

The Assay of Quercetin Solid Dispersion as a Potential Nephron-protector in Acute Renal Failure Induced Mice

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ABSTRACT

Quercetin has been used with other nutraceutical components to improve renal function. Its potential to be developed as an active pharmaceutical ingredient, however, is limited by poor aqueous solubility and low rate of dissolution leading to low bioavailability in rats (< 17%) and in human (1%). Solid dispersion of quercetin with PVP K30 has increased its solubility 13.24 times and the amount dissolved ($95.12 \pm 1.83\%$) in comparison to pure quercetin. This study aimed to determine the nephron-protection effect of the solid dispersion on Acute Renal Failure (ARF) mice. The animals were divided into 6 groups, normal mice as a negative control group (G1), ARF induced mice as a positive control group (G2), ARF induced mice given pure quercetin 50 mg/kg BW (G3), ARF induced mice given solid dispersion containing 10 mg/kg BW (G4), 5 mg/kg BW (G5) and 2.5 mg/kg BW (G6) quercetin respectively. The ARF was induced by injection of gentamycin sulphate 100 mg/kg BW for 7 days consecutively. Renal function was monitored by measuring the serum creatinine at day 8th. The protection effect was also observed from the histopathology score of the nephrons. Results showed that ARF induction increased serum creatinine above normal. Solid dispersion doses variations significantly influence the serum creatinine ($p < 0.05$). The stage of renal impairment based on histopathology score was significantly influenced by the doses of quercetin in solid dispersion ($p < 0.05$). It was concluded that solid dispersion containing quercetin at doses 2.5 and 5.0 mg/kg BW respectively did not effective as a nephron-protector. The solid dispersion containing quercetin 10.0 mg/kg BW was effective to reduce the serum creatinine and showed a nephron-protection effect on the ARF induced mice.

Key words: Quercetin, Solid dispersion, Nephron-protector, Acute renal failure mice.

INTRODUCTION

Quercetin (3,3',4',5'-7 pentahydroxy flavon) is a naturally occurring flavonoid found in fruits and vegetables which have many beneficial effects to the human body. It showed a wide range of biological and pharmacological activities such as antitumor, antibacterial, antioxidant, anti-inflammation, obtains protection against osteoporosis, pulmonary and cardiovascular diseases.¹⁻⁴ Its potential to be developed as an active pharmaceutical ingredient (API), however, is limited by poor solubility and low rate of dissolution leading to low bioavailability in rats (< 17%) and in human (1%).⁵

Several studies reported quercetin as a promising nephron-protector which protect the kidneys from damages due to age related diseases or chronic use of nephrotoxic drugs. Combination of quercetin (500 mg) with other vitamins such as vitamin C, vitamin B3 and folic acid has been reported to improve the renal function of human subjects.⁶ The coadministration of quercetin (50 mg/kgBW) with cisplatin (4 mg/kgBW) to Mat B-III breast adenocarcinoma rats was able to prevent renal impairment caused by the chemotherapeutic agent.⁷ Another study showed that quercetin at oral dose of 10 mg/kgBW per day for 4 weeks showed a beneficial effect in the prevention and the treatment of renal dysfunction caused by diabetes mellitus.⁸

Currently, quercetin is available in the market at a dose 500 mg as food supplement with a claim as antioxidant. In an attempt to develop quercetin as a potential nephron-protector, we formulated Quercetin Solid Dispersion (QSD) to yield submicron quercetin particles which increased its aqueous solubility as well as the dissolution rate.⁹ An increase in dissolution rate correlates with an increase in the bioavailability and in turn a reduction in quercetin effective dose. The aims of this study was to determine the nephron-protection effect of the solid dispersion on acute renal failure (ARF) induced mice.

MATERIALS AND METHOD

Materials

Quercetin (Sigma, Singapore), QSD with PVP K30 (1:9) obtained from the Laboratory of Physical Pharmacy Andalas University, tween 80 (Bratachem[®], Indonesia), NaCl 0.9%(Otsuka[®], Indonesia), formalin solution 10% (PT. Arjuna Utama Kimia, Indonesia), gentamicin injection (Otsuka[®], Indonesia), hematoxylin-eosin stains (Himedia, Indonesia), Mayer's albumin (PT. Indo Aчитama Chemical Industry, Indonesia), paraffin (Bratachem[®], Indonesia), xylene (Bratachem[®], Indonesia), ethanol pro analysis (Merck[®], Germany) and Dyasis[®] (Dyasis Diagnostic System GmbH, Germany).

