Subchronic toxicity test of quercetin and cloxacillin in mice

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ABSTRACT

Quercetin is a naturally occuring flavonol, which is classified in flavonoid family. It occurs ubiquitously in the normal human diet and also exhibits numerous pharmacological properties. However, in vivo toxicity test of quercetin has only few reports. Thus, the purpose of this study is to investigate the subchronic toxicity of quercetin alone and in combination with cloxacillin antibiotic in vivo. The mice were administered intraperitoneally (i.p.) with quercetin either alone at 20 and 40 mg/kg BW/day (group 1 and group 2 respectively) or in combination with cloxacillin at 20 plus 150 (group 3) and 40 plus 300 mg/kg BW/day (group 4) of quercetin plus cloxacillin, respectively twice daily for 90 days. At the end of the experiments, blood and the selected main organs were collected for haematological and histological analysis. There was no significant difference in either the growth rate measured by living body weight or the relative weight of the selected main body organs of mice treated with all doses of either quercetin alone or in combination with cloxacillin, when compared to control. The histology of the liver, spleen, heart, kidney and stomach all exhibited a normal appearance in comparison with the control. Results of blood haematological and chemistry marker indicated that there was no significant changes of RBC, Hb, Hct WBC, MCV AST, BUN, FBS, and Uric acid levels between pre- and post-treatment and compared to control in all groups ($p<0.05$). However, cholesterol level exhibited a significant reduction in the group treated with 20 plus 150 mg/kg BW/day of quercetin plus cloxacillin ($p<0.05$).

Keywords: Quercetin, Cloxacillin, Subchronic toxicity, Haematological, Histological, Mice

1. INTRODUCTION

Bacterial resistance to antibiotics is a serious global problem [1]. New approaches to resolve this problem is needed. Active compounds from medicinal plants have long been isolated to use for multipurpose, including antibiotic purpose.

Flavonoids are polyphenolic compounds generally found in fruit, vegetables, gains, bark, stems, roots, wine, and tea. They are considered as integral components in diets. More than 4,000 varieties of flavonoids have been identified. It is widely accepted that the high intake of foods and beverages rich in polyphenols, especially in flavonoids, has been associated with decreased risk of coronary heart disease and also inhibited the proliferation of various tumor growth. Furthermore, epidemiologic studies suggested a protective role of dietary flavonoids against cancer induction in several human tissues, including colon, lung, prostate and urinary bladder [2]. In addition, the combination of β-lactams (ampicillin, cloxacillin and ceftazidime) with flavonoids (baicalein, galangin and quercetin) showed potential activity against drug-resistant bacterial strains [3].

It is very well established that quercetin is chemically related to a class of flavonoids called (pro) anthocyanins. Quercetin has been indicated from pharmacological studies to possess antihistamine, anti-inflammatory, antiallergic, antiviral properties. Quercetin was also active against *Bacillus cereus* when present in the amount of 2.5 μg/ml [4]. Furthermore, quercertin has shown to cause chromosomal mutations in certain bacteria in *in vitro* study. It was reported that quercetin at the dose of 0.33 mg/kg can protect liver cells and mitochondria from oxidative stress by maintaining normal levels of serum transaminases and preventing lipid peroxidation [5].

Although antibiotics in combination with active compounds isolated from medicinal plants are considered as the new approach for overcoming various resistant bacteria, *in vivo* toxicity test of the some flavonoids, including quercetin, have not been investigated. Therefore, the aim of this study was to investigate subchronic toxicity tests of quercetin alone and in combination with antibiotic, cloxacillin *in vivo*.

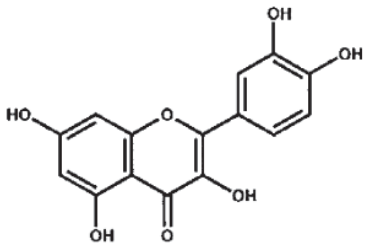

Figure1. Chemical structure of quercetin

2. MATERIALS AND METHODS

Chemicals

Quercetin and Cloxacillin were purchased from Indofine Chemical Company (New Jersy, USA) and dissolved in Normal Saline Solution (NSS) for toxicity test.

