การทดสอบพิษกึ่งเฉียบพลันของกาแลนจินและเซพทาซิดีนในหนูถีบจักร

เกรียงศักดิ์ เอื้อมเก็บ[1*], อภัย ดวงคำ[1]

[1] สาขาวิชาเภสัชวิทยา/ชีววิทยา สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี จ. นครราชสีมา 30000

บทคัดย่อ

กาแลนจินเป็นฟลาโวนอยด์ในกลุ่มของฟลาโวนอลที่เกิดขึ้นตามธรรมชาติ มันเป็นส่วนประกอบที่สำคัญของอาหารที่เรารับประทานเป็นประจำและมีฤทธิ์ทางเภสัชวิทยา อย่างไรก็ตามยังไม่มีการศึกษาถึงพิษของกาแลนจินในสัตว์ทดลอง ดังนั้นวัตถุประสงค์ของการวิจัยครั้งนี้ เพื่อศึกษาพิษของกาแลนจินเมื่อใช้เดี่ยวๆ และผสมกับเซพทาซิดีมในสัตว์ทดลอง วิธีการศึกษาโดยฉีดกาแลนจินเดี่ยวๆ ในขนาด 10, 20 มิลลิกรัมต่อกิโลกรัม (น้ำหนักตัว) ต่อวัน เข้าในช่องท้องของหนูถีบจักรในกลุ่มที่ 1 และ 2 ตามลำดับ ส่วนกลุ่มที่ 3 และ 4 ฉีดกาแลนจินผสมกับเซพทาซิดีนขนาด 10+160 และ 20+320 มิลลิกรัมต่อกิโลกรัม (น้ำหนักตัว) ต่อวัน ตามลำดับ โดยแบ่งฉีดวันละ 2 ครั้ง เป็นเวลา 14 วัน ภายหลังสิ้นสุดการทดลอง ทำการเจาะเลือดและผ่าตัดหนูทดลองเพื่อดูการเปลี่ยนแปลงทางจุลกายวิภาคของอวัยวะสำคัญ ผลการวิจัยพบว่า น้ำหนักสัมพัทธ์และน้ำหนักของอวัยวะสำคัญ (ต่อน้ำหนักตัว) ของหนูทดลอง ไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม จุลกายวิภาคของตับ ม้าม หัวใจ ไตและกระเพาะอาหาร ไม่แตกต่างจากกลุ่มควบคุม ค่าทางเคมีในเลือดไม่มีความแตกต่างระหว่างก่อนและหลังฉีดกาแลนจินเดี่ยวๆ และ/หรือผสมกับเซพทาซิดีน ในทุกๆกลุ่มของหนูทดลอง ยกเว้นระดับของฮีมาโทคริตในหนูที่ได้รับการฉีดสารดังกล่าว มีการลดลงอย่างสัมพันธ์กับขนาดของสารทดลองอย่างมีนัยสำคัญทางสถิติ ที่ระดับความเชื่อมั่น 95%

คำสำคัญ : ฟลาโวนอยด์, กาแลนจิน, เซพทาซิดีน, พิษกึ่งเฉียบพลัน, หนูถีบจักร

***Corresponding author**: ดร. เกรียงศักดิ์ เอื้อมเก็บ

สาขาวิชาเภสัชวิทยา/ชีววิทยา สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี จ. นครราชสีมา 30000

โทร. 044-224260 โทรสาร. 044-224633

E-mail: griang@sut.ac.th

Subacute Toxicity Test of Galangin and Ceftazidime in Mice

Griangsak Eumkeb[1*], Aphai Duangkham[1]

[1]School of Pharmacology/Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand.

