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Effect of pH Variation of Ethanol Solvent in Purple Sweet Potato (*Ipomoea batatas* L.) Extract on Hepatoprotective Activity of White Rats (*Rattus novergicus*)

Mochamad H. Nurzaman[1], Salsabila Adlina[1], Aditya Kuncoro[1], Nitya N. Fadilah[1], Taufik Hidayat[2]*

[1]*Department of Pharmacy, Faculty of Health Science, Perjuangan University of Tasikmalaya 46115, Indonesia.*
[2]*Department of Pharmacy, Faculty of Pharmacy, BTH University of Tasikmalaya 46115, Indonesia.*

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ABSTRACT

In particular, PM 2.5 (air pollution) passes through the alveoli into the blood circulation and can reach the liver for catabolism, leading to liver damage. The hepatoprotective agents may be purple sweet potato tubers (*Ipomoea batatas* L.) with bioactive anthocyanin compounds. Anthocyanins are unstable at pH values ≥7. This study aimed to determine the effect of pH variations of the ethanol solvent (without acidification, pH 2.5 and pH 1.5) in the extraction of purple sweet potato tubers on hepatoprotective activity. For the study, the experimental animals were divided into six different groups, including a standard control group, a negative control group with administered carboxy methyl cellulose (CMC) 1% /kg body weight, a positive control group with 100 mg/kg body weight of Silymarin (SMR), purple sweet potato extract (PSPE) 400 mg/kg body weight group without acidification and two groups with pH 1.5 and pH 2.5 with 400 mg/kg bw of PSPE. Paracetamol (PCM) was administered on the seventh day (except for the standard group) with 3000 mg/kg body weight one hour after the last oral treatment. PSPE has hepatoprotective activity and affects acidic pH in biochemical tests, total protein, albumin, liver index and histology. Results revealed that treatment with pH 1.5 and 400 mg/kg bw of PSPE significantly restored the liver function markers to their average values, including serum enzyme levels, and increased total protein and albumin levels is an indication of the ability to restore synthesis function in paracetamol-induced liver damage slowly.

Keywords: Hepatoprotective, Antioxidants, Paracetamol, Air pollution, *Ipomoea batatas* L.

Introduction

In 2022, Indonesia was ranked as the first country with the worst air quality in the Southeast Asia region, with a PM 2.5 (particulate matter) concentration of 49.6 µg/m^3 which is still far from the annual standard for the concentration of PM 2.5 of 5 µg/m^3. [1] Specifically, PM 2.5 enters the alveoli through the blood circulation and can reach the liver for catabolism[2] which may have an impact on oxidative stress, genotoxicity, and liver damage[3] by disrupting the balance between free radicals and antioxidants,[4,5] then accumulates in the Kupffer cells, which cause inflammation and activate the stellate cells of the liver.[2] The liver is the largest organ in the human body, maintaining the body's balance (homeostasis).[6] It has several functions in the form of metabolic processes (nutrients, bilirubin, and xenobiotics, and supports digestion, biosynthesis, detoxification, and the formation of antibodies.[7] Damage to the liver will completely disrupt its function and even cause death. This shows the importance of the liver in the body.[8,9] The purple sweet potato tuber is a functional plant with bioactive compounds called anthocyanins, which have antioxidant and hepatoprotective activity.[10]

*Corresponding author. E mail: taufikhidayat@universitas-bth.ac.id
Tel: +62-8815227696

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Since anthocyanins are generally unstable at pH ≥ 7, the acidity and basicity of a solvent must be considered when extracting anthocyanin compounds.[11] Studies results[12] reported that the solvents in the acidic pH conditions had a significant effect on the levels of anthocyanins, and variations of pH 1.5, pH 2, pH 2.5, and pH 3 resulted in a total anthocyanin content of 1368 mg/100 g of *Ribes nigrum* extract at pH 1.5. Based on the literature, the hepatoprotective activity of ethanol extract of purple sweet potato tubers at a dose of 400 mg/kg bw in *vivo* shows that it significantly reduces *aspartate aminotransferase* (AST) *and alanine aminotransferase* (ALT) levels and also can repair antioxidant substances that help normalise defense systems such as superoxide dismutase, and glutathione peroxidase in CCl_4-damaged livers.[13] Hepatoprotective activity using natural ingredients that were previously unknown based on scientific research is now being developed, validated, and scientifically evaluated by further studies.[14,15,16] Based on this background, the current study aimed to evaluate the hepatoprotective activity of purple sweet potato tubers (extracted using 96% ethanol with variations in the pH of the solvent) on *Rattus novergicus.*

