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Original Article

Shallot-Peel Extract Supplementation Increases Glutathione Levels in Gastritis Rat Model

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Abstract

Background: Mefenamic acid can cause gastritis. Glutathione is one of the oxidative stress defense mechanisms and nutrient supplementation containing high antioxidants. Peel of shallot (*Allium cepa var. Ascalonicum*) contains higher antioxidant compounds and may increase glutathione levels. This study aimed to analyze the effect of shallot peel extract supplementation on gastric glutathione (GSH) levels in gastritis rats model.

Methods: This was an experimental study using 24 male Wistar rats which were divided into 4 groups, namely group K0 (given 2 ml of aquadest for 14 days); group K1 (given mefenamic acid 23.25 mg/day for the first 7 days, then Na-CMC 2 ml/day for the next 7 days), group P1 and P2 (given mefenamic acid 23.25 mg/day for the first 7 days, then given shallot peel extract for the next 7 days at dose of 600 mg/kgBW/day or 1200 mg/kgBW/day). Gastric GSH levels were determined by the Ellman method. The data was analyzed by ANOVA and post hoc test.

Results: The shallot peel extract supplementation at a dose of 600 mg/kgBW and 1,200 mg/kgBW significantly increased gastric GSH levels in gastritis rats ($p<0.05$), however, GSH levels did not reach normal conditions. Linear regression analysis showed an R coefficient of 0.751.

Conclusion: Shallot peel extract supplementation increases gastric GSH levels in gastritis rats model in a dose-dependent manner, suggesting an effective dose of shallot peel extract. Further study to develop shallot peel extract into phytopharmaca is imperative.

Keywords: Gastric glutathione (GSH), mefenamic acid, stress oxidative

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Introduction

Gastritis is an inflammation of the gastric mucosa with a wide spectrum of etiologies.[1] Based on epidemiological studies, the incidence of gastritis is widespread throughout the world. In Indonesia, gastritis is one of the 10 most common diseases faced by public health services in various regions.[2–4] One of the common etiologies gastritis is the consumption of non-steroidal anti-inflammatory drugs (NSAIDs).[5] One type of NSAIDs that is commonly used is mefenamic acid which is used for self-medication as a pain reliever because it can be purchased without a doctor's prescription.[6]

However, mefenamic acid has side effects because it can induce gastric mucosal damage by inhibitting prostaglandin biosynthesis unselectively.[7] Previous studies also revealed that mefenamic acid has a local irritative effect on the gastric mucose by provoking the ion-trapping mechanism.[8] Another study showed a new mechanism of mefenamic acid-induced gastritis, which is by promoting oxidative stress.[9] Regarding this report, previous studies have shown that antioxidant compounds such as flavonoids can alleviate gaster-histopathological features in gastritis rats.[10–12] However, only a few studies have

been conducted to explain how antioxidants improve gastric histopathological features in gastritis rats.

Shallots or red onion (*Allium cepa var. Ascalonicum*) are herbaceous plants with numerous benefits. However, the utilization of this herb is limited to the tuber only. Meanwhile, previous research has shown that shallot peel contains 3–5 times more phenolic and quercetin compounds than the tubers.[13] These antioxidant compounds are known to increase glutathione as the master antioxidant.[14] Many beneficial activities of shallot peel extract have been studied.[13,15] However, there has been no study on shallot peel as a potential antioxidant for gastritis. By considering the principle of zero-waste and the unknown mechanism of how antioxidants improve gastric histopathological features, this study aimed to observe the effect of shallot peel extract on gastric glutathione (GSH) levels in gastritis rats model.

Methods

This study was an experimental study conducted in April until May 2020 at the Pharmacology and Biochemistry Department of Faculty of Medicine, University of Jember using male Wistar rats and the peel of *Allium Cepa var. Ascalonicum* taken from the waste of a fried shallot company located in Gebang Village, Jember Regency, East Java (Figure 1). The washed shallot peels were dried using an oven at a temperature of 40–45^{0}C.[16] The dried onion peel was then crushed using a blender.

The solvent used for extraction was 70% ethanol. The reagents to measure the gastric glutathione (GSH) levels were Phosphat Buffer Saline, 0.1 M Phosphate Buffer with a pH of 7.0, trichloroacetic acid (TCA) 10%, 0.1 M Phosphate Buffer with a pH of 8.0, and Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB. The TCA reagent was purchased from Sigma-Aldrich, Saint Louis, USA and the Ellman's reagent was purchased from Solarbio Life Sciences, China. Other materials were obtained from the laboratory.

