

# A phase II double-blinded study to evaluate the efficacy of EW02 in reducing chemotherapy-induced neutropenia in breast cancer

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**Abstract.** EW02, a polysaccharide-enriched crude extract from black soybean, has been shown to assist hematopoiesis in chemotherapy-treated animals. The present study aimed to clarify the safety, quality of life (QOL) and efficacy for myelopoiesis of EW02 administration in early breast cancer (EBC) patients receiving adjuvant chemotherapy. A total of 60 eligible EBC patients were enrolled in a randomized, double-blinded trial, 40 of whom were prescribed 700 mg oral EW02 three times daily for 15 days in chemotherapy cycle (C)2. The remainder were prescribed a placebo. All subjects took EW02 in C3 for 15 days. Blood samples were collected at different time-points for determining the blood cell count, and the serum level of granulocyte colony-stimulating factor (G-CSF) and interleukin (IL)-6. All patients tolerated EW02 well without severe side-effects. QOL evaluation showed that only the score of one questionnaire section (QLQ-C30) was significantly increased at C1 day (D)8 to C2D8 when the EW02 and placebo groups were compared ( $P=0.045$ ). No significant myelopoiesis recovery, and no incremental change in IL-6 and G-CSF levels were found in C2. Subgroup analysis showed a slightly lower decrease in absolute neutrophil count (ANC) in the EW02 patients who underwent Adriamycin + cyclophosphamide treatment compared with the placebo group. Although EW02 failed to show efficacy for myelopoiesis in the present study, EW02 was still well tolerated in EBC patients who underwent adjuvant chemotherapy.

## Introduction

EW02 is a polysaccharide-enriched crude extract from black soybean (BS). BS has been used extensively by the Chinese as food, traditional Chinese medicine or animal feed, for hundreds of years (1). References to BS can be found in traditional Chinese medicine pharmacopeias from 200 A.D. onwards (2,3).

Review of the available traditional Chinese medical literature at the China Academy of Chinese Medicine between 1988 and 2001 resulted in 100 references being found on BS in Chinese. These contained safety and efficacy information on over 5,000 Chinese patients, with over 4,000 of these patients receiving BS orally, either as monotherapy or in combination with other foods/herbs. Recent studies, including those by Liao *et al* (1) and Wu *et al* (4), have also described the ability of BS polysaccharides to increase white cell counts, stimulate cytokine production, enhance immunity and inhibit tumor growth.

*In vitro* assays using the enzyme-linked immunosorbent assay method have demonstrated the ability of polysaccharides from BS (PSBS) to stimulate the production of cytokines in U937 cells, and colony-forming unit assays have demonstrated the ability of EW02 to increase the number of hematopoietic progenitor cells (5). *In vivo* studies in mice showed that when administered after 5-FU, PSBS not only reduced the level of neutropenia compared with the control, but also reduced the time for the neutrophil levels to normalize. Bone marrow cells taken from the sacrificed PSBS-treated mice showed increased myeloid colony formation (1).

The aforementioned findings have been replicated in tumor-bearing (CT26 murine adenocarcinoma) and non-tumor-bearing 6-10-week-old BALB/c mice (weight,  $20\pm 5$  g) when PSBS was administered for 5 days following an intraperitoneal injection of 5-FU (6). A clear dose response was observed between the 100-mg/kg and 400-mg/kg oral doses. No deaths and no changes in body weights were observed during the study. Another two studies also used genistein, an isoflavone found in BS, to rescue irradiated mice, which subsequently showed hematopoietic recovery (7,8). The production of granulocyte

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colony-stimulating factor (G-CSF) and interleukin (IL)-6 was also enhanced.