Animals preparation

Male and female mice (ICR mouse), 7 ± 1 weeks of age, were purchased from National Laboratory Animal Centre, Mahidol University, Thailand. The animals were housed in stainless cages, in a light- and temperature-controlled room (light on 06.00-20.00 h) temperature 25 ± 0.5°C at the Animal Care Building at Suranaree University of Technology, Nakhon Ratchasima, Thailand. Mouse chow diet (Pokaphan Animal Feed Co., Ltd, Bangkok, Thailand) and water were provided ad libitum. The experimental protocol was approved in accordance with guideline for the care and use of laboratory animal by Animal Care and Use Committee (ACUC), Suranaree University of Technology.

Subchronic toxicity study in mice

Fifty adult mice of body weight 28-40 g were divided into five groups. Ten mice per group (five males and five females) were classified into group one to four including control. The control group was injected with 0.9 % NaCl intraperitoneally (i.p.) twice a day. The tested one to four groups were administered intraperitoneally (i.p.) with quercetin alone at 20 (group 1) and 40 mg/kg BW/day (group 2), quercetin plus cloxacillin at 20 plus 150 (group 3) and 40 plus 300 mg/kg BW/day (group 4), respectively twice a day for three months. These mice were analyzed the blood composition before and after sample injection. Their body weights were recorded weekly.

Haematology and blood chemistry

At the end of the experiments, blood samples were collected by tail under thiopental sodium anesthesia from 9.00 to 10.00 a.m. and were partly used for haematology. From the remainder blood serum was prepared by centrifugation at 1000 x g for 30 min and kept at -20°C for blood chemistry analysis, including fasting blood sugar (FBS), cholesterol, aspartate aminotransferase (AST), blood urea nitrogen (BUN) and uric acid. The assays were employed with automated analytical systems at the centre for medical and public health service, Suranaree University of Technology. The replications of serum from each of the treated groups and control group were run at the same analysis.

Necropsy

After the blood sampling, the mice were sacrificed under thiopental sodium anesthesia and subjected to necropsy. The heart, liver, spleen, lung, kidney, and stomach were removed and weighed. Body weight measured on the day of necropsy was used to calculate the relative organ weight. All organs were preserved in 10% (w/v) neutral phosphate buffer formaldehyde. Heart, liver, spleen, lung, kidney, and stomach fixed-tissue were embedded in paraffin and prepared for microtome sectioning at 5 μm. Haematoxylin and eosin were used for staining. The pathohistology of the organ tissue slides were examined under light microscope.

Statistical analysis

All data are presented as the mean ± S.E.M. Significant differences between the relative selected organ weight or body weight of control and treatment groups were analyzed by ANOVA. The difference of haematology, blood chemistry, growth rate analysis between pre- and post- treatment groups were calculated by *paired student's t-test*. Then, significant difference between each group was compared using ANCOVA. The Tukey HSD post hoc test at $p < 0.05$ and $p < 0.01$ were also considered statistically significant difference between each group.

3. RESULTS

Animal growth and histology analysis

There was no significant difference in either the growth rate measured by living body weight or the relative weight of the selected main body organs of all groups of treated mice for 90 consecutive days, when compared to the control (Figure 2). The pathohistology of all organs showed normal appearance compared to the control organs (data not shown).

