Post Aloxan	Week-8
	$\overline{X} \pm SD$	$\overline{X} \pm SD$
N	126,6 ± 17,813	194,6 ± 30,163
K+	123,4 ± 19,165	171,2 ± 24,356
K-	125 ± 19,092	181,80 ± 23,91
P1	137 ± 20,037	180,60 ± 17,271
P2	139,4 ± 24,583	186,60 ± 17,271
P3	135,6 ± 10,922	185,40 ± 41,795
P Value (One Way Anova)	.654	.869

N = Normal, K+ = Positive Control, K- = Negative Control, P1 = 200 Intervention, P2 = 400 Intervention, P3 = 800 Intervention

Table 2 shows the mean value of Blood Glucose Post Aloxan and 8-weeks after intervention from each groups. Normality test shows p value < 0,05 which means the blood glucose data distribution is not normal. Data analyze using Kruskal-Wallis shows p value 0,29 and 0,197 which means there are no significant differences.

Table 2. Mean Value and Standard Deviation of Blood Glucose (mg/dL) (Mean ± SD)

Group	Post Aloxan	50-days after Intervention
	$\overline{X} \pm SD$	$\overline{X} \pm SD$
N	118,80 ± 29,895	165 ± 62,837
K+	224,20 ± 154,416	191,40 ± 119,667
K-	117,20 ± 42,133	158,40 ± 33,783
P1	212,60 ± 103,028	134 ± 7,550
P2	127,20 ± 35,024	114,80 ± 28,482
P3	253,60 ± 104,064	138,60 ± 18,889
P Value (Kruskal-Wallis)	.029	0.197

N = Normal, K+ = Positive Control, K- = Negative Control, P1 = 200 Intervention, P2 = 400 Intervention, P3 = 800 Intervention

Table 3 shows the mean value of Malondialdehyde & C-Reactive Protein after intervention from each groups. Normality test shows p value < 0,05 which means the Malondialdehyde and C-Reactive Protein data distribution is not normal. Data analyze using Kruskal-Wallis shows p value 0,059 and 0,870 which means there are no significant differences.

Table 3. Mean Value and SD of Malondialdehyde (nmol/mL) and C-Reactive Protein (mg/L) (Mean ± SD)

Group	MDA	C-Reactive Protein
	$\overline{X} \pm SD$	$\overline{X} \pm SD$
N	14.410,4 ± 8.716,925	0,16000 ± 0,89443
K+	9.073,40 ± 4.468,712	0,14000 ± 0,054772
K-	28.929,00 ± 11.891,547	0,12000 ± 0,044721
P1	10.838,00 ± 7.066,070	0,16000 ± 0,054772
P2	9.143,20 ± 7.361,604	0,16000 ± 0,054772
P3	10.815,20 ± 7.200,407	0,20000 ± 0,173205
P Value (Kruskal-Wallis)	.059	.870

N = Normal, K+ = Positive Control, K- = Negative Control, P1 = 200 Intervention, P2 = 400 Intervention, P3 = 800 Intervention

Table 4 shows the difference between Body Weight Post Aloxan and 8 weeks after within all groups except of the 800 mg/Kg intervention groups. intervention within each groups. This table shows that there are significant difference

Table 4. Paired Sample T-test of Body Weight between Groups

Group	Body Weight Post Aloxan - Week-8
N	,001
K+	,008
K-	,003
P1	,015
P2	,000
P3	,069

N = Normal, K+ = Positive Control, K- = Negative Control, P1 = 200 Intervention, P2 = 400 Intervention, P3 = 800 Intervention

Table 5 shows the difference between control groups and intervention groups of body weight. Independent T-test shows no significant difference between control groups and intervention groups.

Table 5. Independent T-test of Body Weight between Groups

Group	Body Weight Week-8 After Intervention	
	K+	K-
P1	,682	,236
P2	,742	,965
P3	,244	,289

N = Normal, K+ = Positive Control, K- = Negative Control, P1 = 200 Intervention, P2 = 400 Intervention, P3 = 800 Intervention

Table 6 shows the distribution of blood glucose and malondialdehyde between control groups and intervention groups. This table shows that there are significant difference of Malondialdehyde level between negative control groups with P1, P2 and P3 intervention groups ($p < 0,05$). Blood glucose after intervention shows no significant difference between negative control and intervention group except P3 intervention groups.

Table 6. Distribution of Blood Glucose and Malondialdehyde After Intervention Across Categories of Groups

Group	Blood Glucose After Intervention	Malondialdehyde
K- with P1 (Mann-Whitney)	.045	.016
K- with P2 (Mann-Whitney)	.075	.016
K- with P3 (Mann-Whitney)	.402	.028
K+ with P2 (Mann-Whitney)	.141	.917
P Value (Kruskal-Wallis)	.197	.059

N = Normal, K+ = Positive Control, K- = Negative Control, P1 = 200 Intervention, P2 = 400 Intervention, P3 = 800 Intervention

DISCUSSION

Our study did not show significant results in reducing blood sugar levels, CRP or MDA. MDA and CRP were used as indicators, respectively, to determine whether the adminstration of whole extract Porang had an effect on the inflammatory process and oxidative stress in rats.

However, there are some interesting results found in the level of Blood Glucose after intervention between Negative Control and Intervention Groups. We found that there are significant difference between negative control and intervention groups who received 200 and 400 mg/Kg of whole extract porang. This result may mean that 200 and 400 mg/Kg of whole extract Porang might reduce the level of blood glucose after 56 days of intervention even though it is not significant enough to be compared with positive control group who gets 0,2 / 200 mg dose of Acarbose. Similar result were also found at level of Malondialdehyde between negative control and P1, P2, P3 intervention groups.