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Animals

Male white mice (*Mus musculus* strain DDY) weighing 20–30 g were obtained from the Veterinary Office of Bukittinggi. The animals were kept under observation before the experiment for acclimatization. The chosen animals were housed in plastic good aerated cages at the normal atmospheric temperature ($24 \pm 2^\circ\text{C}$) and normal 12 h light/dark cycle. They were given access to water and standard diet of known composition. All animal procedures were undertaken with the approval of the Animal Ethics Committee of the Faculty of Medicine, Andalas University (document no. 208/KEP/FK/2017 on July 3rd 2017).

Experimental method

After 7 days of acclimatization, the animals (n=42) were randomly distributed into 6 groups. Group 1 (G1) was a negative control group, continued to receive water, the normal diet and vehicle (2% tween 80 solution). G2, a positive control group, was Acute Renal Failed (ARF) mice induced with gentamicin sulphate injection 100 mg/kg BW intraperitoneally for 7 days consecutively.¹⁰ G3, G4, G5 and G6 were ARF mice received pure quercetin or QSD orally from day 1st to 7th as in Table 1.

After the 7 days of treatments, all the mice were sacrificed. The blood was collected from the carotid artery and placed in a tube for 15 min, then centrifuged at 3000 rpm for 20 min, the serum was taken and kept frozen until being analyzed for the creatinine level. The serum creatinine level was determined by using Dyasis[®], the absorbance was measured spectrophotometrically (Shimadzu[®]). The kidneys were isolated and kept for histopathological analysis.

Histopathological analysis

The kidneys were fixed in 10% formalin solution for 24 h. It was dehydrated by passing through a graded series of alcohols (96%, 90%, 80%, 70% and 50%) for an h each time. It was then transferred to an alcohol : xylene (1:1) solution and xylene respectively for an h. The tissue was infiltrated at 58-60°C with xylene : paraffin (1:1) for 1 h; followed with paraffin three times for ± 3 h. It was then embedded in liquid paraffin and left frozen. It was sliced with microtome to obtain a tissue section with the thickness of 5 μm .^{10,11} The tissue sections were deparaffinized with xylene for 30 min to an h and then with alcohol : xylene (1:1) solution for 5 min.

The tissue sections were hydrated with decreasing concentration of alcohol baths (96%, 90%, 80%, 70% and 50%) for 3 min each time. They were stained in Hematoxylin Erlich for 1-5 min and washed with

running water for 5 min or less. This was then followed by rehydration with alcohol 70% and 80% for 3 min each, staining in 1% Eosin-alcohol for an h, dehydration with increasing alcohol concentration (90% and 96%) for 1 and 2 min respectively, with alcohol : xylene (1:1) for 2 min and xylene for 30 min. The tissue sections were then mounted and ready for observation under a microscope (Carl Zeis[®], Germany). Histopathological scores were determined based on changes in the features of kidney section mentioned in Table 2.¹²

Statistical analysis

Data were expressed as (Mean \pm Standard of Deviation) and analyzed by two-way ANOVA ($\alpha = 0.05$) to determine the significant differences between means followed by Duncan Multiple Range Test at 5% significance level.

RESULTS

Figure 1 shows the effect of treatments on serum creatinine level of mice. Induction of acute renal impairment on mice significantly increases the serum creatinine of the ARF mice (G2) ($p < 0.05$) which is not significantly different than G5 and G6 (QSD containing 5 mg/kgBW and 2.5 mg/kgBW quercetin) ($p > 0.05$) as in Table 3. There is no significant difference in serum creatinine levels of G3 (pure quercetin 50 mg/kgBW) and G4 (QSD containing 10 mg/kgBW quercetin) ($p > 0.05$). Administration of pure quercetin or QSD significantly increased the serum creatinine level of mice ($p < 0.05$). These results were in accordance with histopathological scores of the renal section presented in Figure 2 and Table 3.

Histopathological examination of the renal section of normal mice (Figure 3, G1) reveals normal features such as a clear nuclei of nephron, normal appearance of glomeruli and bowman capsule; the proximal and distal tubules in good condition with clear cell nuclei. However, there is tubular hypertrophy characterized by narrowing of the lumen, cell infiltration, inflammation of the tubules and glomeruli, tubular and glomeruli necrosis; and apoptosis in the section of ARF mice induced by gentamicin (G2). There is also lysis on the bowman capsule membrane and the bowman space.

