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Antioxidant effects of unpolished Sung-Yod sticky rice on prevention of ethylene glycol-induced renal pathology in rats

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Background : *Kidney stone is a chronic disease that is found worldwide. The high prevalence of the kidney stone in Thailand observed in the northeastern part which was about 16.9%. The pathogenesis of calcium oxalate stone formation originated from crystallization of calcium and oxalate ions in renal tubular fluid, overgrowth, aggregation and adhesion to renal epithelial cells. The adhesion of CaOx crystals to renal tubular cell is a critical step of kidney stone formation. It can cause increased oxidative stress and result in the renal tubular cells damage. Studying in natural products may be an alternative way to prevent the pathogenesis of stone formation.*

Objective : *To study the antioxidant potential of Sung Yod sticky rice extract on the prevention of ethylene glycol-induced renal tissue damage in rats.*

Methods : *Unpolished Sung-Yod sticky rice was extracted. The total anthocyanin and phenolic contents were determined by pH differential method and Follin Ciocalteu reagent assay, respectively. The percentage of antioxidant*

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activity was assessed by DPPH scavenging assay compared with the standard serial concentrations of ascorbic acid. In in vivo experiment, rats were divided into two groups: ethylene glycol control (EGC) group received normal drinking water for two weeks and SY treated group was daily fed SY at the dose of 200 mg/kg by gastric intubation for two weeks. After two weeks, 0.5% ethylene glycol was administered in all groups, and 1% ammonium chloride was fed by gastric intubation for one week. At the end of the experiment, the kidney tissues were collected for histological study. Oxidative stress, 8 hydroxyguanosine (8-OHG), was determined by immunoperoxidase whereas antioxidant biomarkers including superoxide dismutase (SOD) and catalase were observed by Western immunoblotting.

Results : *The mean total anthocyanin and total phenolic compounds were 19.566 μg/g dry weights and 0.295 mg/g dry weight, respectively. The half maximal inhibitory concentration of the Sung-Yod sticky rice extract was significantly increased at the fifth teen folded compared with ascorbic acid ($P < 0.05$). Pathological changes of the kidney including tubular dilation, shrinkage of glomerulus and the flattened renal tubular cells in rat were reduced by treatment with Sung Yod sticky rice extract. The number of 8-OHG -positive cells decreased in the SY treated group. The expression of SOD and catalase significantly increased in the SY treated group, as compared with EGC group.*

Conclusion : *These findings demonstrate that the Sung-Yod sticky rice extract has a protective effect on the ethylene glycol-induced renal pathology. This extract might be beneficial in preventing the cause of kidney stone.*

Keywords : *Sung-Yod sticky rice, antioxidant, ethylene glycol, renal pathology.*

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ทิษฎยา เสมาเงิน, วิทูร ขาวสุข, นัทพล สิมานนท์, ธารารัตน์ สร้อยเพ็ชร, วิรุฬห์วัฒน์ หอมใจ, บัณฑิตา บุญเฉลียว, พรชนก แสงจันทร์, ปริญญาพร หนูอุไร. ฤทธิ์การต้านอนุมูลอิสระของสารสกัดข้าวกล้องข้าวเหนียวสังข์หยดต่อการป้องกันการเกิดพยาธิสภาพในเนื้อไตของหนูที่ถูกเหนี่ยวนำด้วยเอททิลลีน ไกลคอล. จุฬาลงกรณ์เวชสาร 2561 พ.ค. – มิ.ย.; 62(3): 451 – 64

เหตุผลของการทำวิจัย : *โรคนิ่วในไตเป็นโรคเรื้อรังที่พบได้ทั่วโลก ในประเทศไทยส่วนใหญ่พบมากทางภาคตะวันออกเฉียงเหนือ โดยกลไกการเกิดนิ่วประเภทแคลเซียม ออกซาเลต เริ่มจากการตกตะกอนของผลึกแคลเซียมและออกซาเลต ไอออน ในของเหลวภายในท่อของหน่วยไต เกิดการสะสมและการจับกันของผลึกและยึดเกาะที่บริเวณเยื่อบุผิวของท่อหน่วยไต ส่งผลให้เกิดภาวะเครียดภายในเซลล์ และส่งผลให้เซลล์เยื่อบุท่อไตถูกทำลาย การศึกษาการใช้สารจากธรรมชาติอาจเป็นวิธีการหนึ่งในการป้องกันเกี่ยวกับกลไกการเกิดโรคนิ่วในผู้ป่วย*