In patients on myelosuppressive chemotherapy, the white blood cell (WBC) count nadir occurs between 11 and 19 days post-chemotherapy. Blood samples taken around this time-point, with or without EW02, may serve to provide preliminary information on the effect of EW02 on absolute neutrophil count (ANC) and circulatory cytokines (9). Under clinician supervision, observational results on 8 breast cancer (BC) patients for 12 days and 5 cervical cancer patients for 36 days have shown oral EW02 to be safe and well tolerated at the dose of 100 mg three times per day. Furthermore, preliminary results on 4 BC patients observed for 12 days and 3 patients for 36 days also showed this preparation to be safe and well-tolerated at the dose of 300 mg three times per day. None of the three patients with 36 days EW02 therapy experienced leucopenia (9). Doses of BS in previous studies ranged from <10 to 500 g, administered once daily, in 2 to 3 divided doses, or as part of regular meals (10). The dosage of 700 mg EW02 three times a day, which is equal to a daily dose of 300 g BS, is well tolerated in the clinical setting. Furthermore, neutropenia is associated with reduced myelopoietic proliferation (11). Therefore, we designed a randomized, double-blinded trial to assess the efficacy for myelopoiesis, the safety and the quality of life (QOL) conferred by EW02 administered orally in BC patients receiving chemotherapy.

## Materials and methods

**Study design.** The present study is a single-center, double-blinded, placebo-controlled crossover pilot study of oral EW02 in combination with chemotherapy versus placebo in combination with chemotherapy in BC patients (ClinicalTrials.gov identifier, NCT00555516). In total, 700 mg oral EW02 or placebo were administered three times per day at the beginning of cycle (C)2 for 15 consecutive days (Fig. 1). Sample size computation was based on the responder rate, defined as the percentage of subjects who did not develop neutropenia during one specific cycle (C1) of chemotherapy. With the assumption of a 51 and 17% responder rate in the EW02 and placebo groups, respectively, in order to reject the null hypothesis at a two-sided level of significance of 0.05, with a 2:1 randomization (EW02:placebo), a total sample size of 60 would allow the detection of a minimal difference with a power of 70%.

A total of 60 subjects were randomized 2:1 into two groups in permuted blocks of six, with four subjects assigned to group 1 and two subjects assigned to group 2. Group 1 received EW02 for 15 consecutive days during C2 and group 2 received 15 consecutive days of placebo during C2. C3 was designed as the extension phase to collect additional safety data. All patients received EW02 in C3 for 15 consecutive days.

The primary efficacy endpoint was the comparison of surrogate markers, ANC/WBC (expressed as the mean percentage change from baseline), between the two treatment groups during the C2 nadir. Patients underwent either one of the standard adjuvant regimens listed in the inclusion criteria. The nadir of ANC/WBC was defined on day (D)15 of each cycle and was compared with D1 to calculate the net percentage of change. The secondary efficacy endpoint was the intergroup comparison of the QOL survey collected in C2.

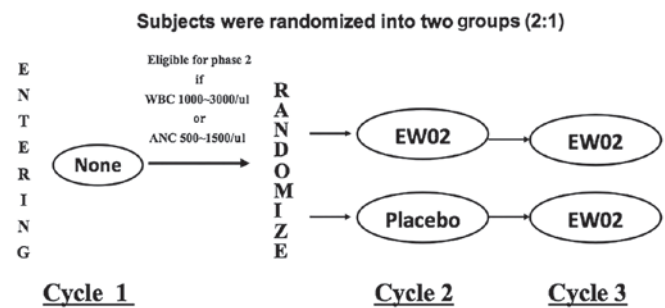


Figure 1. Treatment paradigm of EW02 trial. WBC, white blood cell; ANC, absolute neutrophil count.

The study was performed according to the guidelines of the Helsinki Declaration and was approved by the Institutional Review Board of the Tri-Service General Hospital (TSGHIRB; Taipei, Taiwan, R.O.C; TSGHIRB Approval Number, 094-01-0001-II). All patients provided written informed consent.

**Eligibility criteria.** Eligible patients were aged between 20 and 70 years, and had histological confirmed and completely excised invasive BC of stage I-IIIa, with neutropenia (WBC count of <3,000/mm<sup>3</sup> or ANC of <500/mm<sup>3</sup>) on D8 or D15 of C1 of chemotherapy. The performance status, which was defined by the Eastern Cooperative Oncology Group (ECOG) (12), was ≤2. Patients were treated with chemotherapy as clinically indicated, but were restricted to one of the following regimens: 60 mg/m<sup>2</sup> Adriamycin + 600 mg/m<sup>2</sup> cyclophosphamide (AC) every 3 weeks for 4 courses, or 50 mg/m<sup>2</sup> Adriamycin + 500 mg/m<sup>2</sup> cyclophosphamide + 500 mg/m<sup>2</sup> fluorouracil every 3 weeks for six courses (CAF).

Exclusion criteria included pregnancy or lactation, hypersensitivity to bean products, and any prior systemic therapy or radiotherapy for BC. Patients with an uncontrollable medical or psychiatric disease, a history of myocardial infarction or a secondary malignancy, those who were hepatitis B/C carriers, those with neutropenia (WBC count of <4,000/mm<sup>3</sup> or ANC of <2,000/mm<sup>3</sup> on C1D1) at the time of enrollment, or those who had been part of other investigational drug studies within the past 30 days were also excluded.

**Rescue with G-CSF and withdrawal from study.** A rescue treatment was provided if a patient suffered from febrile neutropenia and if the investigator believed that G-CSF plus empiric antibiotics or equivalent treatment would be necessary to relieve the situation. The criteria for G-CSF rescue were febrile neutropenia and an ANC of <500/mm<sup>3</sup> or a WBC count of <1,000/mm<sup>3</sup>, as defined by the National Health Insurance Bureau, Taiwan.

**Follow-up procedure.** Patients were evaluated at each visit, and blood was drawn for determination of WBC counts (ANC), and serum electrolyte, aspartate transaminase, alanine transaminase, blood urea nitrogen and serum creatinine levels on D1, D8 and D15 of C2-C4. QOL was evaluated at each cycle, using two questionnaires provided by the European Organization for the Research and Treatment of Cancer (EORTC) and the National Health Research Institutes (NHRI),

Table I. Patient demographics and biomarker evaluation.

Factor	EW02 (n=40)	Placebo (n=20)	P-value
Age	48.2±9.9	52.7±6.5	0.068 <sup>a</sup>
ER			
Positive	22	15	0.133 <sup>b</sup>
Negative	18	5	
HER			
Positive	12	4	0.409 <sup>b</sup>
Negative	28	16	
Stage			
I	18	8	0.302 <sup>b</sup>
II	18	7	
III	4	5	
Chemotherapy			
AC	30	12	0.232 <sup>b</sup>
CAF	10	8	

<sup>a</sup>Student' t-test; <sup>b</sup> $\chi^2$  test. AC, Adriamycin + cyclophosphamide; CAF, Adriamycin + cyclophosphamide + fluorouracil.

Taiwan, based on Chinese medicine theory, which included three sections: EORTC QLQ-C30, EORTC QLQ-BR23 and QOL-NCH04 (13).

**Biomarker tests.** The serum of each patient was collected at C1D1, and D15 of C2 and C3 for biomarker assay. Venous blood was drawn, allowed to clot at room temperature for 30-60 min, centrifuged at 1,500 x g for 10 min to collect the serum and then stored at 4°C for no more than 4 h. All sera were then stored at -80°C and only thawed to room temperature immediately prior to the biomarker assays. Serum IL-6 and G-CSF levels were determined using commercial immunoassay kits according to the manufacturer's instructions (RayBiotech Inc., Atlanta, GA, USA) and expressed in pg/ml [intra-assay coefficient of variability (CV), <10%; inter-assay CV, <12%].

**Statistical analyses.** Student's t-test and  $\chi^2$  tests were used to check the baseline characteristics of each group. An intent-to-treat analysis was used. A two-sided Wilcoxon rank-sum test was used for the comparison of percentage change of WBC/ANC from pre-dose (D1) to D15 (nadir) in the two groups. The primary efficacy endpoint was the comparison of surrogate markers, WBC/ANC, using analysis of covariance (expressed as mean percentage change from baseline), during the C2 nadir in the EW02 and placebo groups. The secondary efficacy endpoints include inter-group and individual comparisons of the QOL survey, and serum IL-6 and G-CSF levels.

## Results

**Patient characteristics.** The demographics of the placebo and experimental groups are shown in Table I. A total of 60 patients were enrolled, with 40 patients in the EW02 group

and 20 patients in the placebo group. There was no significant difference in any of the stratified variances.