Renal histopathology of groups given 50 mg/kgBW of pure quercetin (Figure 3, G3) and QSD equivalent to quercetin 10 mg/kgBW (G4) showed improvement in kidney damage caused by gentamicin. Glomeruli looks normal and tubular epithelial cells are still intact, the bowman capsule looks good. These features support the results in Table 3 that there were no significant differences in serum creatinine

Table 1: Treatments given to all groups.

| Group | Treatments | QSD doses (mg/kg BW) | Quercetin content (mg/kg BW) |
|-------|--|----------------------|------------------------------|
| G1 | Normal food, water and vehicle (normal mice) | - | - |
| G2 | Normal food and water + Gentamycin sulphate injection (ARF mice) | - | - |
| G3 | Normal food and water + Gentamycin sulphate injection + pure quercetin | - | 50 |
| G4 | Normal food and water + Gentamycin sulphate injection + QSD | 100 | 10 |
| G5 | Normal food and water + Gentamycin sulphate injection + QSD | 50 | 5 |
| G6 | Normal food and water + Gentamycin sulphate injection + QSD | 25 | 2.5 |

Table 2: Histopathology score of renal impairment.

| Score | Impairment of renal histology | | | |
|-------|-------------------------------|---------------------|--|--|
| | Tubular atrophy | Tubular hypertrophy | Lysis of Bowman capsule and tubular necrosis | Lysis and necrosis of glomeruli and tubular necrosis |
| 1 | + | - | - | - |
| 2 | + | + | - | - |
| 3 | + | + | + | - |
| 4 | + | + | + | + |

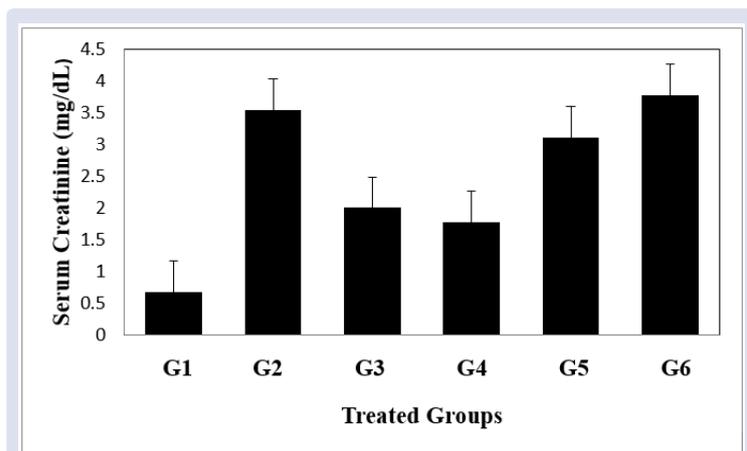


Figure 1: The serum creatinine level of normal mice (G1), ARF mice (G2) and groups treated with pure quercetin 50 mg/kg BW (G3), QSD equivalent to 10 mg/kgBW (G4); 5 mg/kgBW (G5) and 2.5 mg/kg BW quercetin (G6).

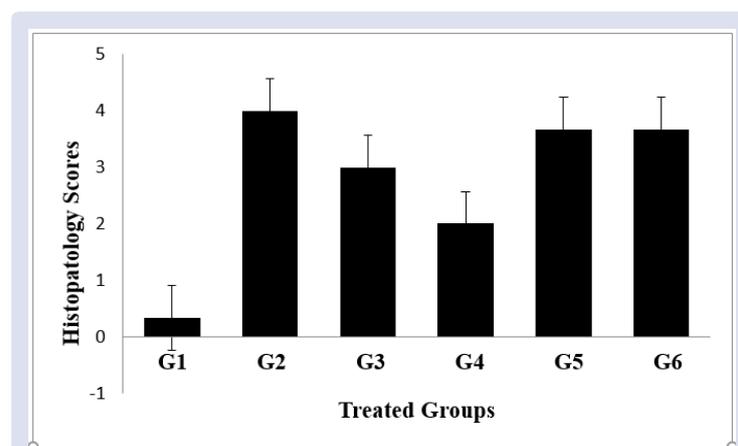


Figure 2: The renal histopathology score of normal mice (G1), ARF mice (G2) and groups treated with pure quercetin 50 mg/kg BW (G3), QSD equivalent to 10 mg/kg BW (G4); 5 mg/kg BW (G5) and 2.5 mg/kg BW quercetin (G6).