วัตถุประสงค์ : *เพื่อศึกษาฤทธิ์การต้านอนุมูลอิสระของสารสกัดข้าวกล้องข้าวเหนียวสังข์หยดต่อการป้องกันการเกิดพยาธิสภาพในเนื้อไตของหนูที่ถูกเหนี่ยวนำด้วยเอททิลลีนไกลคอล*

วิธีการทำวิจัย : *นำข้าวกล้องข้าวเหนียวสังข์หยดมาสกัดเพื่อหาปริมาณแอนโทไซยานินและสารประกอบฟีนอลรวมด้วยวิธี pH differential และ Follin Ciocalteu reagent assay ตามลำดับ จากนั้นนำมาทดสอบความสามารถในการกำจัดอนุมูลอิสระโดยเปรียบเทียบกับค่าความเข้มข้นมาตรฐานของ ascorbic acid ด้วยวิธี DPPH scavenging assay การศึกษาในสัตว์ทดลองใช้หนูขาวเพศผู้โดยแบ่งการทดลองเป็น 2 กลุ่ม คือกลุ่มที่ไม่ได้ให้สารสกัด และกลุ่มที่ให้สารสกัดข้าวกล้องข้าวเหนียวสังข์หยดปริมาณ 200 มิลลิกรัมต่อกิโลกรัม เป็นเวลา 2 สัปดาห์ จากนั้นหนูแต่ละกลุ่มถูกเหนี่ยวนำให้เกิดพยาธิสภาพในเนื้อเยื่อไตด้วย 0.5% ethylene glycol และ 1% ammonium chloride เป็นเวลา 1 สัปดาห์ เมื่อสิ้นสุดการทดลอง ทำการเก็บเนื้อเยื่อไตมาศึกษาลักษณะโครงสร้างทางเนื้อเยื่อวิทยา การแสดงออกของสารอนุมูลอิสระชนิด 8-OHG ด้วยวิธี immunoperoxidase และศึกษาการแสดงออกของsuperoxide dismutase (SOD) และ catalase ด้วยวิธี Western immunoblotting.*

ผลการศึกษา : *การทดลองพบว่าสารสกัดจากข้าวกล้องเหนียวสังข์หยดมีปริมาณแอนโทไซยานินและสารประกอบฟีนอลรวมเฉลี่ย19.566 ไมโครกรัม และ 0.295 มิลิกรัมต่อน้ำหนักแห้งของผงข้าวเหนียวสังข์หยด 1 กรัม และมีความสามารถในการกำจัดอนุมูลอิสระมากกว่าสารละลายมาตรฐาน ascorbic acid ประมาณ 15 เท่า (P < 0.05) เมื่อศึกษาลักษณะทางพยาธิวิทยาของเนื้อเยื่อไตหนูขาวในกลุ่มที่ไม่ได้ให้สารสกัด พบพยาธิสภาพในเนื้อเยื่อไต คือ ท่อไตขยายขนาด เกิดการหดตัวและฝ่อของโกลเมอรูลัส และเซลล์เยื่อบุท่อไตมีขนาดแบนลง ซึ่งการเปลี่ยนแปลงดังกล่าวจะกลับคืนสภาวะใกล้เคียงปกติเมื่อให้สารสกัดข้าวกล้องเหนียวสังข์หยด นอกจากนี้พบจำนวนของเซลล์ ที่มีการแสดงออกของ 8-OHG ลดลง แต่พบการแสดงออกของ SOD และ catalase เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่มที่ให้สารสกัดข้าวกล้องเหนียวสังข์หยด*

สรุป : *สารสกัดข้าวกล้องเหนียวสังข์หยดมีฤทธิ์ป้องกันการเกิดพยาธิสภาพของเนื้อเยื่อไตที่ถูกเหนี่ยวนำด้วยเอทิลีน ไกลคอล ซึ่งสารสกัดดังกล่าวอาจนำไปใช้ป้องกันสาเหตุของการเกิดภาวะนิ่วในไตได้*

คำสำคัญ : *ข้าวกล้องเหนียวสังข์หยด, สารต้านอนุมูลอิสระ, เอทิลีน ไกลคอล, พยาธิสภาพในเนื้อเยื่อไต.*

