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# APPENDIX

# LIST OF PUBLICATIONS

1. Kijkuokool, P., Parhar, I.S. and Malaivijitnond, S. Genistein enhances N-nitrosomethylurea (NMU) - induced tumorigenesis in adult female rats. In proceedings of the fifth congress of AOSCE in conjunction with the annual meeting of JSCE on March 26-30, 2004 at Nara, Japan.
2. Kijkuokool, P., Parhar, I.S. and Malaivijitnond, S. The mechanisms of genistein and tamoxifen on the mammary tumor growth in adult female rats. In proceedings of RGJ-Ph.D. congress VI on April 28-30, 2005 at Chonburi, Thailand.
3. Kijkuokool, P., Parhar, I.S. and Malaivijitnond, S. Genistein enhances N-nitrosomethylurea-induced rat mammary tunorigenesis. Cancer Lett., 2005 (in press).

# GENISTEIN ENHANCES N-NITROSOMETHYLUREA (NMU)-INDUCED TUMORIGENESIS IN ADULT FEMALE RATS

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## Summary

We compared the effects of daily subcutaneous injections of 1mg/kg BW of genistein and vehicle (2% DMSO in peanut oil) for 20 weeks on NMU-induced tumorigenesis in adult female rats. Genistein significantly increased tumor cross-sectional area and tumor multiplicity when compared with the vehicle-treated rats, but not the tumor incidence and latency period. There were no significant differences in body weights, food consumption and weights of livers, uteri and ovaries. Our data shows that exposure to low dose of genistein stimulates NMU-induced tumorigenesis in adult female rats, possibly through estrogenic activity directly at the mammary glands.

## Introduction

Genistein, a phytoestrogen, is of great interest for its implications as an anticancer compound. It has been reported to have weak estrogenic and anti-estrogenic properties, to be an antioxidant and to inhibit the protein tyrosine kinase, topoisomerase II and angiogenesis. The effects of genistein on mammary tumor development are still controversial. Whereas some reports show prevention others show development of breast cancer when exposured to genistein[1, 2, 3]. The purpose of the present study was to determine whether supplement of genistein at the dosage comparable to ordinary human consumption affects NMU-induced tumorigenesis in adult female rats.

## Materials and Methods

Female Spraque-Dawley DC rats, 45-day old, were used. All rats received a single dose of 40 mg/kg BW of freshly prepared NMU, a carcinogen, via tailed vein on day-1 of the study period. The rats were subcutaneously injected daily with either genistein (1 mg/kg BW; n = 20) or vehicle (2% DMSO in 0.1 ml peanut oil; n = 20) for 20 weeks.

Body weight and diet intake of rats were measured weekly. The rats were palpated weekly for mammary tumors until the end of treatment period or until the tumor size reached 3.5 cm in diameter, the examination was

terminated. The tumor tissues, livers, uteri and ovaries were dissected and weighted. Tumor diameters were measured by digital vernia calipers and tumor cross-sectional area was determined according to a formula Length/2 x Width/2 x $\pi$($mm^2$). The data of a) latency of tumor development, b) the percentage of rats with tumors (tumor incidence), c)the number of tumors per rat (tumor multiplicity) and d) tumor cross-sectional area were analyzed. All rats were also examined for metastases.

## Results

NMU treatment with or without genistein supplement had no significant effect on body weights, food consumption and organ weights. At the end of the experiment, all rats in both groups (100%) developed tumors. Tumors appeared mostly at cervical and thoracic regions. No significant difference in the duration to tumor development was observed between the two groups. The total number and the mean tumor weight tended to be higher in the genistein-treated group than the vehicle-treated groups ($p = 0.106$). Female rats treated with genistein developed significantly greater tumor cross-sectional area and tumor multiplicity than vehicle-treated rats ($p < 0.05$) (Fig. 1). One uterine metastases was found in the vehicle-treated groups, whereas four rats of genistein-treated group showed metastases in the uterus (2 rats), spleen (1 rat) and uterus and liver (1 rat).