**Efficacy evaluation.** The WBC count and ANC decreased on D15 after C1 in the two groups (Table II). The WBC count and ANC percentage changes between C2D1 and C2D15 decreased with no significant difference between them (data not shown). There was no significant difference in the increase in WBC count/ANC between the EW02 and control groups in C2 compared with C1 (Table II). Patients receiving AC treatment with EW02 presented with a slightly lower net decrease in ANC than the placebo group (Table III), however this was not statistically significant (P=0.052). Patients in the CAF treatment arm also presented with a lower, but not significant reduction in ANC (P=0.245). Biomarker study comparing IL-6 and G-CSF between each cycle showed no difference between the change in the EW02 group and the placebo group (Table II; Fig. 2).

**QOL.** The score for the QLQ-BR23 and NCH04 sections of the questionnaire showed no significant difference between the two groups in C2 (Table IV). The QLQ-C30 score increased at C2D8 compared with C1D8 (P=0.045), however, the remaining comparisons between different cycles were not significant (data not shown).

**Side-effects.** All patients underwent chemotherapy with no significant grade 3 or 4 side-effects in either group. Only mild asthenia, neutropenia, insomnia and gastrointestinal discomfort were recorded, as listed in Table V. No severe side-effects, including hypotension, were reported to be associated with EW02 in C2 and C3. No long-term side-effects were recorded during follow-up.

## Discussion

The present study showed that oral EW02 was a safe supplement and that it did not aggravate the side-effects of chemotherapy. However, EW02 did not provide a significant increase in WBC count or ANC during C2 when the WBC nadir occurs compared with the control group. Subgroup analysis showed a slightly lower decrease in absolute neutrophil count after chemotherapy with AC regimen in the EW02 group compared with the control group (Table III; P=0.052), although this result was not statistically significant. This trend was not evident in patients who received the CAF regimen, probably due to the small sample size (n=18). All of these findings support the notion that EW02 may partially assist myelopoiesis in EBC patients who undergo adjuvant chemotherapy with the AC or CAF regimens. Further studies with a larger sample size should be performed to validate it.

Biomarker studies showed that the IL-6 and G-CSF levels were not changed between the two groups during C2. Chen *et al* (14) showed that EW02 could increase the serum G-CSF level in cancer patients treated with cisplatin-containing regimens. Possible reasons for this discrepancy include the different target population, the different dosage of EW02 and the different chemotherapeutic agents used. The IL-6 level may have decreased in the patients who underwent chemotherapy while inflammation was inhibited (15). As a result,

Table II. Blood cell count and biomarkers during treatment.

Factor	EW02 (n=40)	Placebo (n=20)	P-value
C1D1			
WBC	6200±1490	6360±1760	0.783
ANC	3860±1320	3700±1430	0.836
IL-6	0.53±8.32	0.43±10.28	0.872
G-CSF	2.24±71.95	3.41±541.9	0.421
C1D15			
WBC	1860±520	1760±950	0.358
ANC	410±270	330±340	0.806
IL-6	4.24±15.22	5.17±20.22	0.550
G-CSF	3.17±57.88	3.35±448.69	0.524
C2D15			
WBC	2140±620	2560±1100	0.424
ANC	694.2±386.8	706.3±665.5	0.932
IL-6	2.60±6.00	2.88±5.76	0.521
G-CSF	2.39±58.23	3.12±370.2	0.541

WBC, white blood cell; ANC, absolute neutrophil count; IL-6, interleukin 6; G-CSF, granulocyte colony-stimulating factor.

Table III. Subgroup analysis of net absolute neutrophil count change between C2D1 and C2D15 in different chemotherapy regimens.

Regimen	n	Δ mean	95% CI	P-value
AC				
EW02	27	-2849	-2980 to -2676	0.052
Placebo	11	-3057	-3346 to -2870	
CAF				
EW02	10	-2730	-3418 to -2780	0.245
Placebo	8	-3284	-3181 to -2465	

AC, Adriamycin + cyclophosphamide; C, cycle; D, day; CI, confidence interval.

Table IV. Quality of life score difference between EW02 and placebo group in C2.