Kidney stone disease is a common global health problem which has different prevalence in various parts of the world.[1] In Thailand, the prevalence of the kidney stone was about 16.9% which observed primarily in the northeastern region of Thailand.[1,2] The most common type in patients are calcium oxalate (CaOx) stone, which originated from crystallization of calcium and oxalate ions in renal tubular fluid, overgrowth, aggregation and adhesion to renal epithelial cells.[3,4] These adherence crystals are endocytosis and induce oxidative stresses within the renal tubular cells.[5,6] The adhesion of CaOx crystals to renal tubular cell is a critical step of kidney stone formation. It induced oxidative stresses which lead to overproduction of free radicals and reactive oxygen species, *i.e.* superoxide and hydrogen peroxide. These free radicals can damage cell membrane and induce apoptosis. In addition, the free radicals can act as mediator in several pathways and result in alteration of renal cell physiology via the up-regulation of genes and proteins such as transcriptional activators, regulator of the extracellular matrix composition and growth factors, OPN, fibronectin and transforming growth factor beta-1.[6] Interestingly, expression and/or synthesis of several macromolecules are up-regulated, that promotes inflammation and leads to fibrosis.[7] Medical treatment or surgical stone removal is the options to manage stone. However, they are expensive, several side effects and cannot prevent the recurrence of the stone formation.[4,8,9] Therefore, there is an interest towards the use of medicinal plants for prevention kidney stones. [10-13]

Several studies in pigmented rice such as black and red rice have shown that they contain antioxidants which can prevent chronic diseases such as cancer, diabetes, and heart diseases.[14,15] The molecules with antioxidant activities include anthocyanins, proanthocyanidins, phenolic acids, flavonoids, tocopherols, tocotrienols, γ-oryzanol, and phytic acid.[16,17] Some studies in flavonoids from plants have shown that they decrease calcium oxalate crystal deposits of stone forming in rats.[13, 18] Moreover, they found the highly expression of antioxidant enzyme, superoxide dismutase (SOD), which can contributed to kidney stone prevention.[13]

Sung-Yod (SY) sticky rice (*Oryza sativa* L.) is a local red colored-sticky rice found in the South of Thailand, particularly, Songkhla province. However, the role of SY sticky rice in antioxidant correlated with kidney disease remains unclear. We, hereby, investigated the antioxidant potential of SY sticky rice extract on the prevention of ethylene glycol induced renal tissue damage in rats.

Methods

1. Preparation of SY sticky rice extract

Unpolished SY sticky rice was ground into powder of 2 kg. The ground rice samples were extracted with 2.5 L of 75% ethanol at 37°C for 24 hours on rotator. The extracted solutions were collected and passed through a sieve with mesh no.4 and kept in well-closed containers. The extracted samples were evaporated in oven at 60°C for two days to eliminate ethanol. Thereafter, the samples were dried in lyophilizer and kept at -20°C until use. Calculation of the extracted yield of the rice was directly in percentage using formula. Weight of dry extracted powder/Weight of raw material × 100.

2. Determination of total anthocyanin

Total anthocyanin was determined by pH differential method. Briefly, the 10% (w/v) extracted concentrate was prepared with 40% acetone. The appropriate dilution factor was determined by diluting the test portion with 0.025 M KCl pH 1.0 buffer, until absorbance at 510 nm which is within the linear range of the spectrophotometer. Using this dilution factor, two dilutions of the test samples were prepared, one with pH 1.0 buffer and the other with 0.4 M sodium acetate pH 4.5 buffer. Absorbance of test portion was diluted with pH 1.0 and pH 4.5 buffers, at both 510 and 700 nm. The diluted test portions were read *versus* a blank cell filled with 40% acetone. Absorbance was measured within 15 - 50 min of preparation. Anthocyanin pigment concentration was calculated and expressed as cyanidin-3-glucoside equivalents, as follows:

Monomeric anthocyanin (mg/L) = $A \times MW \times DF \times 1000 / \varepsilon \times 1$

Where A = (A510nm – A700nm) pH 1.0 – (A510nm – A700nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; l = path length in cm; ε = 26900 molar extinction coefficient, in $L \times mol^{-1} \times cm^{-1}$, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