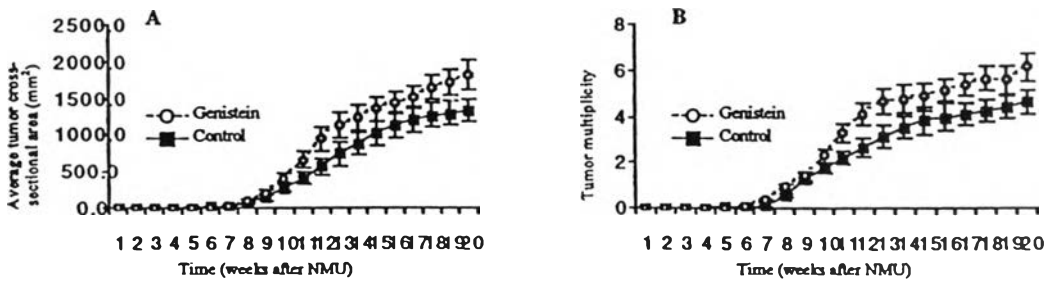


Figure 1. Effects of genistein on average tumor cross-sectional area (A) and tumor multiplicity (B) in adult female rat.

## Discussion

These results demonstrate that supplementation of 1mg/kg BW of genistein can stimulate NMU-induced tumorigenesis in adult female rats by increasing tumor multiplicity and tumor cross-sectional area. In contrast, Hilakivi-Clarke[1] demonstrated that genistein given at the same dosage of 1mg/kg BW protected the mammary gland from DMBA-induced malignant transformation in rats. However, in their study the animals were prepubertal. Thus, it is possible that the chemoprotective action of genistein against breast cancer depends on the timing of exposure, that is, an exposure to genistein during adulthood may increase

the risk of breast cancer. The estrogenic activity of genistein is well documented. *In vitro* studies show an increase in the levels of the estrogen responsive gene pS2 and c-fos when cells are treated with genistein[4]. *In vivo* use of dietary soy, which contains genistein, increases cell proliferation in human breast tissue and pS2 expression in premenopausal women[5]. The present data shows that genistein at the dosage of 1mg/kg BW cannot antagonize the action of endogenous estrogen, but provide the additional estrogenic source to stimulate NMU-induced mammary tumorigenesis in adult female rats.

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# The Mechanism of Genistein and Tamoxifen on the Mammary Tumor Growth in Adult Female Rats

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**Objective**

To determine the mechanism of action of genistein and tamoxifen on mammary tumor growth in adult female rats.

**Methods**

The 45-day old female Sprague-Dawley rats were induced to develop the mammary tumor by a single injection of 40 mg/kg BW of freshly prepared NMU, a carcinogen, via tailed vein. When the tumor developed and reached a diameter of 1 cm, rats were randomized into four treatment groups (10 rats/group): vehicle, tamoxifen, genistein and genistein+tamoxifen in which were subcutaneously injected daily with vehicle (2% DMSO in 0.1 ml peanut oil), genistein (1 mg/kg BW), tamoxifen (100 $\mu$g ) and genistein (1 mg/kg BW) and tamoxifen (100 $\mu$g) for 10 weeks, respectively. Tumor cross-sectional area were measured weekly until the end of treatment period or until the tumor size reached 3.5 cm in diameter and the rats were killed under ether anesthesia thereafter. Five rats were randomly selected from ten rats in each group and assessed the expressions of ER related genes (ER$\alpha$, ER$\beta$ and pS2), growth factor related genes (IGF-1 and *neu*) and metastasis suppressor gene (GPR54) using RT-PCR technique.

**Results**

The average cross - sectional area of mammary tumor tended to be larger in genistein group when compared to that of the vehicle group at the end of treatment period (1,081 $\pm$ 116 and 776 $\pm$ 227 $mm^2$)(p=0.15). Tumors in tamoxifen alone or in combination with genistein were significantly smaller than those of vehicle and genistein groups. Genistein treatment increased the percentage of tumor metastases to liver, uterus and ovary. Tamoxifen treatment suppressed the expression of ER$\alpha$ gene. Genistein treatment increased IGF-1 mRNA levels and decreased the expression of GPR54 gene. The combination of tamoxifen and genistein treatment decreased ER$\alpha$ and pS2 expressions, but increased IGF-1 expression. There were no significant differences in the levels of *neu* mRNA among these four treatment groups.