Materials and Methods

Materials

Purple sweet potato, 96% ethanol (Smart Lab), aquadest, NaCl 0.9% (Kimia Farma), hydrochloric acid (Merck), chloroform (Smart Lab), formalin (Merck), hematoxylin, eosin, reagent kit (ALT, AST, Albumin, Total Protein) (Glory®), CMC (Merck), paracetamol 500mg (Nova), Silymarin (Now), chemical analyser photometer (Mindray BA-88A).

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Experimental animals
The study used male Wistar rats (*Rattus norvegicus*) weighing 150-250 grams, sourced and kept at the Animal House Facility, Department of Pharmacology, Perjuangan University. Rats were acclimatised before the experiment. The rats were kept in polypropylene cages under controlled conditions. The BTH University ethics committee granted ethical approval (approval No. 029/E.02/KEPK-BTH/IV/2024), with all protocols adhering to the committee's guidelines.

Collection of plant material
The fresh mature purple sweet potato (*Ipomoea batatas* L.) was collected in March 2022 from Padakembang district, Tasikmalaya, West Java Province, Indonesia. The plant specimens were authenticated at the School of Life Sciences and Technology, Bandung Institute of Technology (Institut Teknologi Bandung, ITB), Indonesia. The voucher specimen was recorded under the number 113/U/FIK-UP/03/2024.

Extraction of purple sweet potato
Three macerators were prepared, each filled with 300 g of Simplisia powder, and 96% ethanol was added and repeated 3L x 24 hours with stirring every 6 hours, and the solvent changed every 24 hours. After that, the pH was adjusted by adding hydrochloric acid; variations were made to pH 1.5 and 2.5 without adding acid for comparison purposes. The macerate and filtrate were separated on the third day, and the amount of macerate obtained was then recorded and evaporated using a rotary evaporator at a temperature of 50°C until it became viscous.

Grouping of Animals
This study used 24 healthy male Wistar rats weighing 150-250 grams, 8-12 weeks old. The animals were divided into six groups (n=4). Group 1 (standard) and Group 2 (negative) administered CMC (*carboxy methyl cellulose*) 1% /kg body weight, Group 3 (positive control group) with 100 mg/kg body weight of Silymarin (SMR), Group 4 with 400 mg/kg body weight of Purple Sweet Potato Extract/PSPE without acidification, Group 5 with 400 mg/kg bw of PSPE at pH 2.5 and Group 6 with 400 mg/kg body weight of PSPE at pH 1.5.

Hepatoprotective Activity Studies
The animals in each group were acclimated for 7 days. They were fed, watered, and weighed. After acclimation, animals were treated daily for 7 days according to the dose given for each group. Oral administration is consistently effective between 9.30 am and 10.30 am. After 7 days of oral treatment, toxicants were administered on the seventh day (except for the standard group) with 3000 mg/kg body weight paracetamol (PCM) one hour after the last oral treatment. A blood sample was taken 24 hours after the previous dose of the PCM was administered. The blood sample was taken through the heart and then placed in a tube for biochemical analysis, as much as 4-5 ml.

Liver Function Assay
After collecting the blood samples, the serum was extracted using a hematocrit centrifuge Mini Centrifuge Joanlab China at 3000 rpm for 10 minutes and stored at -20°C. Liver function was assessed in serum using biochemical parameters such as ALT, AST, albumin, and total protein using a photometric chemical analyser, Mindray BA 88A China.

