Artikel Penelitian

Can Purple Sweet Potato Water Extract (Ipomoea batatas L.) Induce Steatosis at Toxic Doses in Rat?

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Abstrak

Ubi jalar ungu (Ipomoea batatas L.) memiliki komposisi metabolit sekunder yang kaya antioksidan. Sebagian dari senyawa metabolit sekunder yang terkandung dalam ubi jalar ungu memiliki sifat hepatotoksik. **Tujuan**: Menggambarkan efek toksisitas ekstrak akut air ubi jalar ungu terhadap gambaran steatosis pada tikus. **Metode**: Penelitian deskriptif ini telah dilakukan di Laboratorium Biomedik Fakultas Kedokteran Universitas Islam Bandung dengan menggunakan desain eksperimental murni *in-vivo* dengan teknik random alokasi yang dilakukan terhadap 11 tikus. Satu ekor tikus kontrol tidak diberi perlakuan, sedangkan 10 tikus lainnya diberikan dosis ekstrak air ubi jalar ungu sebesar 50, 200, 400, 800, 1000, 1500, 2000, 3000, 4000, dan 5000 mg/ kgBB/ per oral (p.o). Pengambilan organ hepar dilakukan setelah 24 jam, selanjutnya dibuat sediaan histopatologi di Laboratorium Patologi Anatomi Universitas Padjajaran Bandung. **Hasil**: Pada pemeriksaan histopatologi tidak diperoleh gambaran steatosis baik makrovesikular ataupun mikrovesikular tetapi ditemukan gambaran lain yaitu ballooning degeneration, pelebaran sinusoid, dan inflamasi. **Simpulan**: Steatosis tidak ditemukan pada penelitian ini dapat dikarenakan tingginya kadar antosianin dan tannin dalam ubi jalar ungu.

Kata kunci: steatosis, tannin, ubi jalar ungu

Abstract

Purple sweet potato (Ipomoea batatas L.) has a secondary metabolite that contains a lot of antioxidants. Some of the secondary metabolites in purple sweet potato have a hepatotoxic effect. ***Objective****: To described the toxic effects of purple sweet potato water extract on steatosis in rats.* ***Methods****: This descriptive study was conducted on 29–30 June 2019 in the Biomedical Laboratory of the Faculty of Medicine, Islamic Bandung University, with an in-vivo experimental design with random allocation techniques and using the proposed new method on 11 rats. One of the control rats was not given extract, and ten other rats were given a dose of purple sweet potato water extract 50, 200, 400, 800, 1000, 1500, 2000, 3000, 4000, and 5000 mg/KgBW/PO. The liver organ was harvested after 24 hours, and then slides were made at the Pathology Anatomy Laboratory of Padjajaran University.* ***Results****: The histopathological view did not find any steatosis either in macrovesicular or microvesicular, but other features were found in ballooning degeneration, sinusoidal dilation, and inflammation.* ***Conclusion****: Steatosis was not found because purple sweet potato contains anthocyanins and tannins.*

Keywords*: purple sweet potato, steatosis, tannin*

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INTRODUCTION

The most abundant sweet potato in Indonesia is the purple sweet potato.[1] Purple sweet potato contains secondary metabolite components: anthocyanins, phenolic acids, carotenoids, coumarins, tannins, and alkaloids.[1–5] Purple sweet potato has many benefits for the body but does not cover it is possible that if consumed in excess, unwanted effects may occur, such as the use of drugs that exceed normal limits so that they can cause toxicity to organs, especially the liver (Drug–induced liver injury / DILI). Knowing the undesirable effects of using sweet potatoes on organ function or a toxicity test that can cause organ damage

becomes very important.[6,7] The toxicity test is divided into chronic, sub-acute, and acute. The acute toxicity test is a test to assess the presence of a toxic effect on a substance given in a single dose in a short period to see the LD50 (lethal dose 50), which is a dose that can cause 50% death in experimental animals with parameters that can be seen are hematological examination, biochemistry, and histopathology.[7] Histopathological examination is one of the important examinations, with one example to see the progress of a disease that has no symptoms that the effects of drug administration can cause. The effect of drug administration can cause disturbances in the liver, for example, it can cause fatty liver because the liver is an organ that plays a role in all metabolism and drug and hormone release.[6] Giving coumarin orally in Toxic doses of 88 gm/kg for 42 weeks can cause liver damage, changes in blood and tissue levels, and weight loss. Acute toxicity of coumarins given orally can cause impaired liver function tests in humans, cause severe liver changes, cause hepatitis, and reduce liver function tests in rats.[8] Acute exposure to huge doses (e.g., carbon tetrachloride).[9] This underlies the interest in knowing the effects of acute toxicity of purple sweet potato (Ipomoea batatas L.) aqueous extract on the appearance of steatosis in rats.