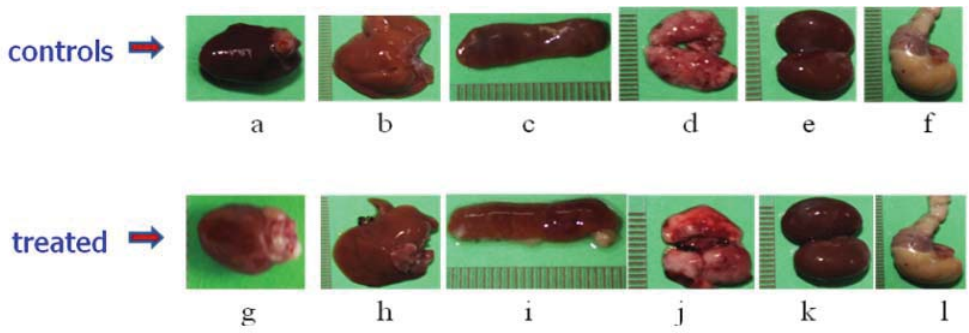

Figure2. Morphology of main body organs of mice treated with quercetin alone and in combination with cloxacillin for 90 consecutive days in comparison with the negative control. Control group; a = heart; b = liver; c = spleen; d = lung; e = kidney; f = stomach. Treated group; g = heart; h = liver; i = spleen; j = lung; k = kidney; l = stomach

Haematology and blood chemistry

Subchronic toxicity study indicated that there was no significant changes of AST, BUN, FBS, Uric acid, WBC, RBC, Hb, Hct, MCV levels between either pre and post treatment in all groups or between all treated groups compared to control ($p < 0.05$) (data not shown). Cholesterol level exhibited a significant reduction in the post-treated group with 20 mg/kg BW/day quercetin plus 150 mg/kg BW/day cloxacillin compared to pre-treated ($p < 0.05$). Also, other post-treated groups displayed cholesterol level lower than pre-treated groups. However, these levels were not significant difference compared to control ($p < 0.05$) (Figure 3).

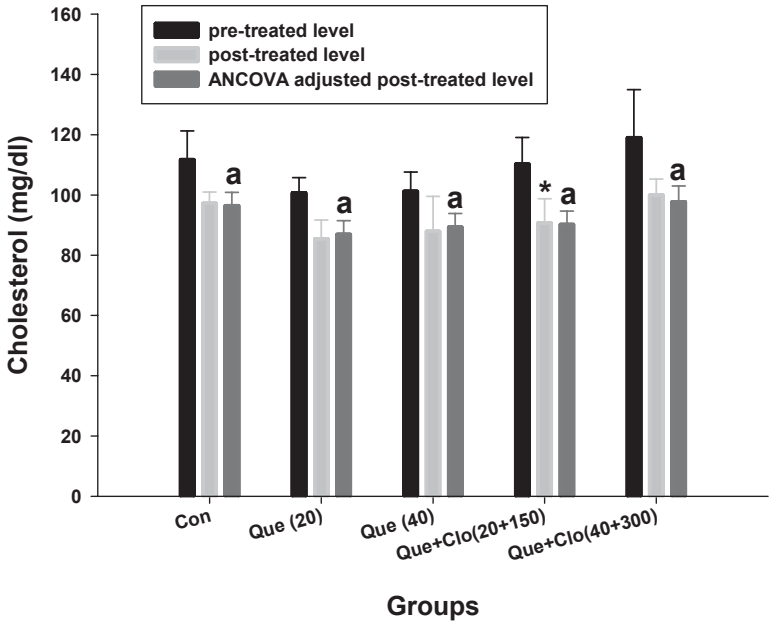

Figure 3. Effects of quercetin, cloxacillin either alone or in combination of mice. Con = control, Que (20) = Quercetin 20 mg/kg BW/day, Que (40) = Quercetin 40 mg/kg BW/day, Que+Clo (20+150) = Quercetin 20 mg/kg BW/day plus Cloxacillin 150 mg/kg BW/day, Que+Clo (40+300) = Quercetin 40 mg/kg BW/day plus Cloxacillin 300 mg/kg BW/day. Significant difference between pre- and post-test in each group was compared using paired student t-test at * $p<0.05$. Significant difference between ANCOVA adjusted post-treated level in each group was compared using ANCOVA and Tukey HSD post hoc test at ^{a} $p < 0.05$.

4. CONCLUSIONS

In conclusion, these findings lead us to believe that quercetin alone and in combination with cloxacilin at these concentrations show no toxicity with blood chemistry, hematology and main organs in mice. Interestingly, these flavonoids provide evidence that its can reduce cholesterol in mice blood when these are taken at high dose for long duration. This study provides essential information for further investigation in other higher mammals, including human.

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