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# Characterization of Crilin and Nanocurcumin's Synergistic Effect on Treatment for 7.12-Dimethylbenz[a]anthracene (DMBA)-Induced Breast Cancer Mice

Tran Gia Buu*, Tran Thi Phuong Nhung, Nguyen Thi Trang

*Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao, Go Vap, Ho Chi Minh City, Vietnam*

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**Abstract**: Breast cancer is the neoplastic disease which is characterized by unregulated ductal and lobular hyperplasia. Some herbal remedies have proved the inhibitory effect on breast cancer, such as crilin-extracted from *Cirnum latifolum* and curcumin-isolated from *Cucuma longa*. However, the synergistic effect of crilin and nanocurcumin has not been studied so far. In this study, we established the mouse model of breast cancer induced by DMBA and evaluated the effectiveness of the combination of crilin and nanocurcumin in treatment of breast cancer. After a 12-week co-administration of crilin and nanocurcumin, the DMBA-induced mice's body weight and the number of erythrocytes and leukocytes in their blood reversed. Furthermore, the synergistic effect of crilin and nanocucumin on reduction in the tumor volume was proven. Histological analysis revealed that co-administration of crilin and nanocurcumin inhibited the expansion of mammary ductal carcinoma cells into surrounding tissues, recovered lobular cells structure, and diminished leukocyte composition. Thereby, the combination of crilin and nanocurcumin helped recover immune system and prevent further development of breast cancer.

*Keywords*: Breast cancer, DMBA, Cirnum latifolum, nanocucumin, synergistic effect.

## 1. Introduction

Breast cancer is major burden to public healthy in worldwide, especially in women. Breast cancer is recognized as the most common invasive cancer in women and accounts for majority of the death from cancer in women. Ferlay et al (2010) estimated that one of ten new cancer patients throughout the world each year are related into breast cancer with more than 1.1 million cases and over 410,000 deaths annually [1]. The unregulated proliferation of breast lobular or ductal cells generates cancer cells, and they invade into surrounding tissue, which leads into breast cancer. Furthermore, cancer cells may metastasize through breast and lymph nodes to other parts of the body. The stage and severity of breast cancer are determined by TMN

* Corresponding author. Tel.: 84- 938983086.
Email: trangiabuu@iuh.edu.vn

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system, which categorizes breast cancer by the size of tumor (T), the spread to lympho nodes near the breast (N) and the spread to other part of body (M). A variety of treatments for breast cancer is available such as surgery, radiation therapy, hormone therapy and chemotherapy. Recently, the combination of folk remedies and synthetic medicine is recognized as a supportive treatment to prevent and cure breast cancer. In 2013, Vinodhini et al proved that bis-carboxy ethyl germanium sesquoxide (Ge-132), an organometallic component of many medicinal plants such as ginseng, could reduce the size and growth of tumor in N-methyl-N-nitrosourea (MNU)-induced mammary carcinoma [2]. Furthermore, the synergistic effect and toxicity reduction of dietary fucoidan extracted from brown seaweed with standard anti-cancer agents, such as oxaliplatin plus 5-fluorouracil/leucovorin, irinotecan plus 5-fluorouracil/leucovorin,
cytarabine, resveratrol, cisplatin, tamoxifen, paclitaxel, and lapatinib, have been well documented [3]

The anti-cancer effect of *Crinum latifolium* and *Curcuma longa* have been well documented in several studies. In 2011, Jenny et al proved that *Crinum latifolium* leaf extract could suppress the proliferation of PC3 cells, highly metastatic human prostate tumor cells, and androgen-sensitive prostate adenocarcinoma LNCaP cells, and benign prostate hyperplasia BPH-1 cells *in vitro* [4]. Moreover, *Crinum latifolium* extracts also recover immune function through the immuno-modulatory effect on indoleamine 2, 3-dioxygenase (IDO) activity of stimulated and resting human peripheral blood mononuclear cells. Although the activation of IDO inhibits the growth of malignant cells and contributes to tumor rejection, IDO also attenuates T-cell proliferation and immune response. Therefore, IDO activity could contribute to development of immunodeficiency, which lead to cancer progression. Antitumor activity of IDO inhibitors, such as 1-methyl tryptophan, methylthiohydantoin-tryptophan, and phytoalexin brassinin was shown in various animal models [4]. Furthermore, Nguyen et al suggested that aqueous extract of *Crinum latifolium* leaf could inhibit the proliferation of EL4-luc2 lymphoma cells and/or activated the tumorcidal activity of macrophages [5]. They showed that aqueous extract of *Crinum latifolium* activated M1 phenotype of macrophages by induction of TNFα, IL-1β, IL-6 mRNA expression. Furthermore, aqueous extract also enhanced NADPH quinine oxido-reductase -1 mRNA expression in polarized macrophages exerting important in cancer chemoprevention. These findings strongly demonstrated antitumor and anti-cancer properties of *Crinum latifolium* extracts.

Moreover, curcumin, the principal polyphenolic constituent (diferuloylmethane) isolated from turmeric rhizome *Curcuma longa* has been long used to treat neoplastic and neurodegenerative diseases. Curcumin possesses strong anti-inflammatory, antioxidant effects, apoptosis as well as modulation of several signal mechanisms, which underlies its therapeutic effect on hepatocellular carcinoma. Several studies on both chemically induced and xenograft preclinical hepatocarcinogenesis models suggested curcumin as an effective remedy to prevent and treat hepatocellular carcinoma [6]. However, bioavailability of curcumin is limited due to its poor absorption and rapid metabolism to glucuronide conjugated form. