Evaluation of in vitro and in vivo genotoxicity of Angelica acutiloba in a standard battery of assays

Jun-Won Yun1, Yun-Soon Kim2, Euna Kwon2, Seung-Hyun Kim2, Ji-Ran You2, Hyeon Hoe Kim1, Jeong-Hwan Che4,*, Byeong-Cheol Kang2,4,5,6,*

1Department of Biotechnology, The Catholic University of Korea, Bucheon, Gyeonggi-do, Korea
2Department of Experimental Animal Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea
3Department of Urology, Seoul National University College of Medicine, Seoul, Korea
4Biomedical Center for Animal Resource and Development, Seoul National University College of Medicine, Seoul, Korea
5Graduate School of Translational Medicine, Seoul National University College of Medicine, Seoul, Korea
6Designed Animal and Transplantation Research Institute, Institute of GreenBio Science Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, Korea

Among three representative species of Angelica found in Asian countries, including Korea, China, and Japan, Angelica acutiloba (AA) has been used as traditional herbal medicine with antitumor, anti-inflammatory, anti-obesity, and anti-diabetes activities. In this study, the potential genotoxicity and mutagenicity of the AA extract were examined in a battery of in vitro and in vivo tests (bacterial reverse mutation assay, in vitro chromosomal aberrations assay, and in vivo micronucleus assay) in accordance with the test guidelines for toxicity testing developed by the Organization for Economic Cooperation and Development. Upon testing in the bacterial mutation assay (Ames test) using five Salmonella typhimurium TA98, TA100, TA102, TA1535 and TA1537, no significant increase the number of revertant colonies in the metabolic activation system and non-activation system was noted in the AA extract groups. Also, in the chromosome aberration test, the AA extract did not cause chromosomal aberration with or without metabolic activation by S9 mix. A bone marrow micronucleus test of mice demonstrated that the incidence of micronucleated polychromatic erythrocytes in the AA extract groups (500, 1000 and 2000 mg/kg BW) was equivalent to that of the negative control group. Based on these results from a standard battery of assays, the AA extract was concluded to have no genotoxicity at the proper dose.

Keywords: Angelica acutiloba, traditional medicine, genotoxicity, mutagenicity

Received 6 July 2017; Revised version received 2 September 2017; Accepted 3 September 2017

Many species Angelica belonging to the family Umbelliferae has long been known as traditional herbal medicines that can be found throughout Asian countries, including Korea, China, and Japan. In particular, Danggui (root of Angelica) or ‘female ginseng’ is widely used as a typical therapeutic for treating gynecological disorders such as menoxenia, menstrual pain, anemia, and fatigue/low vitality [1-5]. Despite similarities in shapes, Danggui has different origin species and scientific names according to geographical locations: Angelica sinensis from China (Chinese Danggui), A. acutiloba (AA) from Japan (Japanese Danggui), and of A. gigas from Korea (Korean Danggui) [6].

Among these three species, AA were reported to exhibit various pharmacological effects including antitumor [7], anti-inflammatory [8-11], immunostimulatory [12],...
anti-complementary [13], and insecticidal [14]. And, the AA could be an effective ethnomedicine for treating diabetic complications and obesity since it has been reported to attenuate fat accumulation in high fat diet-induced obesity through the up-regulation of lipid metabolism [15] and to ameliorate insulin resistance induced by high-fructose diet in rats through the promotion of glucose homeostasis [4]. In addition, it is reported that the AA inhibited the release of histamine from mast cells and the production of pro-inflammatory cytokines, which contributed to the protection against allergic diseases and inflammatory diseases [16-19]. Additional pharmacological effects of the AA include the enhancement of collagen synthesis and suppression of matrix metalloproteinases, resulting in its use as an anti-wrinkle natural agent [1].

The use of medicinal plants as therapeutics, functional foods, or dietary supplements for health-related issues is increasing as self-medication for symptomatic relief because it is believed that these are natural and free from side effects compared with synthetic chemical drugs [20,21]. However, concerns associated with the lack of quality control (plant origin and method of preparation) and potential risks of self-medication (treatment duration) have been raised [4]. Recently, we have identified that long-term ingestion of well-known medicinal herbs available in market can induce potential toxicity that may be hepatotoxicity or nephrotoxicity. In particular, Sophorae radix and vinegar-processed Genkwa flos have been found to have clastogenic and mutagenic potentials, respectively, as evidenced by the chromosome aberration assay and the Ames test [22-24].