ABSTRACT

Galangin is one of several naturally-occuring flavonols, which is a member of flavonoid family. It is important constituents of normal human diet and also has various pharmacological properties. However, *in vivo* toxicity test of galangin have not been studied. Thus, the purpose of this research was to investigate the toxicity of galangin alone and in combination with ceftazidime antibiotic *in vivo*. The mice were administered intraperitoneally (i.p.) with galangin alone at 10 and 20 mg/kg BW/day (group1 and group 2 respectively), galangin in combination with ceftazidime at 10 mg/kg BW/day galangin plus 160 mg/kg BW/day ceftazidime (group3), and 20 mg/kg BW/day galangin plus 320 mg/kg BW/day ceftazidime (group4), twice daily for 14 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 of total body weight or the relative weight of the selected main body organs of mice treated with all doses of galangin alone or in combination with ceftazidime, when compared with the control. The histology of all the liver, spleen, heart, kidney and stomach exhibited a normal appearance in comparison with the control. The blood chemistry marker assayed showed no significant difference ($p<0.05$) in all treated groups except for the decrease in haematocrit level which were significantly difference ($p<0.05$) in all treated groups.

Keywords: Flavonoids, Galangin, Ceftazidime, Subacute toxicity, Mice

***Corresponding author**: Griangsak Eumkeb

School of Pharmacology/Biology, Institute of Science, Suranaree University of Technology, Nakhon-Ratchasima, Thailand

Tel.: +66 44 224260; Fax: +66 44 224633.

E-mail: griang@sut.ac.th

INTRODUTION

Bacterial resistance to antibiotics is a serious global problem.[1, 2] New approaches to resolve this problem are urgently needed. Active compounds from medicinal plants have long been isolated to use for multipurpose, including antibiotic purpose.

Flavonoids are polyphenolic compounds found as integral components in the human diet. They are gene rally found in fruit, vegetables, grains, bark, stems, roots, wine, and tea. 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.[3, 4] In addition, Eumkeb *et al*. (2008) reported that the combination of β-lactams (ampicillin, cloxacillin and ceftazidime) and flavonoids (baicalein, galangin and quercetin) showed considerably activity against bacterial resistant strains.[5]

It is generally accepted that galangin is a flavonol that does not has any hydroxyl group in the B ring (Fig. 1) and has been suggested to be a substrate of cytochromes P_{450} that through the hydroxyl of the B ring, which could metabolise it to more genotoxic products. Galangin has been reported to have anti-gram-positive bacteria with the minimum inhibitory concentration (MIC) ranging from 0.1 to 0.5 µg/ml, and also against *Enterobacter cloacae* which was significantly inhibited at a MIC of 0.1 mg/ml.[6] Eumkeb and Richards (2004) reported that amoxicillin 0.25-2 µg/ml plus galangin 12.5 µg/ml could inhibit twelve clinical isolates of resistance *S. aureus* and four isolates of methicillin-resistance *S. aureus*.[7]

Up to date, the combination between antibiotics and active compounds from medicinal plants are the new approach against many resistant bacteria. However, *in vivo* toxicity test of some potential flavonoids have not been studied. Previous study offered for the development of a valuable galangin adjunct to β-lactam treatments against otherwise resistant strains of currently almost untreatable microorganisms**.** The in vitro results have to be still confirmed in an animal test or in humans.[8] Thus, the purpose of this study was to investigate the toxicity of galangin alone and in combination with ceftazidime *in vivo*. This study may lead us to clarify whether there is the toxicity of galangin *in vivo* or not.

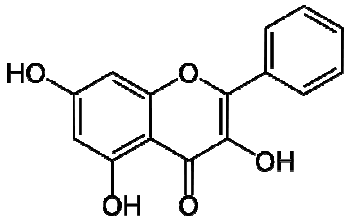


Figure 1 Chemical structure of galangin

2. MATERIALS AND METHODS

2.1 Chemicals

Galangin was purchased from Indofine Chemical Company (New Jersy, USA) and dissolved in 0.1% (v/v) dimethyl sulfoxide (DMSO) for toxicity test. Ceftazidime was obtained from Sigma.

2.2 Animals preparation

Male and female mice (ICR mouse), 6-8 weeks of age, were purchased from the national Laboratory animal center, 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, Nakhonratchasima, 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.