The ethanol extract was prepared by soaking 500 grams of simplicia in 2.5 liters of 70% ethanol for 24 hours and stirring occasionally. The extraction process was repeated three times with a new solvent. The resulting extract was filtered using Whatman No.2 filter paper.[17] The filtrate obtained was then evaporated using a water bath at a temperature of 60^{0}C to obtain a thick extract.

Twenty-four males Wistar strain Rattus

Figure 1 Peel of Shallots (*Allium Cepa var. Ascalonicum*)

norvegicus were divided into four groups namely K0, K1, P1, and P2. The K0 group received aquadest 2 mL/day for 14 days. The K1 group received mefenamic acid 23.25 mg/day from the 1st day to 7th day then received Na-CMC 2 mL/day from the 8th day to 14th day. The P1 group received mefenamic acid 23.25 mg/day from the 1st day to the 7th day, then shallot peel ethanol extract 600 mg/kgBW/day from the 8th day to 14th day. The P2 group received mefenamic acid 23.25 mg/day, from the 1st day to the 7th day then shallot peel ethanol extract 1200 mg/kgBW/day from the 8th day to the 14th day. All administrations were carried out by gavage. The capacity of the rat stomach was approximately 3.5 mL.[18] On the 14th day, Wistar rats were euthanized by cervical dislocation after being anesthetized using diethyl ether. After that, the rats were fixed with pins on

Figure 2 Surgical Procedure for Removing the Wistar Rat Gaster

a fixation board and dissected using curved scissors starting from the abdomen towards the thorax (Figure 2). The surgical procedure was conducted to take the stomach out for glutathione determination. This research was approved by the Research Ethics Committee, Medical Faculty, University of Jember with reference number 1.411/H25.1.11/KE/2020.

The glutathione (GSH) determination in the gastric tissue was measured using the Ellman's method with some modifications.[19] The gastric tissue was washed with phosphate buffer saline solution and then 200 mg of tissue was taken as a sample. The sample was homogenized in 2 mL of 0.1 M phosphate buffer with pH 7.0 and 500 L of 10% TCA. The homogenate was then centrifuged at 10,000 rpm for 10 minutes at 4^0C. The supernatant from each homogenate was taken as much as 100 L and added 1,775 L 0.1 M Phosphate buffer with pH 8.0 then vortexed. Then after being homogeneous, add 25 L of dTNB solution and incubate at room temperature for one hour (protected from light). The absorbance was measured using a spectrophotometer with a wavelength of 412 nm.[20]

The data was analyzed using SPSS 23.0 for Windows. The ANOVA test was used to compare groups, then post hoc analysis was used to determine differences between groups with a significance level described as $p<0.05$. A linear regression test was obtained to analyze the dose-response relationship related to the dose of shallot peel extract supplementation.

Results

The mean of gastric glutathione (GSH) levels in each group can be seen in Figure 3. The normal gastric GSH level was shown by the K0 group which was 3.714±0.424 mM/g. This group only received aquadest during treatment. This GSH level was also becomes the highest among other groups. This study also indicated that mefenamic acid supplementation significantly decreased gastric glutathione level as seen in the K1 group (Figure 3).

Meanwhile, this study showed that the shallot peel extract supplementation at a dose of 600 mg/kgBw (P1 group) and 1,200 mg/kgBw (P2 group) could significantly increase gastric GSH level compared to the gastritis group (K1) but could not restore GSH levels to normal conditions (K0 group).

Linear regression analysis showed an R coefficient of 0.751, meaning that shallot peel extract supplementation had a relatively strong and positive correlation with gastric

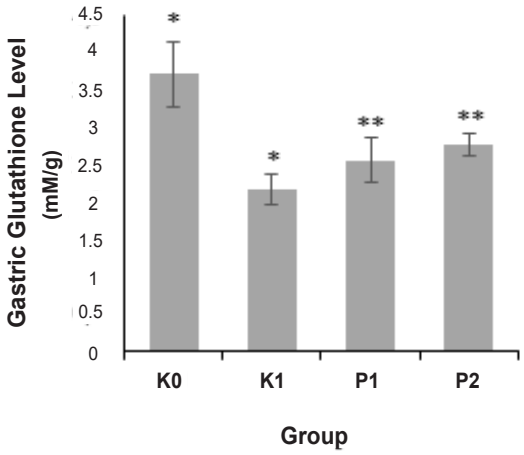

Figure 3 Mean of Gastric Glutathione (GSH) Levels (mM/g)