**Conclusion**

Tamoxifen alone or in combination with genistein inhibited the mammary tumor growth by downregulaing ER$\alpha$ expression. Genistein induced IGF-1 expression and stimulated the mammary tumor growth, possibly via this pathway. The stimulation of tumor growth was, however, obscured when genistein was treated to the rats in combination with tamoxifen. The decrease in expression of GPR54 in genistein treated tumors may play a role in the metastatic potential of cancer cells to other organs. This study raises concern about the consumption of genistein supplements in premenopausal women with E-dependent breast cancer.

**Keywords:** genistein, tamoxifen, mammary tumor, adult female rat

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# Genistein enhances *N*-nitrosomethylurea-induced rat mammary tumorigenesis

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**Abstract**

Genistein is of great interest for its implications as an anticancer compound. We compared the effects of daily subcutaneous injections of 1 mg/kg BW of genistein and vehicle (2% DMSO in peanut oil) for 20 weeks on *N*-nitroso-*N*-methylurea (NMU)-induced tumorigenesis in adult female rats. Genistein significantly increased tumor cross-sectional area and tumor multiplicity but not the tumor incidence and latency period when compared with the vehicle treated group. The serum $E_2$ levels of genistein treated group were significantly higher than those of the vehicle treated group at 1 and 2 months after treatment which is the time when most of the rats developed tumors. There were no significant differences in the length of the estrous cycle, food consumption and weights of body, livers, uteri and ovaries between the two groups. Our data shows that supplementation of genistein at a dosage comparable to the isoflavone consumption in humans did not affect the reproductive system but resulted in enhancement of NMU-induced tumorigenesis in adult female rats. Thus, the supplementation of soy isoflavone in premenopausal women may potentially potentiate the risk of breast cancer.
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*Keywords:* Genistein; NMU; Mammary tumor; Phytoestrogen; Rats

## 1. Introduction

Breast cancer is the second leading cause of cancer related death among women [1]. Apparently, women consuming diet high in soy products, containing large amounts of phytoestrogens especially genistein, have a low incidence of breast cancer [2,3]. In contrast, supplementation of soy for 2 weeks in premenopausal women increased progesterone receptor expression and the proliferation rate of breast lobular epithelium [4].

Genistein (major isoflavone in soya) has been reported to have weak estrogenic and anti-estrogenic properties [5,6], to be an antioxidant [7] and to inhibit the protein tyrosine kinase and topoisomerase II activity [8,9] and also angiogenesis [10]. It is interesting that animals treated neonatally and prepubertally with genistein have reduced incidence and multiplicity of 7,12-dimethylbenzanthracene (DMBA)-induced mammary adenocarcinomas, decreased numbers of terminal end buds but increased numbers of lobular structures [11–13]. On the contrary, in adult animals, the effects of soy isoflavones on mammary tumor development remains controversial. For example, in adult mice, genistein increases mammary tumorigenesis [14] but in adult rats exposure to soy protein either prevented [15] or did not inhibit

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chemically induced mammary tumors [16,17]. Therefore, the present study was designed to determine the effects of genistein on *N*-nitroso-*N*-methylurea (NMU)-induced tumorigenesis in adult female rats.

## 2. Materials and methods

### *2.1. Animals*

Forty female Sprague–Dawley rats, 30 days of age, and 120–140 g body weight (BW) were obtained from the National Laboratory Animal Center, Thailand. They were housed (5 animals per cage) in a room with controlled lighting (lights on 06.00–20.00 h) and temperature (25 ± 1 °C) at the Primate Research Unit, Chulalongkorn University, Thailand. The animals were fed with rat chow diet (lot no. 070, Pokaphan Animal Feed Co, Ltd, Thailand). Diet and water were supplied ad libitum. The rats were acclimated for 2 weeks before the start of the study. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guidelines for the care and use of laboratory animals prepared by Chulalongkorn University.

### *2.2. The preparation of NMU and genistein solutions*

The *N*-nitroso-*N*-methylurea (NMU) (Sigma, St Louis, MO) solution was dissolved in a few drops of 3% acetic acid and diluted with distilled water to give a stock concentration of 20 mg/ml. Within 2 h after preparation, the NMU solution (40 mg/kg of rat) was administered to each rat [18]. The NMU-induced rat mammary tumor was used as a model because mammary tumors induced by NMU are hormone dependent and closely resemble human breast cancer [19].