Along with the current use as an herbal medicine available on the market in Asian countries, many studies have reported various beneficial activities of the AA extract, but little information on risk and safety has been reported. Therefore, we carried out the battery of regulatory genotoxicity studies, including in vitro bacterial reverse gene mutation assay (Ames test), in vitro chromosome aberration assay, and in vivo micronucleus assay in mice, to assess the mutagenicity and clastogenicity of the AA extract according to test guidelines for toxicological studies developed by the Organization for Economic Cooperation and Development (OECD) and the Ministry of Food and Drug Safety (MFDS).

Materials and Methods

Test substance and animals

A hot water AA extract was provided by the National Institute of Food and Drug Safety Evaluation (Osong, Korea). AA roots were purchased from an Oriental medicine market in Korea, and an extract of AA was obtained according to a method described previously [25]. In brief, dried AA roots were ground by a mixer, and incubated with distilled water (DW) at 100°C. After filtration through filter paper, the filtrate was freeze-dried and dissolved in DW for oral administration. The extraction yield of the hot water AA extract was 0.153 g of freeze-dried AA extract/g of dried AA root.

ICR mice ( Orient Bio, Seongnam, Korea) were used after a week of quarantine and acclimatization. During the studies, the animal facility was maintained under standard conditions (22±2°C, 40-60% humidity, and 12 h light/dark cycle). The animals were fed a rodent diet (LabDiet 5002 Certified Rodent Diet, PMI Nutrition International, St. Louis, MO, USA) and tap water ad libitum. All of the animal experiments were approved by the Institutional Animal Care and Use Committee of the Biomedical Research Institute at the Seoul National University Hospital, and this study was performed in compliance with the guidelines published by the OECD as well as the guidance for the GLP for toxicity tests issued by the Ministry of Food and Drug Safety [26].

In vitro bacterial reverse gene mutation assay (Ames test)

Five characterized histidine-dependent strains of Salmonella typhimurium (TA98, TA100, TA102, TA1535, TA1537; MFDS, Osong, Korea) were utilized for bacterial reverse mutation assay (Ames test) in accordance with OECD guideline 471 [27]. S. typhimurium strains were incubated with the AA extract with or without an S9 mix in the dark at 37°C for 48 h. The standard mutagens (2-nitrofluorene, sodium azide, mitomycin C, 9-aminoacridine, and 2-aminoanthracene; Sigma-Aldrich, St. Louis, MO, USA) were used as positive controls. The extract was considered to be positive if there was a two-fold increase relative to negative control or a dose-dependent increase in the number of revertant colonies.
**In vitro chromosomal aberration assay**

An *in vitro* chromosomal aberration test using Chinese hamster lung (CHL) fibroblast cells was conducted in accordance with OECD guideline 473 [28]. The cells were incubated in a CO\(_2\) incubator (5% CO\(_2\), 37°C, high humidity) with the AA extract in the presence or absence of an S9 mix for 6 h or 24 h. Mitomycin C and cyclophosphamide (Sigma-Aldrich) were used as positive controls. After colcemid (0.2 µg/mL, Gibco, Carlsbad, CA, USA) was added for 2 h, the cells were treated with hypotonic solution, fixed in 3:1 methanol/glacial acetic acid, and stained with 4% Giemsa.

**In vivo bone marrow micronucleus assay**

An *in vivo* bone marrow micronucleus test was conducted in accordance with OECD guideline 474 [29]. 8-week-old male ICR mice were orally treated with the AA extract at 0, 500, 1000, and 2000 mg/kg of body weight (BW) once daily for 4 d. Mitomycin C (2 mg/kg BW) served as a positive control and was intraperitoneally injected. The clinical signs and body weight were evaluated once daily. The mice were euthanized at 24 h after the last dose. The femoral bone marrow cells were isolated, centrifuged, smeared on the slides, and dried. After fixation with methanol and 5% Giemsa staining, the number of micronucleated polychromatic erythrocytes (MNPs) was counted from 2000 PCEs. In addition, the PCE/(PCE+NCE) ratio, where the NCEs indicate the normochromatic erythrocytes, was calculated to detect the possibility of cytotoxicity [30].

**Statistical analysis**

All of the values are expressed as mean±SD. The statistical analysis was performed using a one-way ANOVA, followed by a multiple comparison procedure with a Tukey/Duncan test using SPSS software version 19 (SPSS Inc., Chicago, IL, USA). *P* values of less than 0.05 were considered to be statistically significant.

**Results and Discussion**

**In vitro bacterial reverse gene mutation assay (Ames test)**

Among the battery of three genotoxicity tests, the Ames test is known as the most accurate and commonly used *in vitro* method developed by Ames and coworkers in the early 1970s for an initial detection of genotoxicants that induce mutations in the DNA, particularly point mutation induction [31,32]. The mutagenic activity of the AA extract was investigated by Ames test using Table 1.

| S9 Chemical       | Dose (µg/plate) | TA98 | TA100 | TA102 | TA1535 | TA1537 |
|-------------------|-----------------|------|-------|-------|--------|--------|
| Distilled water\(^a\) | 0.0             | 22.0±5.3 | 279.3±30.6 | 487.0±45.6 | 21.3±2.5 | 73.7±18.4 |
| 2-nitrofluorenone\(^b\) | 10              | 285.7±13.3* | - | - | - | - |
| Sodium azide\(^b\)          | 5               | - | 1578.0±321.5* | - | - | - |
| Mitomycin C\(^b\)          | 0.5             | - | - | 1906.7±179.9* | - | - |
| Sodium azide\(^b\)          | 0.5             | - | - | - | 453.3±47.2* | - |
| 9-aminoacridine\(^b\)       | 80              | - | - | - | - | 2440.0±322.0* |
| Angelica acutiloba         | 312.5           | 24.0±2.6 | 255.7±16.7 | 463.7±18.0 | 19.7±1.5 | 59.0±1.0 |
| 625               | 26.0±7.2   | 283.0±22.3 | 452.7±12.9 | 21.7±5.5 | 61.0±11.4 |
| 2,500             | 28.7±6.4   | 309.0±19.1 | 533.0±29.5 | 20.7±3.5 | 71.3±9.2 |
| 5,000             | 23.0±4.8   | 366.7±42.2 | 598.7±96.2 | 28.0±2.6 | 59.7±6.0 |
| Distilled water\(^a\) | 0.0             | 31.0±4.6 | 213.3±22.9 | 543.0±15.9 | 10.3±1.2 | 61.7±8.5 |
| 2-aminoanthracene\(^b\)  | 2               | 263.3±18.8* | 641.7±25.0* | - | - | - |
| 5                 | -             | - | 1438.0±244.2* | 239.0±18.4* | 866.3±64.7* |
| Angelica acutiloba         | 312.5           | 29.0±7.2 | 214.7±15.0 | 531.7±28.6 | 9.3±0.6 | 59.3±5.5 |
| 625               | 31.7±2.1   | 235.0±7.8 | 534.0±8.7 | 14.3±0.6 | 60.0±8.5 |
| 2,500             | 29.7±7.2   | 214.0±18.2 | 557.7±49.9 | 9.7±4.5 | 51.7±5.5 |
| 5,000             | 31.7±6.1   | 219.7±30.0 | 587.3±80.8 | 14.7±6.7 | 53.0±2.6 |
| 5,000             | 34.7±3.8   | 186.7±60.4 | 653.7±71.7 | 11.3±1.5 | 46.7±4.6 |

\(^a\)Negative control
\(^b\)Positive control
\(^\ast\)Significantly different from negative control group (*P*<0.05).
histidine requiring strains of *S. typhimurium*, such as the frameshift tester strain TA98 and TA1537, the base-pair substitution tester strain TA100 and TA1535, or oxidative and cross-linking tester strain TA102 [33]. The AA extract treatments exerted no significant increases in the number of revertants per plate in the absence and presence of the metabolic activation system at all concentration tested (312.5, 625, 1250, 2500 and 5000 µg/plate) relative to the negative control group (Table 1). In contrast, large increase in the number of revertant colonies were seen for the various mutagens (positive controls) with or without of S9 mix, indicating that the test system conducted in the present study responded appropriately under acceptable experimental condition. Therefore, the results of this assay indicated that the AA extract did not show mutagenicity in the tester strains under these experimental conditions.

**In vitro chromosomal aberration assay**

The chromosomal aberration test is an *in vitro* screening assay that has been used to detect chromosomal damage in cultured mammalian cells [34-36]. At first, the effect of the AA extract on the proliferation of CHL cells were analyzed using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay (Table 2), and we decided to set 5000 µg/mL of the AA extract as the highest exposure level for *in vitro* chromosomal aberration assay based on the MTT assay resulting to 98.1% viability at 24 h incubation. The positive controls (mitomycin C without the S9 mix and cyclophosphamide with the S9 mix) resulted in significant increases in the incidence of structural chromosome aberrations, supporting the validity of the study (Table 3). In contrast, the number of cells with chromosomal aberrations (breaks, fragments, and exchanges) in the AA extract groups was not significantly different from the negative control group after short-term treatment for 6 h with or without the S9 mix and after continuous treatment for 24 h without the S9 mix. Our current data show that the AA extract did not induce chromosomal aberrations in CHL cells under the conditions used in this assay.