Questionnaire section	C2D1 vs. C1D1		C2D8 vs. C1D8		C2D15 vs. C1D15	
	Difference	P-value	Difference	P-value	Difference	P-value
EORTC QLQ-BR23	3.097	0.087	0.965	0.749	1.295	0.523
EORTC QLQ-C30	1.264	0.644	7.515	0.045	-1.172	0.745
NCH04	-0.807	0.674	0.502	0.867	2.685	0.344

C, cycle; D, day; EORTC, European Organization for the Research and Treatment of Cancer.

EW02 may not have been able to stimulate an increase in IL-6 in the present patients. Although EW02 was found to increase IL-6 in a mouse model (1), the present study failed to show the same results in humans.

Patients in the present study were prescribed 700 mg EW02 three times a day, which is equals to a daily dose of 300 g BS,

which is equivalent to that used in the literature (10). Previous studies had reported that BS increased the neutrophil count and induced differentiation in the U937 leukemic cell line (5). Two study groups also showed that BS could assist in the myeloproliferation of irradiated rats (8,16). Administering BS in rats with or without condition medium can assist in reticulocyte count

Table V. Side-effects.

Symptom	EW02 (n=40)	Placebo (n=20)	P-value <sup>a</sup>
Asthenia	16	9	0.711
Digestive system			
Anorexia	16	7	0.707
Nausea	19	13	0.200
Vomiting	18	7	0.459
Hematological system			
Leucopenia	7	5	0.494
Neutropenia	8	6	0.388
Cardiovascular system	1	0	0.476
Insomnia	6	3	1.000
Skin	8	2	0.327

<sup>a</sup> $\chi^2$  test.

proliferation (1,17). BS was also found to promote myelopoiesis in mouse splenocytes and bone marrow cells in a previous study. Granulocyte/monocyte colony formation increased in culture with BS plus splenocyte-conditioned medium, but not when BS was used alone (1). It was concluded that BS indirectly assisted hematopoiesis through increasing growth factors via T lymphocytes, macrophages, fibroblasts and endothelial cells. The dosage of EW02 in the present study was adequate, but did not assist myelopoiesis in C2. Although previous studies showed successfully increasing myelopoiesis in cell lines and rat models, the present study did not find any increase in neutrophil count. We postulated that the primary endpoint of this study should be able to be met with a longer duration of usage and a larger study cohort in the future. Another possible reason to explain why no effects on myelopoiesis were observed in C2 is perhaps due to the fact that no co-stimulating agents were used. Prolonged EW02 usage may be required to achieve a stimulating effect to generate more hematopoietic growth factors. We hypothesize that adding EW02 along with another growth factor may further enhance the myeloproliferative effect of these factors.

There were no observed adverse side-effects attributable to EW02 in the present patient cohort. Although soybean has been reported to cause hypotension in healthy menopausal women, this was not found in the present study (18). Other side-effects, including anorexia, nausea and insomnia, were experienced equally by the two groups, and were attributed to the chemotherapy. EW02 only transiently ameliorated the symptoms and discomforts of the patients in C2D8; however, the QOL was equivalent for the majority of the time in the two groups. Systematic meta-analysis had shown that soy products ameliorate menopausal symptoms in post-menopausal women (19). However, BS does not reduce the hot flash symptoms in BC patients, which may relate to the short duration of usage or the placebo effect (20,21).

In conclusion, in the present study, EW02 was found to be a relatively safe alternative supplement for BC patients who underwent adjuvant chemotherapy. However, it did not meet the primary endpoint successfully, with only a slightly lower