Genistein (98% of purity, lot no.049H0521, Sigma, St Louis, MO) was dissolved in 2% dimethyl sulfoxide (DMSO), then mixed with peanut oil to give a stock concentration of 3 mg/ml, and kept in the dark bottle at 4 °C. Genistein stock solution was adjusted with 2% DMSO in peanut oil to a concentration of 1 mg/kg BW/0.1 ml before administration to rats.

### *2.3. Experimental procedure*

To induce mammary tumors, 45-days old rats were injected a single dose of 40 mg/kg BW of freshly prepared NMU via tailed vein at the start of the study. They were then randomized into two groups (20 rats/group) and given a daily subcutaneous injection of 1 mg/kg BW of genistein (test group) or 2% dimethyl sulfoxide (DMSO) in 0.1 ml peanut oil (vehicle group) between 0930–1000 h, for 20 weeks. The body weight and diet intake of rats were measured on a weekly basis. The rats were euthanized at the end of the study (20 weeks) or when the tumor size reached 3.5 cm in diameter.

Each week, the rats were palpated at the breast and the abdomen to detect tumors. The date of first appearance of each tumor, the tumor location and size were recorded for each animal. The tumor sizes were measured once a week by recording the tumor diameters with a digital vernia calipers (Starrett, England); determining the length of the longest axis and the width perpendicular to the longest axis [20]. The latency of tumor appearance, tumor incidence, tumor multiplicity and the tumor cross-sectional area in each treatment group were recorded on a weekly basis.

At the end of the study (20 weeks), rats in diestrous phase were euthanized. The tumor tissues, livers, uteri and ovaries were dissected and weighed. The tumor tissues were fixed in 10% neutral buffered formalin. All rats were also examined for metastases of the tumor to the other organs, e.g. thoracic and abdominal cavities. Tissues for microscopic examination of histopathology were trimmed, embedded in paraffin, cut into sections of 5 μm thickness, and stained with hematoxylin and eosin. Forty specimens of tumor tissues were randomly selected for histopathological study from each treatment group. The histopathological criteria for the identification of mammary tumor type and subtype were as described by Russo et al [21].

### *2.4. Vaginal smears*

The estrous cycle was monitored daily by vaginal cytology assay performed at 0800–0900 h. A glass-rod was inserted into the vagina and gently touched against the vaginal wall. The vaginal cells were then smeared onto a drop of 0.9% normal saline placed on the glass slide. The smears were observed under a light microscope (Olympus, Tokyo, Japan). The criteria used to determine the phase of the estrous cycle have been described previously [22].

### *2.5. Blood collection and serum estradiol determination*

Each month, during the diestrous phase, rats were anesthetized using ether and 1-ml of blood was sampled by cardiac puncture between 0900–0930 h. Blood sera were separated immediately by centrifugation

(2000 rpm) for 20 min at 4 °C. Sera were then aliquoted and stored at -20 °C until estradiol ($E_2$) assay. Serum $E_2$ concentration was analysed by a double-antibody RIA system using $^{125}$I-Labeled radioligands (Diagnostic Systems Laboratories, Inc, Texas, USA). To minimize the inter-assay variation, all samples in each group were run in the same assay. The intra-assay coefficients of variations were 8.9, 6.8 and 8.9%, respectively, for the high, medium and low $E_2$ concentration.

### 2.6. Statistical analysis

Statistical analysis of tumor incidence (number of rats with tumors per total number of rats $\times 100$) was performed by chi-square analysis. The median tumor latency period, tumor multiplicity (number of tumors per rat), tumor weight, tumor cross-sectional area (tumor length/2width/2$\pi$ (mm$^2$)) [15], body and organ weights and food intake between groups were determined by unpaired $t$-test using SPSS, a statistical analysis program. Data were expressed as mean $\pm$ SEM. The statistical significance was considered at $P<0.05$.

## 3. Results

### 3.1. Diet intake, body and organ weights

The average diet intake of rats treated with genistein or vehicle did not differ throughout the study (approximately 13–15 g/rat/day). The body, liver, uterine and the ovarian weights were not significantly different between genistein and vehicle treated groups (Table 1).