3. Determination of total phenolic compound

Total phenolic content was investigated by Follin Ciocalteu reagent assay. An aliquot 50 μl of standard solution of gallic acid 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10 mg/ml and rice extract solution 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/ml were prepared. Each aliquot was added with 100 μl Follin Ciocalteu reagent and shaken. After 5 minutes, 1.5 mL of 5% sodium carbonate solution was added to the mixture and incubated at room temperature in the dark for 60 minutes. The absorbance against the reagent blank (40% acetone) was determined at 760 nm with spectrophotometer. Total phenolic content was expressed as mg gallic acid equivalents (GAE)

4. 1,1-Diphenyl-2-picryl-hydrazyl radical (DPPH) scavenging activity

The percentage of antioxidant activity of crude SY sticky rice extract was assessed by DPPH scavenging assay. The samples were reacted with the stable DPPH radical in 40% acetone solution. An aliquot of standard serial concentrations of ascorbic acid (0.01-0.06 mg/ml) and the various concentrations of SY sticky rice extract (0.1 - 1.0 mg/ml) were analyzed. The reaction mixture consisted of adding 0.1 mL of sample with 1.4 mL of 60 μM DPPH radical solution in 40% acetone. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color were read [Absorbance (Abs)] at 515 nm after 30 min in the dark of reaction using a UV VIS spectrophotometer. The percentage of scavenging activity was determined according to the formula:

$$\%\text{Scavenging activity} = \frac{A_C - A_S}{A_C} \times 100$$

Where Ac = Absorbance of control, As = Absorbance of sample

The results were expressed as percentages of scavenging activity. The half maximal inhibitory concentration (IC_{50}) of both ascorbic acid and SY crude extract were reported.

5. In vivo experimental design

The animal experimental protocol has been approved by the Animal Research Committee of Burapha University. Seven-week-old male Sprague-Dawley rats (*n* = 8), of body weight 180 - 200 g, were acclimated and rested for one week before the start of the experiment. During the study, the animals were fed with standard commercial diet and water *ad libitum*. The animal experimental protocol was performed as described by Akanae *et al* [13] with some modification. Briefly, the animals were randomly divided into two groups of 4. Group I, ethylene glycol control (EGC) group received normal drinking water for two weeks. Group II, SY sticky rice extract group was daily fed SY at the dose of 200 mg/kg by gastric intubation for two weeks. After two weeks, both groups received drinking water containing 0.5% ethylene glycol (EG) and was fed three times (every other day) of 1% ammonium chloride (NH_4Cl) (v/v) by gastric intubation for one week to induce crystal deposition.

At the end of the experiment, the rats of both groups were euthanized with anesthesia, killed and their kidneys were collected. One kidney from each animal was immediately kept at -80°C for further study. The other kidney of each animal was fixed in 4% paraformaldehyde, washed in 0.1 M phosphate buffer saline (PBS) pH 7.4, incubated in 30% sucrose, embedded in tissue tex, and cut into 5-μm sections for histological study.

6. Histopathological study

Five randomly frozen sections from each animal were rehydrated through a graded series of ethanol (100% - 70%) for 5 min each and stained with hematoxylin and eosin solution. The pathological change was observed under light microscope of ten regions thoroughly the cortex and medulla regions of the section.

7. Immunohistological study

Oxidative stress biomarker, 8 Hydroxy-guanosine (8-OHG), was determined by immuno-peroxidase. Five randomly tissue sections from each animal were dried and placed in ice-cold acetone for 10 min. Endogenous peroxidases were quenched by treatment with 0.3% hydrogen peroxide (H_2O_2) in methanol for 30 min. The sections were washed (3×) in 0.1 M PBS, pH 7.4 containing 0.4% Triton X-100 (PBST), and then incubated in 0.1% glycine, 0.1 M PBS for 15 min. Non-specific antibody binding was prevented by incubation in blocking solution (4% bovine serum albumin; BSA, 4% Normal goat serum; NGS and PBST) for 1 h. Sections were incubated in primary antibody (8-oHdG mouse monoclonal antibody) containing diluent solution (2% BSA, 2% NGS and PBST) at the dilution of 1:200 for 12 - 18 h at 4°C. After incubation, sections were washed (3x), and then incubated with HRP-conjugated goat anti-mouse IgG containing PBST solution (1:500) for 100 min. After washing, the sections were further incubated with 3',3'-diaminobenzidine tetrahydrochloride (DAB) substrate. The sections were counterstained with Mayer's hematoxylin in order to visualize the nucleus; and then observed under a Nikon Eclipse E600 microscope fitted with Nikon digital camera DXM1200.