2.3 Subacute toxicity study in mice

Thirty adult mice of body weight 35-40 g were divided into five groups. Ten mice (five both males and females) were used as control. Five mice per group (two males and three females) were classified into group one to four. The control group was injected 0.02 ml of 0.1% (v/v) DMSO plus 0.9% NaCl intraperitoneally (i.p.) twice a day. The tested dissolved in 0.1% (v/v) DMSO one to four groups were administered intraperitoneally with 10 mg/kg BW/day galangin, 20 mg/kg BW/day galangin, 10 mg/kg BW/day galangin plus 160 mg/kg BW/day ceftazidime and 20 mg/kg BW/day galangin plus 320 mg/kg BW/day ceftazidime respectively. The dose of galangin alone or in combination with ceftazidime were divided for injection twice a day for two weeks (fourteen days). These mice were analyzed the blood composition before and after sample injection. Their body weights were recorded weekly.

2.4 Haematology and blood chemistry

At the end of the experiments, blood samples were collected by cardiac puncture 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). The assays were employed with automated analytical systems at the center for medical and public heath service, Suranaree University of Technology. The replications of serum from each of the treated groups and control group were run at the same analysis.

2.5 Necropsy

After the blood sampling, the mice were sacrificed under thiopental sodium anesthesia and subjected to necropsy. The heart, liver, spleen, 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, kidney, and stomach fixed-tissue were embedded in paraffin and prepared for microtome sectioning at 5 μm and haematoxylin and eosin were used for staining. The histopathology of the organ tissue slides were examined under light microscope.

2.6 Statistical analysis

All data are presented as the mean ± S.E.M. Significant differences between the relative main organ weight and body weight of control and treatment groups were analyzed by independent student's *t-test*. The difference of haematology and blood chemistry between pre- and post treatment groups were calculated by paired student's *t-test*. The *P* values < 0.05 was considered statistically significant.

3. RESULTS

3.1 Animal growth and histology analysis

There was no significant difference in either the growth rate measured by living body weight (Fig. 2) or the relative weight of the selected main body organs of all groups of treated mice, when compared to the control (Table 1). The histopathology of the heart, liver, spleen, kidney, and stomach revealed normal appearance compared to the control organs.

3.2 Blood chemistry

The blood chemistry marker assayed exhibited that there was a small decrease of AST and cholesterol level after treatment with galangin alone or in combination with ceftazidime compared to pre-treatment groups. However, the AST and cholesterol reduction were not significantly different at $p<0.05$. There was no clear dose-dependent or significant changes ($p<0.05$) of BUN and FBS between pre- and post-treatment in all groups (Table 3).

3.3 Haematology

Results of haematology demonstrated that there were no significant change of White blood cell (WBC), Red blood cell (RBC), Hemoglobin (Hb), and Mean corpuscular volume (MCV) in of post-treatment groups compared to pre-treatment groups. Conversely, the post-treatment haematocrit (Hct) level exhibited clear dose-dependent and significant reduction ($p<0.05$) with increasing galangin and ceftazidime doses in comparison with pre-treatment in all groups (Table 2).

4. DISCUSSION

From the results, it can be concluded that 10 and 20 mg/kg BW/day galangin, i.p. injection, showed discernable adverse effect upon mice growth rates and the selected main body organs changes of all mice. The results from blood chemistry exhibited no hazard on this parameter. Similary, the toxicity of galangin at this concentration on WBC, RBC, Hb, and MCV is not concern. The results of galangin on WBC are presumably understanding with those of Hoe *et al.* (1994) that galagin suppressed the induction of lymphocytes chromosome aberrations by bleomycin as dose-dependent manner.[9] In contrast, Galangin revealed significant reduction ($p<0.05$) of Hct after treatment in all groups as dose-dependent way. These results seem consistent with previous research that galangin in the lower doses of treatment (0.1 mg/kg) showed more potent anticlastogenic effect than in higher dose (10 mg/kg) from both single and multiple pre-treatment.[10] These findings provide evidence that high dose and long duration administration of galangin may result in Hct reduction.