### 3.2. Effect of genistein treatment on mammary tumorigenesis

The first palpable tumors appeared at 34 days in vehicle group and 42 days in genistein group. The median latencies were 59.5 and 51.0 days in vehicle and genistein treated groups, respectively. All rats in the study developed tumors and there was no significant difference in the duration of tumor development between the two treatment groups. The mean tumor weight in the genistein group tended to be higher than that of vehicle group ($P=0.106$) (Table 2). Female rats treated with genistein developed significantly greater tumor cross-sectional area than those of vehicle group at 11, 12, 13 and 20 week (Fig. 1(A)). At the end of the study, the average tumor cross-sectional areas were $1343.59 \pm 150.82$ mm$^2$ and $1811.85 \pm 203.68$ mm$^2$ for vehicle and genistein groups, respectively (Table 2). A significantly high tumor multiplicity was observed in the genistein group at 12, 13, 17, 18 and 20 week of the study period (Fig. 1(B)). Tumor multiplicities at the end of the study were $4.65 \pm 0.49$ and $6.21 \pm 0.75$ tumors/rat for vehicle and genistein treated groups, respectively (Table 2). Five percent of rats (1 of 20 rats) in the vehicle group showed metastases, only in the uterus, whereas 21% of rats (4 of 19 rats) in the genistein group showed metastases, in the uterus (3 rats), spleen (1 rat) and liver (1 rat). One rat in the genistein group showed metastases both in the uterus and liver. Histopathological examination revealed that all of 40 mammary tumors, which were randomly selected in the genistein treated group, were adenocarcinoma type (100%). In the vehicle treated group, 38 of 40 tumors were adenocarcinoma type and the other two tumors were mammary fibroadenoma and tubular adenoma type.

### 3.3. Effect of genistein treatment on the estrous cycle

Vaginal smears taken during the first few months of the study period showed that all rats had regular estrous cycles of 4 to 5 days; the first 3 months for the vehicle group and the first 2 months for the genistein group. Thereafter, the length of the estrous cycle increased significantly for both groups (vehicle: $6.25 \pm 2.6$ days; genistein: $6.82 \pm 2.7$ days, mean $\pm$ SD). There was no significant difference in the length of the estrous cycle between the two treatment groups throughout the study period.

Table 1
Body and organ weights of vehicle and genistein treated adult female rats

| Treatment | Body weight (g) | | Liver weight (g) | Uterine weight (mg) | Ovarian weight (mg) |
|---|---|---|---|---|---|
| | 1 week | 20 week | | | |
| Vehicle ($n=20$) | $169.5 \pm 1.7$ | $292.2 \pm 4.8$ | $9.2 \pm 0.6$ | $459.2 \pm 30.1$ | $129.4 \pm 6.6$ |
| Genistein ($n=19$) | $167.6 \pm 2.1$ | $292.0 \pm 4.5$ | $9.6 \pm 0.3$ | $495.4 \pm 37.5$ | $124.1 \pm 4.7$ |

Table 2
Tumor parameters after vehicle and genistein treatment for 20 weeks of adult female rats

| Treatment | Tumor bearing rat | Tumor incidence (%) | Median latency (days) | Tumor multiplicity (tumors) | Tumor weight (g) | Tumor cross-sectional area ($mm^2$) | Percentage of rat showing metastases |
|---|---|---|---|---|---|---|---|
| Vehicle ($n=20$) | 20 | 100 | 59.5 | 4.65±0.49 | 14.8±2.0 | 1343.59± 150.82 | 5 |
| Genistein ($n=19$) | 19 | 100 | 51.0 | 6.21±0.75* | 18.9±2.5 | 1811.85± 203.68* | 21 |

*, Significantly different from vehicle group ($P<0.05$).

### 3.4. Effect of genistein treatment on serum estradiol concentration

The serum $E_2$ concentrations at the start of the experiments were not significantly different between vehicle (33.9±1.9 pg/ml) and genistein treated group (33.5±1.6 pg/ml, Fig. 2). Serum $E_2$ levels in genistein and vehicle treated groups decreased significantly during the first month and then recovered to the basal level and remained constant throughout the