8. Western blot analysis

Evaluation of antioxidant biomarkers, superoxide dismutase (SOD) and catalase was

performed by Western immunoblotting. Kidney samples from each group were homogenized and ice-cold extracted with 10 ml of 25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% EG, 0.5 M EDTA, 1% SDS, 1% PMSF inhibitor, then centrifuged at 10,000×g at 4°C for 10 min. The supernatant was collected and measured for protein concentration by Bradford protein assay. As for immunobloting analysis, 30 μg of protein were separated by 12.5% SDS-PAGE, and then transferred to a nitrocellulose membrane. The membrane was washed with 0.1% Tween 20 in 0.1 M Tris buffer saline (TBS), pH 8.0 and non-specific binding was blocked with 5% skim milk for 1 h at room temperature, with agitation. It was then incubated with primary antibody (i.e., anti-SOD diluted 1:2000, anti-catalase diluted 1:1000) overnight at 4°C, with gentle shaking. After three washes, it was incubated with goat anti-rabbit IgG HRP-conjugated or goat anti-mouse IgG HRP-conjugated (1:5000), for 1 h at room temperature with agitation, and then washed three times. Detection of bound HRP was made with TMB substrate until optimal purple color development. The membranes in all groups were quantitatively analyzed by Image J version 1.49.

9. Statistical analysis

Relevant data are presented as mean and standard deviation ($\overline{X} \pm$ SD.). Statistical significance was evaluated by Student *t*-test. A difference was considered significant if $P < 0.05$.

Results

1. The contents of total anthocyanin and phenolic compounds in SY sticky rice extract

The percentage of SY sticky rice extract yield was 0.589 which based on the weight of dried and ground rice materials. The SY sticky rice extract was further quantified the total anthocyanin by pH differential method. The mean total anthocyanin pigment was 19.566 ± 1.269 μg/g dry weights. The mean total phenolic compound was 0.295 ± 0.040 mg gallic acid/g dry weight.

2. Antioxidant activity of SY sticky rice extract

Antioxidant activity of SY sticky rice extract was determined as the percentage of scavenging activity. The higher concentration of SY sticky rice extract was more effective in scavenging activity (Figure 1). The IC_{50} of the standard ascorbic acid and SY sticky rice extract were 0.023 and 0.35 mg/ml, respectively. Interestingly, the IC_{50} of SY sticky rice extract was significantly increased at the 15 folded compared to ascorbic acid ($P < 0.05$).

3. Histopathological examination

Pathological change of the kidney in all groups is shown in Figure 2. The severe renal damage was observed in the EGC group that contained cystic-liked spaces in the cortex and multiple tubular calculi correlated with tubular dilation. Glomerulus appeared to be a shrinkage created the increase in the distance of Bowman's space. The height of tubular epithelial cell was reduced and become flat. Epithelial cell abrasion can be observed along the dilated renal tubule (Figure 2B). The severity of renal tissue damage in rat was diminished by treatment with SY sticky rice extract as shown in Figure 2C. The glomerular structure appeared to be normal while the slightly swelling in renal epithelial cells are shown in proximal and distal tubules (Figure 2D).

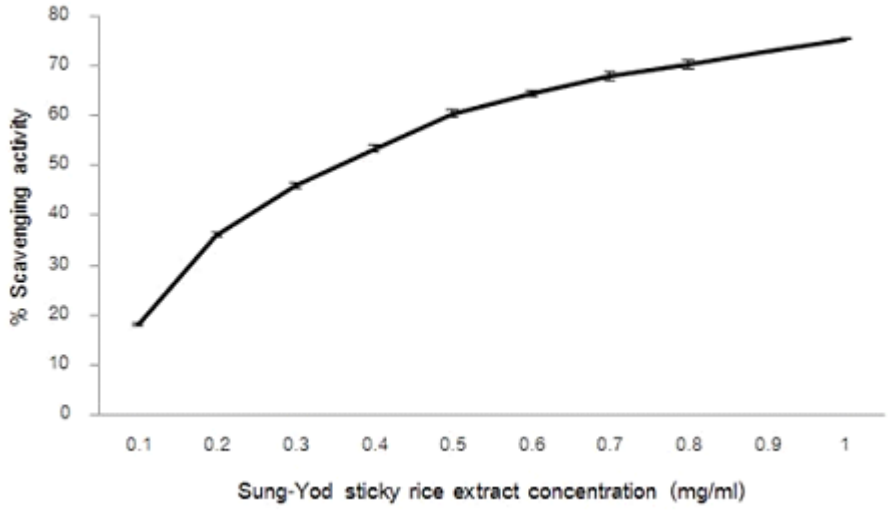

Figure 1. The percentage of scavenging activity of SY sticky rice extract at various concentrations.