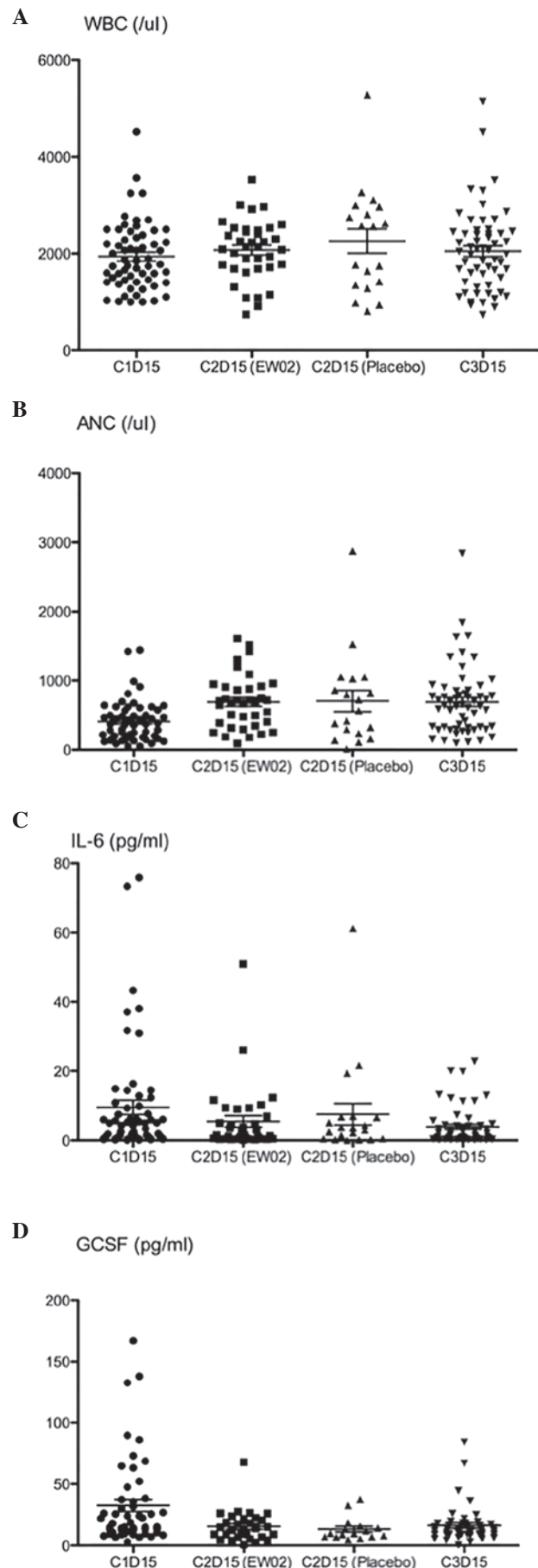


Figure 2. Distribution of the biomarkers of (A) white blood cell (WBC) count, (B) absolute neutrophil count (ANC), (C) interleukin (IL)-6 and (D) granulocyte colony-stimulating factor (G-CSF) on day (D)15 of different cycles (C).

net decrease in ANC percentage in the subgroup analysis. The present study population was small and the duration of EW02 usage was short. Prolonged EW02 usage and a larger study cohort may represent a potential strategy for future clinical trial design.