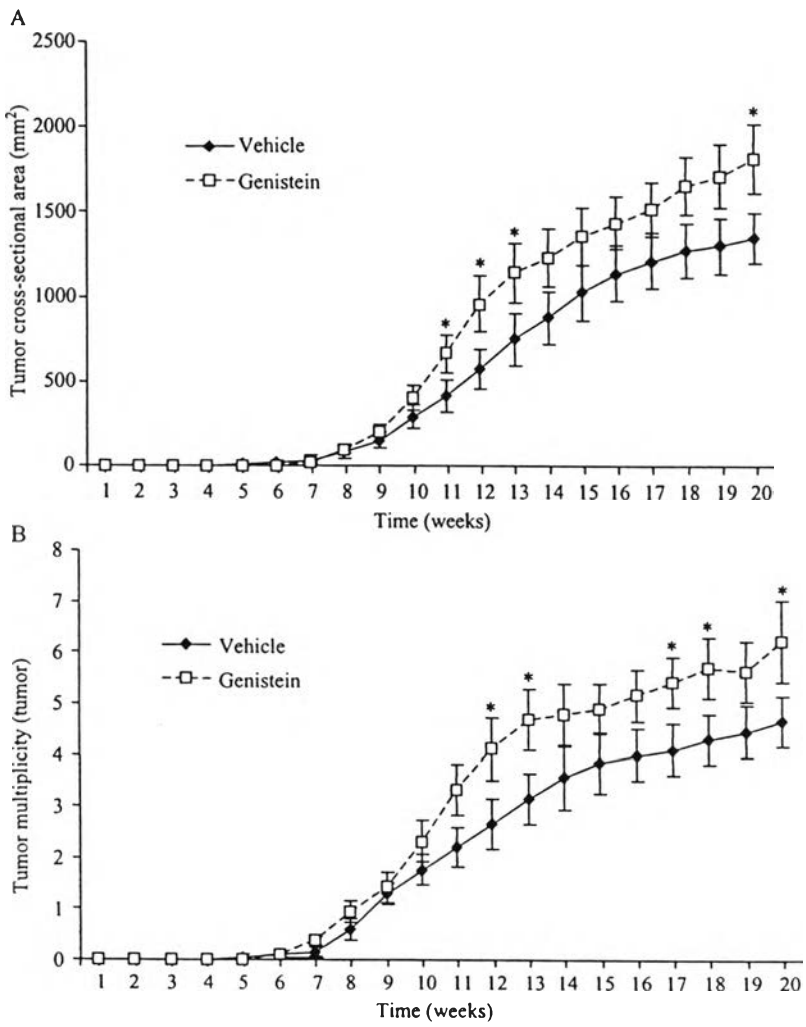


Fig. 1. Tumor cross-sectional area (A) and multiplicity (B) of adult female rats treated with 2% DMSO in peanut oil (vehicle) or genistein. *, Significantly different from vehicle group ($P<0.05$).

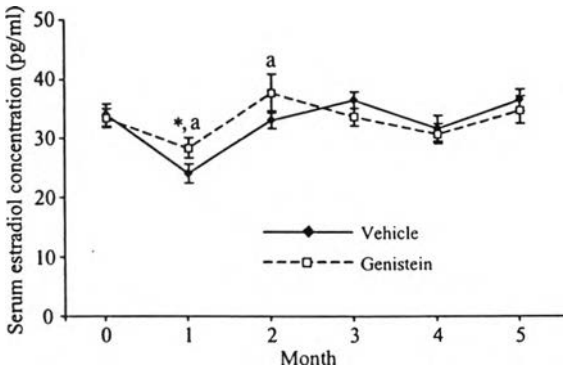


Fig. 2. Changes of serum estradiol levels in adult female rats treated with genistein or 2% DMSO in peanut oil (vehicle). a, Significantly different from vehicle group ($P < 0.05$); *, significantly different from 0 month ($P < 0.05$).

experimental period. Serum $E_2$ levels in rats treated with genistein were significant higher than those of vehicle treated group during the first 2 months of treatment.