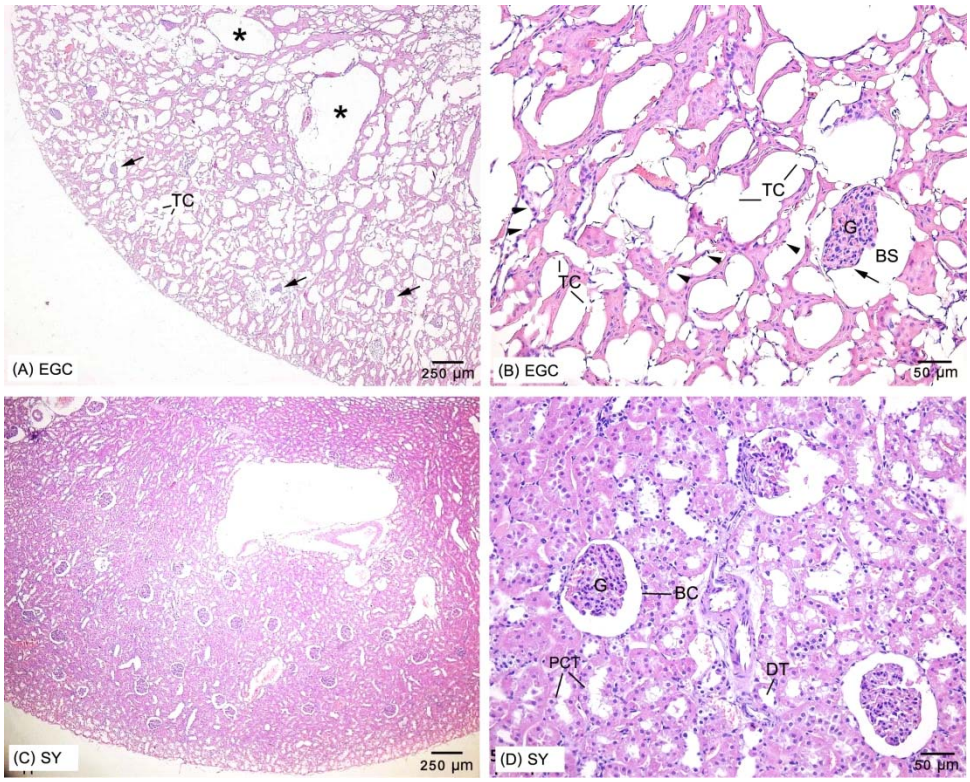

Figure 2. Low (A and C) and medium (B and D) micrographs of the renal histology stained with hematoxylin-eosin. A-B) The EGC groups showing cystic-liked spaces (asterisks) in the cortex, tubular calculi (TC), shrinkage of glomerulus (arrows) and epithelial cell abrasion (arrowheads). C–D) the group treated with SY sticky rice extracted showing slightly pathological change in the renal structure compared with EGC group (G, glomerulus; BS, Bowman's space; BC, Bowman's capsule; PCT, proximal tubule; DCT, distal tubule).

4. Immunohistological study

The 8-OHG immunoreactivitiy in the kidney tissues is shown in Figure 3. Numerous 8-OHG -positive cells were observed in the renal tubule of the EGC group whitelist the SY treated group tended to have fewer 8-OHG -positive cells than EGC group.

5. Western blot analysis

The expression of SOD and catalase in rat kidney is demonstrated in Figure 4. The EGC group showed weakly positive bands of SOD and catalase. Simultaneously, the SOD and catalase bands have been distinctly observed in the SY-treated group (Figure 4A). For quantitative analysis, the fold-change of SOD and catalase levels of SY-treated group was significantly increased at the 3.89 and 2.49, respectively compared with EGC group (Figure 4B and C).