METHODS

This research method uses a pure in-vivo experimental design with a random allocation sample selection technique, defined as a design with a random selection of samples according to the number of samples. The design used in this study was the post-test only with a randomized control group design, or the measurements were made after the experimental animals were given treatment. This research has three stages. The first stage used four rats, each given a 50, 200, 400, and 800 mg/kgBW/PO dose. If no rats die during stage one, then proceed to stage two. The second stage used three different rats from the previous stage, with each rat being given a dose of 1000, 1500, or 2000 mg/kg BW/PO. If no dead rats are found, proceed to the third stage. The third stage used three different rats from the previous stage, with each rat being given a dose of 3000, 4000 or 5000 mg/gBW/PO. No dead rats are found at all stages, and a confirmation test is carried out using two different or new rats. One is given the lowest dose that can cause death, and the other rat is given the highest dose that can cause death. After 24 hours, all rats were operated on to take their liver organs and then sent to the Anatomical Pathology Laboratory of Padjadjaran University to be used as preparations. Results This research was conducted on rats with the Wistar strain with 11 experimental animals that met the inclusion criteria. The rats were then acclimatized for seven days, and then the rats were given purple sweet potato water extract according to a predetermined dose. Liver organ sampling was carried out after 24 hours by surgery and put into a plastic bottle containing formalin to be sent to the Anatomical Pathology Laboratory of Padjadjaran University for preparations to be made. This research was conducted after obtaining approval from the Ethics Commission of Universitas Islam Bandung Number 054/Komite Etik FK/ IV/2019.

RESULTS

The results of observations made in this study were that in all rats, both control and rats that had been given purple sweet potato water extract at a certain dose, no macrovesicular and microvesicular features were found. The results of this study were viewed using a microscope with a magnification of 400x. They found other features, namely, ballooning degeneration, widening of the sinusoids, and inflammation. In rats given purple sweet potato aqueous extract at high doses, namely 4000mg/KgBW and 5000mg/KgBW, it was found that ballooning degeneration and widening of the sinusoids were found. Control rats and rats were given doses of 50mg/kgBW, 200 mg/kgBW, 400 mg/kgBW, 800 mg/kgBW, 1000mg/kgBW, 1500mg/kgBW, 2000 mg/kgBW, 3000 mg/kgBW, 4000mg/kgBW, and 5000mg /kgBW each found a picture of inflammation.

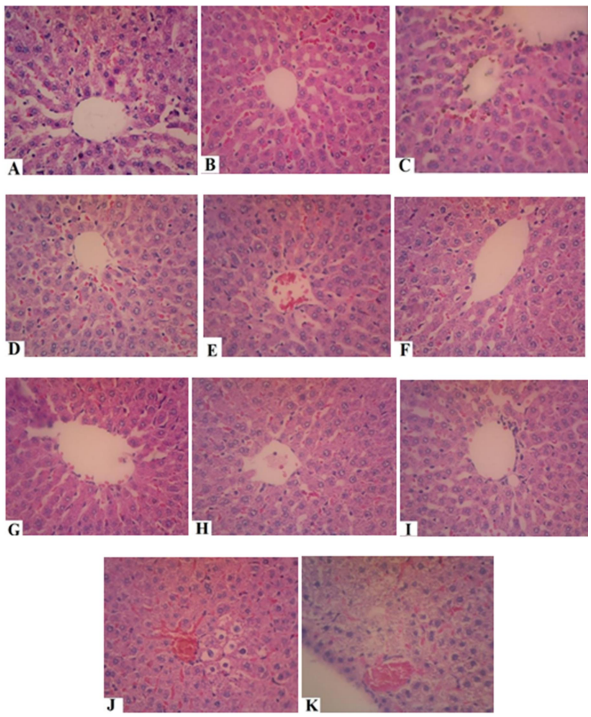

Figure 1. Histopathological view of the liver (400x magnification with HE staining)