## 4. Discussion

In the present study, supplement of genistein stimulated NMU-induced tumorigenesis in adult female rats by increasing tumor multiplicity and tumor cross-sectional area. In contrast, Hilakivi-Clarke et al. [13] demonstrated that genistein given at the same dosage (1 mg/kg BW in rats) protected the mammary glands from 7,12-dimethylbenzanthracene (DMBA)-induced malignant transformation. One explanation for these contrasting results is the reproductive stage of the rats used in both studies. While we used adult female rats, Hilakivi-Clarke et al. [13] used rats of prepubertal stage. Indeed, it has been reported that neonatal and prepubertal treatment of genistein suppressed the development of DMBA-induced mammary adenocarcinomas in rats [11,12] and provides a protective effect against DMBA-induced mammary cancer. Exposure to phytoestrogen before puberty could altered the ontogeny of the mammary glands and cause precocious maturation of breast terminal end buds to more differentiated lobules and subsequent breast cancer protection [23,24]. In contrast, increase of post-pubertal exposure to phytoestrogen may potentially induce breast cancer risks and render adult animals to be more susceptible to chemically-induced mammary cancer. Similarly, exposure to estrogen during adulthood increases mammary cancer risk in animals [25]. It has been demonstrated that genistein stimulates MCF-7 cell growth in vitro, in the presence of estradiol [9,26]. Day et al. [14] found that genistein (1 g/kg diet) fed during adulthood increased the number of DMBA-induced malignant tumors in the mice. Furthermore, the use of dietary soy, which contains genistein, increases cell proliferation in human breast tissue, progesterone receptor and pS2 expressions, indicative of an estrogenic stimulus in premenopausal women [4,27]. Thus, our data support that genistein does not antagonize the action of endogenous estrogen, instead it provides additional estrogenic source to stimulate NMU-induced mammary tumorigenesis in adult female rats.

We found that serum $E_2$ levels in both genistein and vehicle groups decreased at 1 month when compared to 0 month or the 1st day of study period. The reduction of $E_2$ levels could not have been caused by the genistein treatment because it was found in both vehicle and genistein groups. However, it may be due to stress during the tumor development. We found that most rats developed tumors during the first 2 months of NMU injection. The median latency periods were 59.5 and 51.0 days in vehicle and genistein groups, respectively. A relation between stress and reproductive dysfunction is well established [28,29]. Stress increases the hypothalamic-pituitary-adrenal (HPA) axis activity and concomitantly reduces the hypothalamic-pituitary-gonadal (HPG) axis activity. Briski et al. [30] showed that physical stress induced an increase in plasma corticosterone but decreased plasma luteinizing hormone in rats. In rhesus monkeys, an induction of a 5-day inflammatory stress episode can activate the adrenal axis and increase cortisol secretion but decrease progesterone secretion [31]. The recovery of serum $E_2$ levels after 1 month in both genistein and vehicle groups may be due to adaptation. The HPA axis adapts its activation in the face of a repeated stressor. For example, repeated exposure to restraint cause a reduction in the elevation of adrenocorticotrophic hormone and corticosterone levels in female and male rats [32]. Thus, the suppression on the hypothalamic-pituitary axis was attenuated [30].

The serum $E_2$ levels of genistein group were significantly higher than those of the vehicle groups at 1 and 2 month of treatment period. In the present study, we did not determine the effect of genistein on the cortisol level, the indicator of stress. However, some studies have shown that genistein decreased cortisol production of H295 cells, human adrenal cells, in vitro [33,34]. Therefore, it is possible that the reduction of cortisol production by genistein may result in the higher serum $E_2$ levels in the genistein group than those of the vehicle group.

Both groups of rats showed a comparable gain in body weight and food consumption that reflects no

effect of genistein on rat growth. This result is consistent with the study of Hilakivi-Clarke et al. [13], which demonstrated that at low dosage genistein did not affect the body weight gain.

Since there was no significant difference in the length of estrous cycle, uterine and ovarian weights between genistein and vehicle group, this suggests that genistein at the dose of 1 mg/kg BW did not affect female reproductive organs and function. Our results is supported by the study of Santell et al. [35] which reported that dietary genistein (0.375 mg or 0.75 mg/kg feed) did not effect the uterine weight of intact rats. However, the length of the estrous cycle increased significantly in both groups (vehicle: $6.25 \pm 2.6$ days; genistein: $6.82 \pm 2.7$ days, mean $\pm$ SD) during the last 3 months of the study. These changes in the estrous cycles may be due to the transition period to middle-age, which eventually leads to an acyclic reproductive state in rats [36].

In this study, we have used genistein at a dose of 1 mg/kg BW which is comparable to the approximately daily consumption in Asian people on mg/kg BW basis [37,38]. It can be concluded that long-term exposure to genistein at the dosage comparable to the ordinary human consumption did not affect reproductive system but resulted in enhancement of NMU-induced tumorigenesis in intact female adult rats. Although NMU-tumor induction cannot be compared directly with human tumor development, the results of this study suggest that the supplementation of soy isoflavone to premenopausal women may potentially potentiate the risk of breast cancer.

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