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### References

- Liao HF, Chen YJ and Yang YC: A novel polysaccharide of black soybean promotes myelopoiesis and reconstitutes bone marrow after 5-fluorouracil- and irradiation-induced myelosuppression. *Life Sci* 77: 400-413, 2005.
- Yang SZ (ed): *The Divine Farmer's Materia Medica*, 200 A.D. Blue Poppy Press, Boulder, CO, 1998.
- Li S (ed): *Compendium of Materia Medica (Bencao Gangmu)*. Foreign Languages Press, Beijing, 2004.
- Wu MH, Lee YC, Tsai WJ, Yang WB, Chen YC, Chuang KA, Liao JF, Wang CC and Kuo YC: Characterized polysaccharides from black soybean induce granulocyte colony-stimulated factor gene expression in a phosphoinositide 3-kinase-dependent manner. *Immunol Invest* 40: 39-61, 2011.
- Liao HF, Chou CJ, Wu SH, Khoo KH, Chen CF and Wang SY: Isolation and characterization of an active compound from black soybean [*Glycine max* (L.) Merr.] and its effect on proliferation and differentiation of human leukemic U937 cells. *Anticancer Drugs* 12: 841-846, 2001.
- Inagaki S, Morimura S, Gondo K, Tang Y, Akutagawa H and Kida K: Isolation of tryptophol as an apoptosis-inducing component of vinegar produced from boiled extract of black soybean in human monoblastic leukemia U937 cells. *Biosci Biotechnol Biochem* 71: 371-379, 2007.
- Singh VK, Grace MB, Parekh VI, Whitnall MH and Landauer MR: Effects of genistein administration on cytokine induction in whole-body gamma irradiated mice. *Int Immunopharmacol* 9: 1401-1410, 2009.
- Zhou Y and Mi MT: Genistein stimulates hematopoiesis and increases survival in irradiated mice. *J Radiat Res* 46: 425-433, 2005.
- Martin M, Villar A, Sole-Calvo A, Gonzalez R, Massuti B, Lizon J, Camps C, Carrato A, Casado A, Candel MT, *et al*: GEICAM Group (Spanish Breast Cancer Research Group), Spain: Doxorubicin in combination with fluorouracil and cyclophosphamide (i.v. FAC regimen, day 1, 21) versus methotrexate in combination with fluorouracil and cyclophosphamide (i.v. CMF regimen, day 1, 21) as adjuvant chemotherapy for operable breast cancer: A study by the GEICAM group. *Ann Oncol* 14: 833-842, 2003.
- Liu YC: Clinical experiences on therapeutic usage of black soybean. *J He Bai TCM* 1: 40, 1984.
- Wriedt K, Kauder E and Mauer AM: Defective myelopoiesis in congenital neutropenia. *N Engl J Med* 283: 1072-1077, 1970.
- Sørensen JB, Klee M, Palshof T and Hansen HH: Performance status assessment in cancer patients. An inter-observer variability study. *Br J Cancer* 67: 773-775, 1993.
- Chie WC, Chang KJ, Huang CS and Kuo WH: Quality of life of breast cancer patients in Taiwan: Validation of the Taiwan Chinese version of the EORTC QLQ-C30 and EORTC QLQ-BR23. *Psychooncology* 12: 729-735, 2003.
- Chen YM, Whang-Peng J, Liu JM, Kuo BI, Wang SY, Tsai CM and Perng RP: Serum cytokine level fluctuations in chemotherapy-induced myelosuppression. *Jpn J Clin Oncol* 26: 18-23, 1996.
- Jiang XP, Yang DC, Elliott RL and Head JF: Reduction in serum IL-6 after vaccination of breast cancer patients with tumour-associated antigens is related to estrogen receptor status. *Cytokine* 12: 458-465, 2000.
- Shinomiya K, Tokunaga S, Shigemoto Y and Kamei C: Effect of seed coat extract from black soybeans on radial maze performance in rats. *Clin Exp Pharmacol Physiol* 32: 757-760, 2005.
- Hagiwara A, Imai N, Numano T, Nakashima H, Tamano S, Sakaue K, Tanaka K, Yasuhara K and Hayashi SM: A twenty eight-day repeated dose toxicity study of black soybean extract in Sprague-Dawley rats. *J Toxicol Sci* 35: 87-96, 2010.
- Bloedon LT, Jeffcoat AR, Lopaczynski W, Schell MJ, Black TM, Dix KJ, Thomas BF, Albright C, Busby MG, Crowell JA, *et al*: Safety and pharmacokinetics of purified soy isoflavones: Single-dose administration to postmenopausal women. *Am J Clin Nutr* 76: 1126-1137, 2002.
- Shams T, Setia MS, Hemmings R, McCusker J, Sewitch M and Ciampi A: Efficacy of black cohosh-containing preparations on menopausal symptoms: A meta-analysis. *Altern Ther Health Med* 16: 36-44, 2010.
- Quella SK, Loprinzi CL, Barton DL, Knost JA, Sloan JA, LaVasseur BI, Swan D, Krupp KR, Miller KD and Novotny PJ: Evaluation of soy phytoestrogens for the treatment of hot flashes in breast cancer survivors: A North Central Cancer Treatment Group Trial. *J Clin Oncol* 18: 1068-1074, 2000.
- Van Patten CL, Olivotto IA, Chambers GK, Gelmon KA, Hislop TG, Templeton E, Wattie A and Prior JC: Effect of soy phytoestrogens on hot flashes in postmenopausal women with breast cancer: A randomized, controlled clinical trial. *J Clin Oncol* 20: 1449-1455, 2002.