**In vivo bone marrow micronucleus assay**

Micronuclei, also known as Howell-Jolly bodies, are chromatin particles from damaged chromosome fragments that were not incorporated into the nucleus after mitosis [37]. In this study, the presence of micronuclei, which is regarded as an indirect indicator of quantitative and structural chromosomal aberrations [38], was evaluated to determine the genotoxic property of the AA extract. During the study, no abnormal clinical signs of toxicity in general appearance were detected in any of the mice following the AA extract administration. And, there were no statistically significant differences in body weights across all groups (Table 4). The mean ratio of PCEs to total erythrocytes were 50.9, 51.6, 47.4, 53.9, and 28.5% for the negative control, at 500, 1000, 2000

### Table 2. Results of MTT assay in CHL cells treated with *Angelica acutiloba* extract

| Substance        | Dose (µg/mL) | Absorbance | Survival rate (%) |
|------------------|--------------|------------|-------------------|
| Distilled water  | 0.0          | 0.53±0.07  | 100.0             |
|                  | 39.1         | 0.51±0.16  | 96.5              |
|                  | 78.1         | 0.50±0.05  | 93.9              |
|                  | 156.3        | 0.50±0.09  | 94.3              |
| *Angelica acutiloba* | 312.5   | 0.80±0.10  | 113.7             |
|                  | 625.0        | 0.49±0.17  | 93.5              |
|                  | 1250.0       | 0.42±0.06  | 79.3              |
|                  | 2500.0       | 0.51±0.06  | 96.6              |
|                  | 5000.0       | 0.52±0.06  | 98.1              |

Table 3. Results of chromosomal aberration induced by *Angelica acutiloba* extract

| Substance     | Dose (µg/mL) | Number of cells scored | No. of cells with aberrations |
|---------------|--------------|------------------------|-------------------------------|
|               |              | 6h         | 24h         | 6h                 |
| MEM*          | 0            | 4.5±2.1    | 2.5±3.5    | 5.5±0.7            |
| Mitomycin C*  | 0.1          | 38.0±7.1*  | 66.5±9.2*  |                   |
| Cyclophosphamide† | 10     | -          | -          | 98.0±1.4*          |
| Distilled water | 1,250    | 4.5±3.5    | 2.5±0.7    | 7.0±2.8            |
| *Angelica acutiloba* | 2,500        | 1.5±2.1    | 2.5±3.5    | 2.0±2.8            |
|                  | 5,000.0      | 3.5±5.0    | 2.5±0.7    | 5.0±1.4            |
|                 |              | 4.0±2.8    | 7.5±5.0    | 6.0±4.2            |

*Minimum essential medium (negative control)
†Positive control
*Significantly different from negative control group (P<0.05).
mg/kg BW of the AA extract, and the positive control, respectively (Table 5), indicating that this study was valid since no decrease in the PCE/(PCE+NCE) ratio (greater than 20%) reflects a lack of toxic effects of the AA extract (Heddle et al., 1984). The incidence of micronuclei showed no significant difference between the negative control group and the AA extract groups (500, 1000, or 2000 mg/kg BW). In contrast, the number of micronuclei in the mitomycin C-treated groups was significantly higher than that of the negative control group.

In conclusion, these fundamental toxicology data from the Ames assay, in vitro chromosome aberration assay, and in vivo micronucleus assay clearly suggest that the AA extract is safe in terms of genotoxicity. To our knowledge, this is the first comprehensive study assessing the potential genotoxic effects of the AA extract as a traditional medicine with beneficial activities in accordance with the OECD and the GLP Regulations.

**Conflict of interests**  The authors declare that there is no financial conflict of interests to publish these results.