Picture A = not given purple sweet potato water extract (control); B = dose of 50 mg/kgBW/PO; C= dose of 200 mg/kgBW/PO; D = dose of 400 mg/kgBW/PO; E = dose of 800 mg/kgBW/PO; F = dose of 1000 mg/kgBW/PO; G = dose of 1500 mg/KgBW/PO; H = dose of 2000 mg/KgBW/PO; I = dose of 3000 mg/kgBW/PO; J = dose of 4000 mg/kgBW/PO; K = dose of 5000 mg/kgBW/PO

Steatosis is a cytoplasmic condition in hepatocyte cells that accumulates lipids with a composition consisting of triglycerides, phospholipids, and cholesterol.[10] Steatosis can occur due to an increase in fatty acids due to increased lipogenesis from visceral and subcutaneous adipose tissue and decreased removal of fatty acids so that it can cause accumulation of lipid droplets and hepatic steatosis.[11] Observations of liver cells were observed at 400x magnification (Table 1). The results of observations made in this study were that in all rats, both control and rats that had been given purple sweet potato water extract at a specific dose, no macrovesicular and microvesicular features were found.

Steatosis appears when there are supporting risk factors such as metabolic syndrome, type 2 diabetes mellitus, hypertension, dyslipidemia, hepatitis, low HDL cholesterol, and high LDL cholesterol.[12] Steatosis can occur in the chronic and acute phases, for example, when acute exposure to huge doses (e.g., carbon tetrachloride).[9] Purple sweet potato contains metabolites, namely anthocyanins, phenolic acids, carotenoids, coumarins, tannins, and alkaloids. In this study, the maximum acute dose of purple sweet potato did not cause steatosis, either macrovesicular or microvesicular. It is estimated that the tannins contained in purple sweet potatoes can reduce lipogenesis.[13]

Table 1. Histopathological appearances of rat liver induced by purple sweet potato water extract

Group		Doses (mg/kg BW)	Histopathological Appearances	
			Macro-vesicular	Micro-vesicular
	Control	-	-	-
Stage 1	1.1	50	-	-
	1.2	200	-	-
	1.3	400	-	-
	1.4	800	-	-
Stage 2	2.1	1000	-	-
	2.2	1500	-	-
	2.3	2000	-	-
Stage 3	3.1	3000	-	-
	3.2	4000	-	-
	3.3	5000	-	-

Table 2. Additional data

Group		Doses (mg/ kgBW)	Histopathological Appearances		
			Ballooning Degeneration	Sinusoid Dilatation	Inflammation
	Control	-	-	-	+
Stage 1	1.1	50	-	-	+
	1.2	200	-	-	+
	1.3	400	-	-	+
	1.4	800	-	-	+
Stage 2	2.1	1000	-	-	+
	2.2	1500	-	-	+
	2.3	2000	-	-	+
Stage 3	3.1	3000	-	-	+
	3.2	4000	+	+	+
	3.3	5000	+	+	+

This is supported by the research of Min Yu Chung *et al.* in 2019, which said that tannin acid (TA) can reduce lipogenesis and attenuate lipid accumulation.[13] Other secondary metabolites, namely anthocyanins, can also reduce lipogenesis. This is supported by research conducted by Luca Valenti et al in 2013 which stated that anthocyanins are suitable for the treatment of fatty liver diseases.[14] In this study, high doses given to rats did not show signs of steatosis. However, other features were found, such as ballooning degeneration, widening of sinusoids, and inflammation. Ballooning degeneration is when hepatocytes experience enlargement, are round, and have cytoplasm with Mallory hyaline or inclusion bodies found in the cytoplasm of hepatocyte cells. Biologically, ballooning degeneration is an initial event. Leading to progressive disease. This can occur due to the accumulation of fluid in the intracellular.[10]