Determination of Liver Index
Determination of liver organ index was performed after euthanasia of all rats on the eighth day by chloroform anaesthesia to facilitate the cervical dislocation. The animals were dissected, and the liver organs were harvested and weighed using Equation 1:

$$Liver\ index = \frac{\text{Weight of liver (g)}}{\text{Final body weight (g)}} \times 100\% \quad \ldots\ldots (1)$$

Histological Analysis of the Liver
The first step is the fixation of the liver tissue biopsy in 10% formalin solution for 24 hours at 4°C to ensure the stability of the specimen during its storage. Samples were then prepared to a thickness of 3 to 5 mm. They were hydrated with ethanol, cleaned with xylene, and embedded in paraffin wax. The sample was then placed on a glass slide and stained with hematoxylin and eosin for analysis under a microscope (Boeco BM-180 Germany).

Statistical analysis
Descriptive statistics with one-way analysis of variance (ANOVA), with a significance level set at $p<0.05$, followed by post hoc analysis. The data were analysed to determine significant differences in each group using SPSS version 24 for Windows.

Results and Discussion

The yield of the extract obtained (Table 1) was higher when compared to that reported by Hasanah,[17] which produced an extract yield of only 4.49% (without acidification).[16] The pH variation in the purple sweet potato tubers extraction is directly proportional to the yield, where the lower or more acidic the pH level, the higher the yield. A research study states that solvents at acidic pH will affect yield and anthocyanin content.[18] This may be due to the electronic rearrangement of the molecular structure caused by the protons surrounding it.[19]

Table 1: Liver Function Results

Group	AST (U/L)	ALT (U/L)	TP (g/L)	ALB (g/L)
Normal	65.67 ± 5.03	65.67 ± 9.60	73.467 ± 2.37	56.267 ± 10.59
CMC 1% + PCM	125.67 ± 6.50*	78.33 ± 1.52	65.833 ± 6.48	33.100 ± 6.76
SMR100 + PCM	79.67 ± 11.84**	45.00 ± 9.64**	72.900 ± 4.12	40.300 ± 9.50
PSPE pH 2.5 + PCM	82.67 ± 11.84**	41.33 ± 7.23**	67.533 ± 6.46	34.067 ± 9.34
PSPE pH 2 + PCM	77.67 ± 21.07**	36.33 ± 10.11**	71.400 ± 4.03	45.533 ± 9.51
PSPE pH 1.5 + PCM	67.00 ± 9.84**	29.67 ± 8.08**	72.657 ± 2.97	49.500 ± 8.80

*Significant compared to group 1 ($p<0.05$), **Significant compared to group 2 ($p<0.05$)

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This makes the anthocyanins more stable at low pH, allowing for the formation of flavylium cations more soluble in polar solvents.[20] Under normal conditions, serum AST and ALT are found in the cytosol of hepatocytes, with a small amount synthesised. However, if the liver is damaged, these enzymes will increase, allowing them to enter the circulatory system because they are lysing hepatic cells.[21,22] The ability of PSPE to reduce AST and ALT levels (Table 2) significantly (p<0.05) indicates the role of PSPE as a hepatoprotector with the best dose in group 6 (pH 1.5). Protein and albumin are produced in the liver.

Table 2: Liver Index Determination Result

Group	Body Weight (g)	Liver Weight (g)	Liver Index (g/g,%)
Normal	261.23 ± 12.19	7.00 ± 0.86	2.67 ± 0.11**
CMC 1% + PCM	162.16 ± 27.89	8.57 ± 2.13	5.49 ± 0.92*
SMR100 + PCM	156.19 ± 1.73	4.38 ± 0.33**	2.79 ± 0.16**
PSPE pH 2.5 + PCM	182.81 ± 1.86	5.32 ± 0.12**	4.43 ± 0.14*
PSPE pH 2 + PCM	172.86 ± 39.01	5.86 ± 0.85**	3.44 ± 0.40**
PSPE pH 1.5 + PCM	205.67 ± 15.53	5.88 ± 0.87**	2.90 ± 0.77**

*Significant compared to group 1 (p<0.05), **Significant compared to group 2 (p<0.05)