**References**

1. Park MA, Sim MJ, Kim YC. Anti-Photoaging Effects of Angelica acutiloba Root Ethanol Extract in Human Dermal Fibroblasts. Toxicol Res 2017; 33(2): 125-134.
2. Sheng YX, Li L, Wang Q, Guo HZ, Guo DA. Simultaneous determination of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid in Si-Wu decoction by high-performance liquid chromatography DAD method. J Pharm Biomed Anal 2005; 37(4): 805-810.
3. Wen KC, Huang CY, Lu FL. Determination of baicalin and puerarin in traditional Chinese medicinal preparations by high-performance liquid chromatography. J Chromatogr A 1993; 631: 241-250.
4. Liu IM, Tzeng TF, Liou SS, Chang CJ. Angelica acutiloba root attenuates insulin resistance induced by high-fructose diet in rats. Phytother Res 2011; 25(9): 1283-1293.
5. Huntley AL, Ernst E. A systematic review of herbal medicinal products for the treatment of menopausal symptoms. Menopause 2003; 10(5): 465-476.
6. Jeong SY, Kim HM, Lee KH, Kim KY, Huang DS, Kim JH, Seong RS. Quantitative analysis of marker compounds in Angelica gigas, Angelica sinensis, and Angelica acutiloba by HPLC/DAD. Chem Pharm Bull (Tokyo) 2015; 63(7): 504-511.
7. Yamada H, Komiyama K, Kiyohara H, Cyong JC, Hirakawa Y, Otsuka Y. Structural characterization and antitumor activity of a pectic polysaccharide from the roots of Angelica acutiloba. Planta Med 1990; 56(2): 182-186.
8. Yoon TS, Cheon MS, Lee DY, Moon BC, Lee HW, Choo BK, Kim HK. Effects of root extracts from Angelica gigas and Angelica acutiloba on inflammatory mediators in mouse macrophages.