Discussion

Many studies have shown that the red rice contains a variety of bioactive molecules, including phenolic compounds, γ-oryzanol, tocopherols, and tocotrienols.[16,17] In this study, the anthocyanin and total phenolic compounds were determined as antioxidant compounds. These antioxidants are organic molecules that protect the cells from damage caused by free radicals and reactive oxygen species. Using pH differential analysis, the SY sticky rice extract contained monomeric anthocyanin pigments with amount of 19.566 ± 1.269 μg/g dry weights. Anthocyanin is a type of flavonoid pigment in plants which exist as O-glycosides (mono, di or tri) and acylglycosides of anthocyanidins in plants. The characters of anthocyanin structure make them highly reactive toward reactive oxygen species.[16] Total phenolic compounds in SY sticky rice extract was 0.295 ± 0.040 mg gallic acid/g dry weight. The phenolic acids are substances containing a phenolic ring and an organic carboxylic acid function. The phenolic ring can stabilize and delocalize unpaired electrons, conferring an antioxidant property to phenolic acids.[16] To measure the antioxidant activity of SY sticky rice extracts, DPPH radical scavenging activity was used. The IC_{50} value of ~0.35 mg/ml in DPHH radical scavenging assay was higher antioxidants than standard ascorbic acid.

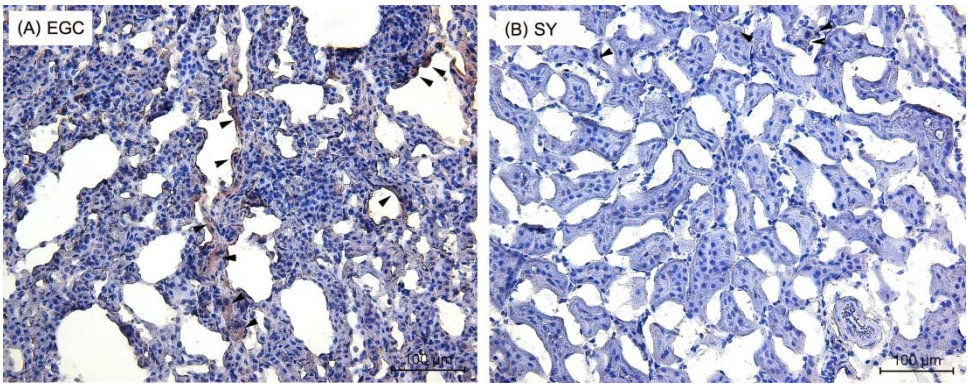

Figure 3. Immunohistochemical localization of a 8-OHG -like structure in the renal tissue of rat. Sections were counterstained with Mayer's hematoxylin. A) The EGC groups showing numerous, few immunoreactive cells (arrowheads) present in the renal tubule. B) The SY–treated group (G, glomerulus; BS, Bowman's space).