Note: K0= received aquadest 2 ml/day for 14 days, K1= received mefenamic acid 23.25 mg/day for the first seven days, then Na-CMC for the remaining days, P1= received mefenamic acid 23.25 mg/day for the first seven days, then shallot peel ethanol extract 600 mg/kgBW/day for the remaining days, P2= received mefenamic acid 23.25 mg/day for the first seven days then shallot peel ethanol extract 1200 mg/kgBW/day for the remaining days, *p<0,05 compared to other groups, **p<0,05 compared to K0 and K1

GSH levels. The higher the dose of shallot peel extract, the higher the gastric GSH level. This indicated a dose-response relationship. On the other hand, the R-squared coefficient of 0.565, implied that the effect of shallot peel extract supplementation on the gastric GSH level was 56.5%. The regression equation obtained was $y= 2.208 + 0.000x$ (y: glutathione level; x: the dose of shallot peel extract).

Discussion

This study shows that gastric glutathione levels decreased due to administration of mefenamic acid. This is in accordance with the previous research regarding the administration of mefenamic acid at a dose of 23.25 mg/day for 7 days which resulted in gastric histopathologic changes.[12] Mefenamic acid is a weak organic acid that is easily ionized and hydrophilic at neutral pH; however, when it interacts with gastric acid, mefenamic acid becomes lipophilic, allowing it to be easily trapped and accumulated in gastric-mucosa cells, particularly mitochondrial organelles, causing cell injury.[21] This will result in mitochondrial uncoupling process and failure of electron transport in the inner

mitochondrials membrane. Mefenamic acid forms a leakage gap in the inner mitochondrial membrane, allowing H+ ions to enter the matrix without passing through the protein complex V. This leakage prevents the ATP formation, resulting in cell apoptosis and also changes the mitochondrial transmembrane potential (MTP), causing electrons to return to the matrix and form free radicals.[8] Excessive levels of free radicals can induce oxidative stress.[22]

Oxidative stress is an imbalance between free radical formation and antioxidant defence mechanisms.[22] In this study, oxidative stress due to mefenamic acid exposure was noticeable in decreased gastric glutathione level in the K1 group. Glutathione is a tripeptide (cysteine, glycine, and glutamic acid) endogenous antioxidant found in surprisingly high concentrations in most cells (5 millimolar).[23] Glutathione is exclusively produced in the cytosol and actively pumped into mitochondria. GSH is available in cells in three ways, the first is de novo synthesis is accomplished through a two-step process catalyzed by the enzymes glutamate cysteine ligase (GCL) and glutathione synthetase (GS) (requires ATP). The second, glutathione reductase converts oxidized GSSG to reduced GSH (requires NADPH). and the third, Cysteine recycling from conjugated glutathione via GGTP (requires NADPH). Glutathione depletion is associated with many diseases, including gastritis.[22] Therefore, GSH is a critical target for health enhancement, disease prevention, and therapy.[24] Further research is needed to find nutrients that promote glutathione production.[17]

The peel of shallot (*Allium Cepa var. Ascalonicum*) contains high concentrations of quercetin, terpenoid, and tannins.[17,25,26] Our study showed that the shallot peel extract supplementation at doses of 600 mg/kgBW (group P1) and 1,200 mg/kgBW (group P2) significantly increased gastric GSH level compared to the mefenamic acid group (K1). These results are in accordance with a previous study, showing that quercetin can stimulate the synthesis of glutathione (GSH), which functions as an antioxidant to fight reactive oxygen species (ROS).[27] Furthermore, shallot peel extract contains terpenoids, which can increase GSH synthesis and inhibit free radicals formation, thereby reducing cell damage.[25] Tannins in shallot peel extract can inhibit the Fenton reaction and thus reduce oxidative stress.[26]

However, the gastric GSH concentration in the P1 and P2 groups was still lower than the normal group (K0). This implies that the shallot peel extract supplementation at a dose of 600mg/kgBW and 1,200 mg/kgBW has not been able to restore GSH levels to normal conditions as in the K0 group. This might be due to the relatively small content of antioxidants active compounds in shallot peel extract so that a higher dose is needed to restore gastric GSH levels to normal concentration. This is consistent with the result of the linear regression analysis of this study which showed a positive dose-response relationship.

The limitation of this study is that the dose range of shallot peel extract used was only three, so the effective dose could not be determined. Besides, it is difficult to determine the safety of the extract using this dose range.

To conclude, administration of shallot peel extract to gastritis rats model increases gastric GSH levels in a dose-dependent manner. Further study is needed to find the effective dose of shallot peel extract.

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