**Acknowledgments**

This research was supported with a grant (05122MFDS472) from the Ministry of Food and Drug Safety.
J Appl Biol Chem 2007; 50(4): 264-269.
9. Tanaka S, Kano Y, Tabata M, Konoshima M. [Effects of “Toki” (Angelica acutiloba Kitagawa) extracts on writhing and capillary permeability in mice (analgentic and antiinflammatory effects)]. Yakugaku Zasshi 1971; 91(10): 1098-1104.
10. Tanaka S, Ishiburo Y, Tabata M, Konoshima M. Anti-neoplastic substances from the roots of Angelica acutiloba. Arzneimittelversuch 1977; 27(11): 2039-2045.
11. Lee K, Sohn Y, Lee MJ, Cho HS, Jang MH, Han NY, Shin KW, Kim SH, Cho BH, Bu Y, Jung HS. Effects of Angelica acutiloba on mast cell-mediated allergic reactions in vitro and in vivo. Immunopharmacol Immunotoxicol 2012; 34(4): 571-577.
12. Kumazawa Y, Nakatsuji Y, Fujisawa H, Nishinami C, Mizanoe K, Otsuka Y, Nomoto K. Lymphocyte activation by a polysaccharide fraction separated from hot water extracts of Angelica acutiloba. J Pharmacobiodyn 1985; 8(6): 417-424.
13. Kiyohara H, Yamada H, Cyong JC, Otsuka Y. Studies on polysaccharides from Angelica acutiloba. V. Molecular aggregation and anti-complementary activity of arabino-galactan from Angelica acutiloba. J Pharmacobiodyn 1986; 9(4): 339-346.
14. Miyazawa M, Tsukamoto T, Anzai J, Ishikawa Y. Insecticidal effect of phthalides and furanocoumarins from Angelica acutiloba against Drosophila melanogaster. J Agric Food Chem 2004; 52(14): 4401-4405.
15. Liu J, Tzeng TF, Liou SS, Chang CJ. Regulation of obesity and lipid disorders by extracts from Angelica acutiloba root in high-fat diet-induced obese rats. Phytother Res 2012; 26(2): 223-230.
16. Lee K, Sohn Y, Lee MJ, Cho HS, Jang MH, Han NY, Shin KW, Kim SH, Cho BH, Bu Y, Jung HS. Effects of Angelica acutiloba on mast cell-mediated allergic reactions in vitro and in vivo. Immunopharmacol Immunotoxicol 2012; 34(4): 571-577.
17. Joo SS, Park D, Shin S, Jeon JH, Kim TK, Choi YJ, Lee SH, Kim JS, Park SK, Hwang BY, Lee DI, Kim YB. Anti-allergic effects and mechanisms of action of the ethanolic extract of Angelica gigas in dinitrofluorobenzene-induced inflammation models. Environ Toxicol Pharmacol 2010; 30(2): 127-133.
18. Sarker SD, Nahar L. Natural medicine: the genus Angelica. Curr Med Chem 2004; 11(11): 1479-1500.
19. Uto T, Tung NH, Tanjyana R, Miyanowaki T, Morinaga O, Shoyama Y. Anti-inflammatory Activity of Constituents Isolated from Aerial Part of Angelica acutiloba Kitagawa. Phytother Res 2015; 29(12): 1956-1963.
20. Markman M. Safety issues in using complementary and alternative medicine. J Clin Oncol 2002; 20: 39-41.
21. Shin SH, Koo KH, Bae JS, Cha SB, Kang IS, Kang MS, Kim HS, Heo HS, Park MS, Gil GH, Lee JY, Kim KH, Li Y, Lee HK, Song SW, Choi HS, Kang BH, Kim JC. Single and 90-day repeated oral dose toxicity studies of fermented Rhus verniciflua stem bark extract in Sprague-Dawley rats. Food Chem Toxicol 2013; 55: 617-626.
22. Che JH, Yun JW, Cho EY, Kim SH, Kim YS, Kim WH, Park JH, Son WC, Kim MK, Kang BC. Toxicologic assessment of Paeolcosmeyces tenuepis in rats: renal toxicity and mutagenic potential. Regul Toxicol Pharmacol 2014; 70(2): 527-534.
23. Che JH, Yun JW, Kim YS, Kim SH, You JR, Jang JJ, Kim HC, Kim HH, Kang BC. Genotoxicity and subchronic toxicity of Sophora radyx in rats: hepatoxic and genotoxic potential. Regul Toxicol Pharmacol 2015; 71(3): 379-387.
24. Yan JW, Kim SH, Kim YS, You JR, Kwon E, Jang JJ, Park IA, Kim HC, Kim HH, Che JH, Kang BC. Evaluation of subchronic (12-week) toxicity and genotoxicity potential of vinegar-processed Genkwa Flos. Regul Toxicol Pharmacol 2015; 72(2): 386-393.
25. Yun JW, Che JH, Kwon E, Kim YS, Kim SH, You JR, Kim WH, Kim HH, Kang BC. Safety evaluation of Angelica gigas: Genotoxicity and 13-weeks oral subchronic toxicity in rats. Regul Toxicol Pharmacol 2015; 72(3): 473-480.
26. Ministry of Food and Drug Safety (MFDS), 2014. Good Laboratory Practice Regulation for Non-clinical Laboratory Studies (Notification No. 2014-67). MFDS, Korea. Available [October 2014], http://www.mfds.go.kr/
27. OECD, 1997. OECD guideline for testing of chemicals, Test No. 471: Bacterial Reverse Mutation test.
28. OECD, 1997. OECD guideline for testing of chemicals, Test No. 473: in vitro mammalian chromosome aberration test.
29. OECD, 1997. OECD guideline for testing of chemicals, Test No. 474: mammalian erythrocyte micronucleus test.
30. Heddle JA, Stuut E, Salamone MF. 1984. The bone marrow micronucleus test in Handbook of mutagenicity test procedures. In: Kilbey, B.J., Legator, M., Nichols, W., Ramel, C. (eds.). Elsevier, Amsterdam; pp 441-457.
31. Hakura A, Suzuki S, Sato T. Advantage of the use of human liver S9 in the Ames test. Mutat Res 1999; 438(1): 29-36.
32. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res 1983; 113(3-4): 173-215.
33. Kaleeswaran S, Sriram P, Prabhu D, Chinnathambi; Vijayakumar, Mathuram LN. Anti- and pro-mutagenic effects of silymarin in the Ames bacterial reverse mutation assay. Phytother Res 2009; 23(10): 1378-1384.
34. Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. Mutat Res 1985; 147(1-2): 29-36.
35. Perry P, Evans HJ. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. Nature 1975; 258(5351): 121-125.
36. Tucker JD, Auletta A, Cimino MC, Dearfield KL, Jacobson-Kram D, Tice RR, Carrano AV. Sister-chromatid exchange: second report of the Gene-Tox Program. Mutat Res 1993; 297(2): 101-108.
37. Luzhna L, Kathiria P, Kovalchuk O. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Front Genet 2013; 4: 131.
38. Akyıl D, Konuk M. Detection of genotoxicity and mutagenicity of chlorothiophos using micronucleus, chromosome aberration, sister chromatid exchange, and Ames tests. Environ Toxicol 2015; 30(8): 937-945.