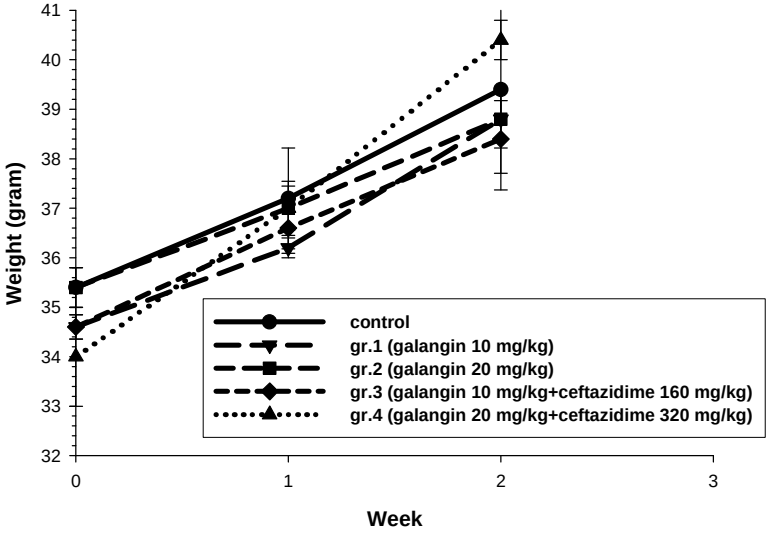


Figure 2 Body weight of mice treated with galangin alone and in combination with ceftazidime for 14 consecutive days in comparison with the negative control.

REFERENCE

1. Kyle JA, Sharp L, Little J, *et al.* Dietary flavonoid intake and colorectal cancer: a case control study. *Br J Nutr* 2010; 103: 429-36.
2. Mulligan ME, Murray-Leisure KA, Ribner BS, *et al.* Methicillin-resistant Staphylococcus aureus: a consensus review of microbiology, pathogenesis, and epidemiology with implications for prevention and management. *Am J Med* 1993; 94: 313-28.
3. Le Marchand L. Cancer preventive effects of flavoids: a review. Biomed Plarmacother 2002; 56: 296-301.
4. Heo MY, Sohn SJ, Au WW. Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. *Mutat Res/Rev Mutat Res* 2001; 488: 135-50.
5. Eumkeb G, Chaisena N, RojtinnakornN, *et al*. Reversing ß-Lactam Antibiotic Resistant Bacteria with Flavonoids. In: Srichairat S, eds. The 15th Dr. Chiravat Sadavongvivad Memorial Lecture. Proceeding of 30th Pharmacological and Therapeutic Society of Thailand Meeting; 2008 March 27-28; Bangkok: Ruen Kaew Press; 2008, 117-26.
6. Afolayan AJ, Meyer JJ. The Antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of Helichrysum aureonitens. *J Ethnopharmacol* 1997; 57; 177-81.
7. Eumkeb G, Richards RME. Reversing beta-lectam antibiotic Resistance in Gram-positive bacteria by Some Flavonoids. Suranaree *J Sci Technol* 2004; 11: 143-50.
8. Eumkeb G, Sakdarat S, Siriwong S. Reversing beta-lactam antibiotic resistance with galangin isolated from smaller galangal (Alpinia officinarum Hance) in Staphylococcus aureus and synergism with Ceftazidime. Phytomedicine 2010; 18: 40-5.
9. Heo MY, Lee SJ, Kwon CH, *et al.* Anticlastogenic effects of galangin against bleomycin induced chromosomal aberrations in mouse spleen lymphocytes. *Mutat Res.* 1994; 311: 255-9.
10. Sohn SJ, Huh IH, Au WW, *et al.* Antigenotoxicity of galangin against N-methyll-N-nitrosourea. Mutat Res. 1998; 402: 231-6.

Table 1 Effects of intraperitoneally administered galangin alone and in combination with ceftazidime for 14 days on relative weight of the selected main organ (per 100g body weight) in mice.

Organ	Control	Group 1	Group 2	Group 3	Group 4
Heart	0.0049 ± 0.0005	0.0050 ± 0.0002	0.0052 ± 0.0002	0.0046 ± 0.0001	0.0045 ± 0.0004
Liver	0.0514 ± 0.0029	0.0498 ± 0.0032	0.0514 ± 0.0048	0.0474 ± 0.0012	0.0523 ± 0.0035
Spleen	0.0041 ± 0.0005	0.0045 ± 0.0004	0.0063 ± 0.0011	0.0040 ± 0.0005	0.0042 ± 0.0006
Lung	0.0062 ± 0.0008	0.0062 ± 0.0001	0.0062 ± 0.0003	0.0061 ± 0.0001	0.0062 ± 0.0003
Kidney	0.0172 ± 0.0033	0.0170 ± 0.0014	0.0163 ±0.0023	0.0165 ± 0.0023	0.0177 ± 0.0027
Stomach	0.0199 ± 0.0035	0.0133 ± 0.0010	0.0223 ± 0.0037	0.0286 ± 0.0043	0.0231 ± 0.0042