Table 3: Effects of treatments on serum creatinine level and renal histopathology score.

| Groups | Treatments | n | Serum Creatinine (mg/dL) | Histopathology score |
|--------|---|---|--------------------------|--------------------------|
| G1 | Normal mice (control negative) | 3 | 0.67 ± 0.00 ^a | 0.33 ± 0.58 ^a |
| G2 | ARF mice (control positive) | 3 | 3.55 ± 0.39 ^b | 4.00 ± 0.00 ^b |
| G3 | Pure quercetin 50 mg/kgBW | 3 | 2.00 ± 0.67 ^c | 3.00 ± 1.00 ^c |
| G4 | QSD equivalent to quercetin 10 mg/kgBW | 3 | 1.78 ± 0.39 ^c | 2.00 ± 1.00 ^c |
| G5 | QSD equivalent to quercetin 5 mg/kgBW | 3 | 3.11 ± 0.38 ^b | 3.67 ± 0.58 ^b |
| G6 | QSD equivalent to quercetin 2.5 mg/kgBW | 3 | 3.78 ± 0.39 ^b | 3.67 ± 0.58 ^b |

QSD: Quercetin solid dispersion; ^{a,b,c}: Data with different superscripts showed significant differences (p < 0.05).

levels of the two groups. It can be concluded that this dose is effective in improving kidney function in ARF induced mice.

Histopathology of groups treated with QSD 5 mg/kgBW (Figure 3, G5) and 2.5 mg/kgBW (G6), shows narrowing of Bowman space, tubular hypertrophy, cells lysis and apoptosis. These features are similar if compared to G2 which can be said that there has not seen an improvement in renal impairment which may be due to ineffective doses.

DISCUSSION

The kidneys and liver are vital organs that are at high risk of damage under exposure to drugs, other chemicals and comorbidities.

Hepatoprotective compounds are familiar to people, such as curcumin or curcuma rhizome extract which is prescribed with anti-tuberculosis drugs to prevent liver damage caused by the hepatotoxic drugs. Yet there are no compound called nephron-protector that protect or prevent kidneys from damage due to adverse effects of nephrotoxic drugs or from nephropathy caused by diabetes mellitus. In an effort to develop quercetin as a potential nephron-protector, we formulated QSD which increased its aqueous solubility as well as the dissolution rate. Furthermore, studies of the acute toxicity and the effects of QSD on renal protection are needed to ensure the safety and effectiveness of the formulation. Our previous study on acute toxicity of QSD showed that it was practically non-toxic with the LD₅₀ > 16 g/kg BW.¹³

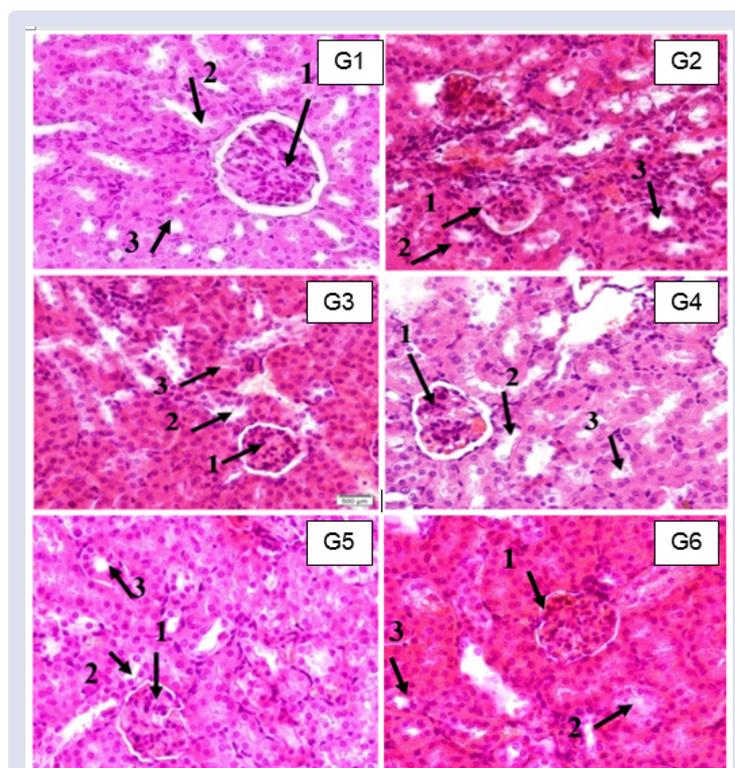


Figure 3: Hematoxylin and eosin staining of the kidney sections of control negative (G1), control positive (G2) and treated mice with pure quercetin 50 mg/kgBW (G3), quercetin solid dispersion equivalent to quercetin 10 mg/gkgBW (G4), 5 mg/kgBW (G5) and 2.5 mg/kgBW (G6). (1 = glomerulus; 2 = Tubulus proximal and 3 = Tubulus distal; 400x).