Ballooning degeneration or hydropic degeneration is a cell injury that is reversible or can be corrected if the cause (for example, hypoxia, exposure to chemical agents, infectious agents, immunological reactions, genetic factors, unbalanced nutrition, and aging) disappears so the cell can return to normal life. Cell injury is when cells experience severe stress so that cells can no longer adapt. Ballooning degeneration occurs due to the failure of energy-dependent ion pumps on the plasma membrane, resulting in cells being unable to maintain ion and fluid homeostasis.[12] Secondary metabolites such as coumarins, tannins, and alkaloids have a hepatotoxic effect. However, until now, no one has revealed a mechanism. The formation of ballooning degeneration due to secondary metabolites such as coumarins, tannins, and alkaloids. This study shows a picture of the widening of the sinusoids. Dilated sinusoids are widening of the hepatic capillaries.[14] Sinusoids are wide, irregular blood vessels lined only by fenestrated endothelial cells.[15] Sinusoids regulate the contribution of blood in the liver to maintain portal pressure.[16] Dilated sinusoids are often associated with impaired venous outflow due to obstruction, which can lead to congestion of the vessels and increased venous pressure. Clinical signs of sinusoidal dilation associated with obstruction in the acute form include hepatomegaly, right upper quadrant pain, and ascites. In a few days or weeks, the symptoms can persist and, if left unchecked, will become a further complaint where collagen deposits can surround the venules.[17] Obstruction of the veins can occur after drug or toxin exposure, causing a picture of sinusoidal dilatation.[18]

One of the toxic substances present in purple sweet potato is alkaloid. Alkaloids produced by plants are secondary metabolites that increase reproduction in plants and act as a defense mechanism against insects. Alkaloids are hepatotoxic, and it has been reported that alkaloids can cause hepato-sinusoidal obstruction syndrome and vena-occlusive disease. Alkaloids induce liver damage by binding to active metabolites and deoxyribonucleic acid (DNA)/protein.[19] Apart from alkaloids, there are substances contained in sweet potatoes that cause hepatotoxicity, namely datesrin and tannins. Coumarin is an aromatic organic chemical compound that is colorless and has a sweet smell but tastes bitter. Coumarins are found in plants that function as a chemical defense against predators.[8] The way coumarins damage the liver is by inhibiting cytochrome P45. Cytochrome P45 is an enzyme involved in the metabolism of endogenous and exogenous compounds whose primary purpose is detoxification.[20]

Tannins or tannic acid are a group of pale yellow to light brown amorphous substances in the form of powder, flakes, or spongy masses. Functionally, tannins function to bind proteins. The ability to bind proteins from tannins is very important in defense against insect pests.[21,22] Although tannins are beneficial for plants and humans, tannins can cause harm to the body, which can cause stomach irritation, nausea, and damage to the liver.[23] Mechanisms of tannins that can damage the liver are currently unknown. This study found inflammation in hepatocyte cells. Inflammation is an immune response to various factors such as pathogens, toxic compounds, and damaged cells.[24] Inflammatory processes play a role in homeostatic processes (which act to resolve inflammation to avoid pathological consequences or excess inflammation) and pathology. In healthy individuals, regulation of systemic homeostasis of the liver is the production of serum proteins such as albumin, complement proteins, fibrinogen, clotting factors, and lipoproteins. These proteins will play a role in the regulation of blood osmotic pressure and act as inactive precursors of several innate immune mediators. Individuals who are infected will have an acute response in the liver. The liver will secrete antimicrobial proteins, coagulation factors, opsonins, and inflammatory mediators such as cytokines (e.g. IL-6) to increase the acute response which will later change thermoregulation and induce leukocytosis.[25] Purple sweet potato has secondary metabolites, which are rich in antioxidants that function to prevent disease. Antioxidants can turn into prooxidants when antioxidants are catalyzed by metals. This will cause prooxidants to produce free radicals which will damage DNA and mutagenesis.[26] The occurrence of inflammation in this study is thought to be due to the conversion of antioxidants into prooxidants, causing inflammation in hepatocytes.

CONCLUSION

Administration of purple sweet potato aqueous extract at toxic doses did not cause steatosis, either microvesicular or macrovesicular, in rat livers.

ACKNOWLEDGMENTS

Thank you to all parties who have played much role in this research so that it can be carried out as it should.

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