(Group 1 = galangin 10 mg/kg BW/day, Group 2 = galangin 20 mg/kg BW/day Group 3 = galangin 10 mg/kg BW/day plus ceftazidime 160 mg/kg BW/day, Group 4 = galangin 20 mg/kg BW/day plus ceftazidime 320 mg/kg BW/day.)

Table 2 Complete blood count study on mice before and after subacute treatment with galangin alone and in combination with ceftazidime for 14 days.

Parameter	Control		Group 1		Group 2		Group 3		Group 4	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
WBC ($x10^9$/L)	7.66±0.45	6.96±0.58	8.08±0.55	7.16±0.77	7.26±1.12	7.60±1.76	5.94±0.54	4.78±0.35	6.58±0.33	5.68±0.99
RBC ($x10^6$ /µL)	8.58±0.09	8.50±0.07	8.96±0.78	7.72±0.18	8.59±0.27	8.40±0.21	8.46±0.43	7.40±0.20	8.20±0.26	7.28±0.37
Hb (g/dl)	13.79±0.29	12.98±0.91	13.94±0.60	12.66±0.27	12.72±0.24	12.00±0.26	14.06±0.75	12.66±0.60	14.54±0.81	12.20±0.65
Hct (%)	54.64±0.63	52.26±1.70	54.80±1.34	45.56±0.63*	51.52±2.49	33.26±1.67*	51.36±0.61	38.80±2.02*	53.18±1.77	36.74±2.45*
MCV (fL)	53.64±1.29	57.48±1.58	50.74±5.75	57.44±0.62	53.50±1.37	50.40±1.43	53.72±2.08	51.80±1.65	53.18±1.77	49.60±0.50

[1] (Group 1 = galangin 10 mg/kg BW/day, Group 2 = galangin 20 mg/kg BW/day, Group 3 = galangin 10 mg/kg BW/day plus ceftazidime 160 mg/kg BW/day, Group 4 = galangin 20 mg/kg BW/day plus ceftazidime 320 mg/kg BW/day.)

[2] Pre- = Pre-treatment, Post- = Post-treatment

[3] Mean ± SEM (n=5), *p < 0.05 (Student's t–test). Significant relative to pre-treatment value

Table 3 Blood Chemistry study on mice before and after subacute treatment with galangin alone and in combination with ceftazidime for 14 days.

Parameter	Control		Group 1		Group 2		Group 3		Group 4	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
AST (U/L)	148.60±4.40	148.80±5.01	150.80±4.07	149.40±3.98	139.20±8.25	109.40±12.33	153.00±24.26	111.40±5.96	118.40±13.30	97.20±4.40
BUN (mg/dl)	23.40±0.42	23.44±0.36	21.40±1.42	15.80±2.13	16.52±1.03	22.05±4.13	25.37±1.48	24.45±1.24	22.47±2.16	16.72±0.44
FBS (mg/dl)	111.40±2.78	111.60±3.20	108.40±19.13	154.00±8.29	109.60±22.76	87.20±10.13	114.80±20.91	115.80±5.07	103.20±25.97	139.80±3.49
Cholesterol (mg/dl)	184.20±1.49	183.80±1.82	166.40±2.23	164.20±1.59	187.20±16.41	141.00±7.76	182.00±14.88	174.00±14.79	185.20±12.53	145.20±9.51

[1] (Group 1 = galangin 10 mg/kg BW/day, Group 2 = galangin 20 mg/kg BW/day, Group 3 = galangin 10 mg/kg BW/day plus ceftazidime 160 mg/kg BW/day, Group 4 = galangin 20 mg/kg BW/day plus ceftazidime 320 mg/kg BW/day.)

[2] Pre- = Pre-treatment, Post- = Post-treatment

[3] Mean ± SEM (n=5), *$p < 0.05$ (Student's *t*–test). Significant relative to pre-treatment value