This insignificant result could be caused by (1) the type of Porang used, (2) the form of the Porang extract and (3) the concentration of the Porang extract.

Several studies that we found showed significant results using Porang extract derived from the type of *Amorphophallus konjac*, this may be due to the effect of glucomannan on *Amorphophallus konjac*_which has been proven thorugh research and has properties for lowering blood glucose levels and anti-oxidant effects. In-vitro and in-vivo studies on male Wistar rats aged 12 weeks showed that glucomannan from *Amorphophallus konjac* had hypoglycemic and anti-oxidant effects as measured by glucose diffusion asessment, enzymatic assay inhibition, oral glucose tolerance test and oral starch tolerance test. Based on the glucose diffusion test, it was found that the rats that received konjac showes a decrease in glucose concentration by 36 and 19% when compared to the positive and negative control groups, respectively. The results of the comparison of the effect of konjac with Acarbose measured using α-amylase inhibition examination showed that konjac has a lower percentage when compared to Acarbose. At the highest concentration of 100 µg Konjac showed an inhibition of 14% compared to Acarbose, which was 21%. Konjac also showed a maximum mean inhibitory concentration (IC_{50}) of 357 µg/µL, while Acarbose was 238 µg/µL. In addition, the inhibitory effect on α-glucosidase Konjac was similar to Acarbose with an inhibition percentage above 90% with the IC_{50} values of Konjac and Acarbose of 52 & 53 µg/µL. This study used Konjac derived from *Amorphophallus konjac* with a soluble tuber powder preparation, the concentration of Konjac used was 102 mg/Kg body weight which is equivalent to 1 gram / dose in humans(16,17).

The inflammatory process in Diabetes Mellitus has been widely studied

after its relationship to the process of insulin resistance and pancreatic beta cell defects associated with inflammatory reaction and hyperglycemia. Many studies on the hypoglycmic effect of konjac glucomannan show the same thing. One study using konjac glucomannan showed that konjac glucomannan inhibited inflammatory reactions through the process of inhibiting the production of Interleukin-10, Interleukin-4 and Tumor Necrosing Factor-α (TNF-α). Other studies also showed that administration of low or high concentration of konjac glucomannan can increase lymphocyte proliferation in mice that have decreased immune system reactions after being induced by Cyclophosphamide (CTX) accompanied by a significant increase in serum levels of TNF- α, IgG and Interleukin-2. Konjac glucomannan inhibits the inflammatory process and regulates the nuclear factor-kappa B (NF-κB) pathway and reduce oxidative stress through the regulation of the *nuclear factor erythroid 2-related factor 2* (Nrf2)(16,18–20).

A study conducted by Haihong et.al. using rats as experimental animals showed a significant decrease in blood glucose levels from the adminstration of soluble konjac glucomannan at a dose of 80 mg/Kg measured using an oral glucose tolerance test (OGTT) and glycated serum protein (GSP) in addition, insulin resistance was also lower when measured using HOMA-IR than the control group in the study. This study also evaluated the effect of glucomannan on oxidative stress using superoxide dismutase (SOD) and serum Malondialdehyde indicators, the results of the study found a more significant decrease in SOD enzymatic activity in the control group than the group receiving soluble konjac glucomannan but giving soluble konjac glucomannan showed decrease in MDA levels although not significant. Administration at a dose of 80 mg/Kg also considered more effective than a dose of 40 mg/Kg or 160 mg/Kg, this is probably due to an increase in viscosity accompanied by an increase inn glucomannan concentration, causing a decrease in post-prandial glucose levels and insulin secretion which in the long run will improve insulin sensitivity(21).

Another study conducted by Akihiro Yoshida et.al compared blood glucose levels in Japanese people who had received 75 grams of oral glucose following rice-gruel administration with or without glucomannan. The study found that the group that had received rice-gruel with glucomannan showed lower blood glucose levels 30 minutes-post-loading and lower insulin levels. This study divided the research subject into 3 groups, namely the

group that was only given rice-gruel without glucomannan, with 0,4% glucomannan and 0,8% glucomannan. Glucomannan used in powder form weighing 250 grams each(22).

Research conducted by Ngatirah et.al found that giving glucomannan Iles-Iles (*Amorphophallus onchophyllus*) and *L.casei* within effervescent synbiotic tablets can reduce the blood glucose / sugar levels of white rats but do not affect the weight of white rats. Ngatirah et.al found that 0,18 mg/200 mg body weight of glucomannan give the biggest decrease of blood glucose compared to half and normal dose (0,045 mg and 0,09 mg) of glucomannan for 1 month(23).

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CONCLUSION

The use of whole extract Porang with *Amorphophallus onchophyllus* did not significantly give hypoglycemic, anti-oxidant and anti-onflammatory effects after sub-chronic adminstration (50 days). Porang with the type of *Amorphophallus onchophyllus* can't be used directly, but requires further processing to obtain the active substance Glucomannan which has shown positive results in several studies. This can be one of the reasons why the price of Porang that has been further processed becomes expensive.We suggest that further research needs to be carried out to determine the content of other active substance from Porang (*Amorphophallus onchophyllus*) which can inhibit the action of glucomannan and to collaborate with the Faculty of Food Engineering to increase the glucomannan of Porang (*Amorphophallus onchophyllus*) efficacy with a simpler and more affordable method.

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