The liver's ability to synthesise protein and albumin can be assessed by checking total protein and albumin levels.[15] The administration of PSPE to rats affects the synthesis of protein and albumin, although it is insignificant. However, the increase in total protein and albumin levels indicates the ability to slowly restore synthesis function in the rat liver. The liver index aims to determine the health status of the internal organs using the increase in the liver index[13] and to observe the effectiveness of PSPE treatment following liver damage in experimental rats.[7] The toxicity of PCM is characterised by a drastic loss of body weight in rats until death.[23,24] In the literature, there is a correlation between the increase in liver haemoglobin due to blood flow impairment and changes in liver weight index, which shows a significant increase in the weight of the liver 1.5 hours after the administration of a toxic dose of PCM to rats which gradually doubles at 6 hours and then decreases at 24 hours, accompanied by an increase in AST and ALT enzyme levels.[25,26] The administration of PSPE shows a mechanism for preventing the rise in rat liver index (Table 3) induced by PCM, with the best results in group 6 (PSPE at pH 2.5 treated) 400 mg/kg body weight has a liver index of 2.90 ± 0.77** which is comparable to group 1 (Normal) (2.67 ± 0.11**). Hepatotoxicity of PCM may occur due to reduced mitochondrial glutathione levels caused by free radicals and oxidative stress, which can damage cell membranes and induce apoptosis. This leads to cell death and acute inflammation.[25] Damage in the form of necrosis (N) of hepatocyte cells, apoptosis (AP), and inflammation (IF) was observed in the rats administered PCM-only in group 2 (Figure 1b). While the administration of PSPE to rats in groups 4, 5, and 6 showed improvement or renewal of damaged hepatocyte cells and prevented inflammation, and in group 6 (Figure 1f), the histological structure was comparable to group 1 (standard) (Figure 1a).

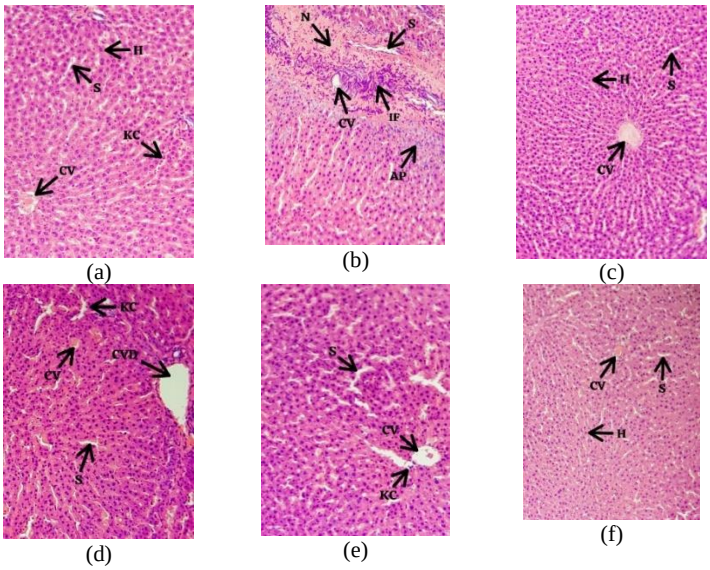

Figure 1: Histology of the liver (a) Group 1 (Normal) (b) Group 2 (Negative) (c) Group 3 (Positive) (d) Group 4 (PSPE non-acidified treated) (e) Group 5 (PSPE at pH 2.5 treated) (f) Group 6 (PSPE at pH 1.5 treated). Description: CV (Central Vein), H (Hepatocyte), KC (Kupffer Cell), S (Sinusoid), N (Necrosis), IF (Inflammation), AP (Apoptosis). Magnification 100×.

Conclusion

Purple Sweet Potato Extract (PSPE) has shown potential as a hepatoprotective agent in biochemical tests and can reduce the levels of AST and ALT and increase the levels of total protein and albumin and hepatoprotective ameliorative effects on the liver index and liver histology in PCM-induced liver damage. Varying the pH of the solvent in the extraction of purple sweet potato tuber affects the hepatoprotective activity in group 4 (without acidification), group 5 (pH 2.5), and group 6 (pH 1.5), where the lower pH of the solvent during extraction increased the hepatoprotective activity of PSPE.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors affirm that this article is original and accepts full accountability for any associated claims.

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