Gentamicin and other aminoglycoside antibiotics are known as nephrotoxics. They induced severe damage to the nephron (Figure 3, G2) which leads to acute renal failure. Gentamicin toxicity is caused by oxidative stress because the free radicals produced, especially hydroxyl radicals from hydrogen peroxide and superoxide, cause inhibition of the electron transport chain, suppression of cell respiration and ATP production, stimulating the release of cytochrome C from mitochondria which causes DNA damage and cessation of cell cycle known as apoptosis.¹⁰

The administration of pure quercetin or QSD significantly increased the serum creatinine level of mice ($p < 0.05$) indicating that quercetin itself affected kidney function. The pharmacokinetic study of quercetin showed that 46.7% - 106.2% of the doses given were renal excreted.^{14,15} This shows that quercetin has high renal affinity and binds to receptors in nephrons. Administration of this compound at certain doses will increase renal workload, resulting in an increase in serum creatinine levels, but at a lower level than those caused by nephrotoxic drugs such as gentamicin. This fact may correspond to the phrase that drugs are toxicants in smaller doses. Changes in serum creatinine levels were in accordance with observations of nephron damage (Figure 2) as well as histopathological scores of the renal section (Table 3).

Data shows that administration of quercetin (50 mg/kgBW) or QSD (equivalent to 10 mg/kgBW quercetin) improves renal function of the ARF mice. The effect of improvement by QSD may be due to the antioxidant effect of quercetin. Antioxidants stabilize free radicals and inhibit chain reactions of free radical formation that can cause oxidative stress. Excessive free radicals in the body are captured by antioxidants by donating hydrogen atoms so that cells that have been damaged by free radicals can regenerate themselves.¹⁶ One study reported that, quercetin

prevents tubular necrosis or tubular apoptosis which is usually caused by cisplatin. In addition, quercetin also prevents inflammation of the kidneys due to the use of cisplatin.¹⁷

These results indicate that, QSD at a dose of one fifth of pure quercetin provides a comparable effect in nephron-protection. Our previous data supports that formulation of QSD increases the amount of quercetin dissolved which consequently can increase the amount absorbed and thus, as expected, causes a decrease in the effective dose of quercetin. Lower quercetin doses as a nephron-protector will be useful to avoid increasing renal workload in patients and slowing down the progress of kidney damage.

CONCLUSION

The solid dispersion containing quercetin 10.0 mg/kg BW is effective to reduce the serum creatinine and showed a nephron-protection effect on the ARF induced mice. Further studies on the mechanisms of nephron protection of QSD are needed.

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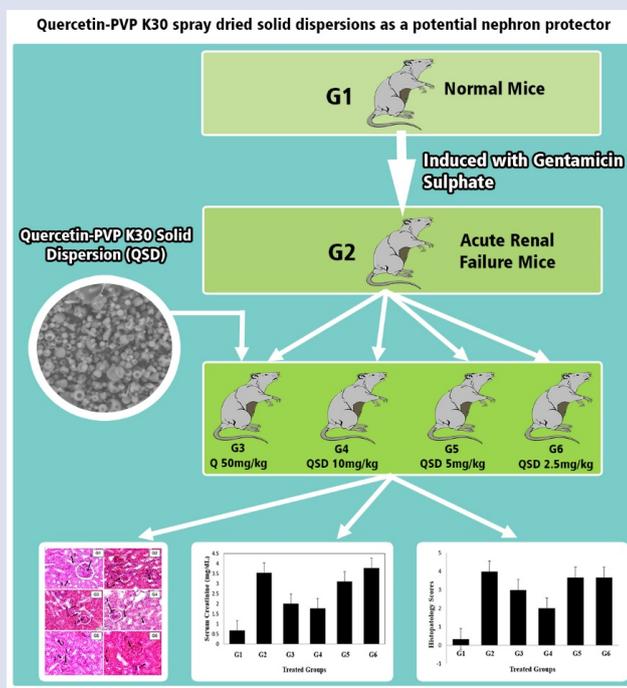
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GRAPHICAL ABSTRACT



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