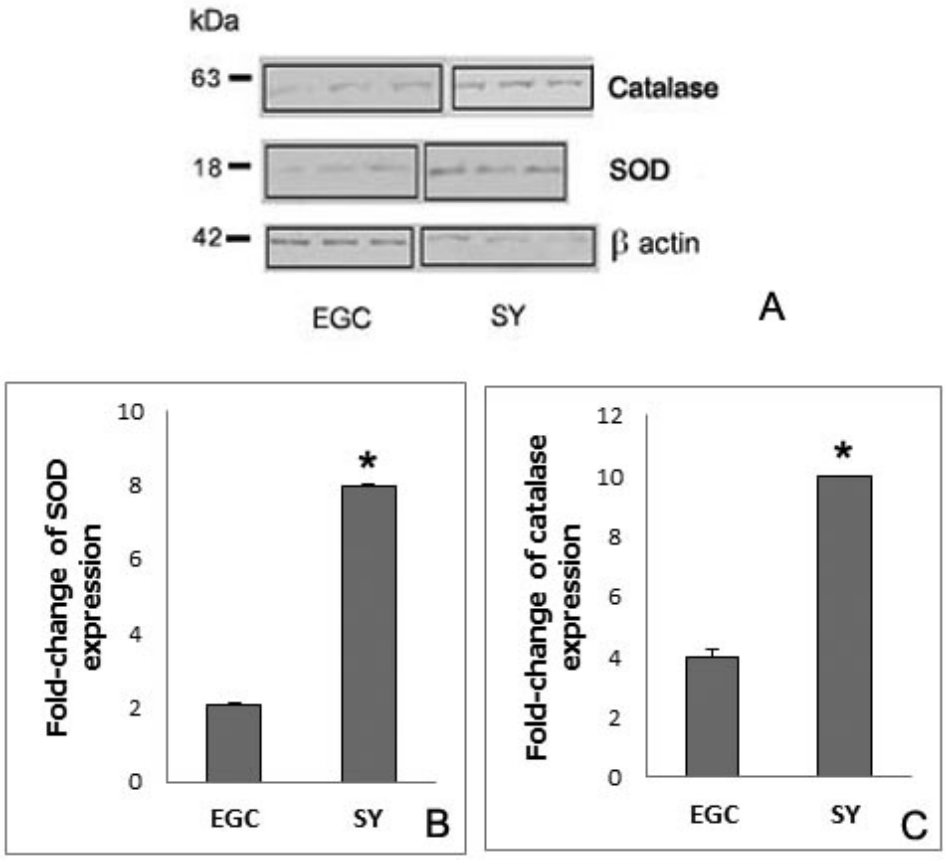

Figure 4. A. Immunoblotting showing the expression of the SOD and catalase in EGC and SY groups. B and C. Quantitative analysis showing the fold-change of SOD and catalase expressions in EGC and SY groups, respectively.

Several studies in red pigmented rice extracts exhibit potent antioxidation, anti-inflammation, anti-cancer cell invasion. [15, 19, 20] In addition, some study demonstrated that antioxidant supplementation decreased ethylene glycol-induced calcium oxalate stone formation.[21] A previous study showed that, ethylene glycol has generally been used in combination with ammonium chloride or vitamin D3 to induce hyperoxaluria and calcium oxalate crystal deposition in the kidney.[13,22] It has been suggested that, ethylene glycol being oxidized into oxalic acid by non-specific dehydrogenase lead to hyperoxaluria.[11] However, no calcium oxalate crystal deposition in the renal tissue observed during this study. It may be a result from the size of the crystals was smaller than that in the renal tubular lumen. In addition, it is necessary to establish an appropriate condition for calcium oxalate crystal formation in the renal tissue. In the present study, we demonstrated the EGC group had high oxidative stress and increased renal tubular and glomerular damages, whilst the structure of renal tubular cells and glomerulus appeared to be normal in the SY sticky rice extract group. One study proposes that oxalate-induced renal epithelial cell injury is mediated by the formation of reactive oxygen species.[23] Oxidative stress may

promote the pathogenesis of kidney stones.[24] In this study, the level of oxidative stress biomarker, 8-OHG, in the EGC group was higher than that in the SY treated group, suggesting that the SY sticky rice extract has ability to decrease renal injury from oxidative stress. In addition, the enhanced activity of antioxidant enzyme SOD and catalase was observed in the SY treated group which can contribute to nephrolithiasis prevention via direct effects on renal epithelial cells. Similar result was demonstrated in the Java tea-treated oxalate-induced stone formation.[13] Several studies reported the antioxidant activity of natural products involved in the pathogenesis of the kidney stone such as the *Herniaria hirsuta* extract can reduce the size of CaOx crystals nucleation and inhibit CaOx crystals aggregation and adhesion.[25, 26] The 1,2,3,4,6-penta-O-galloyl-beta-D-glucose (PGG) from gallnut of *Rhus chinensis* MILL reduced CaOx supersaturation, renal CaOx crystal deposition and oxidative renal cell injury by its antioxidant activity.[27] The polysaccharides from the brown seaweed *Sargassum graminifolium* (Turn.) inhibited CaOx crystal nucleation and aggregation by *in vitro*.[28] Another previous study suggested that antioxidant molecules such as anthocyanin and flavonoids decrease oxidative injury in renal tubular cells and calcium oxalate crystal deposition in the rat kidney.[12, 29, 30] However, the further study is necessary to investigated for the kidney stone formation.

In conclusion, the SY sticky rice extract can prevent the ethylene glycol-induced renal tissue injury. It contains substances that increased SOD and catalase expression which might be beneficial in decrease the cause of kidney stone formation. Our study provides the first evidence to demonstrate the protective effects of SY sticky rice on the ethylene glycol-induced renal pathology in rats. Moreover, these findings may provide the information which leading to the development of new chemoprevention agents for kidney stone.

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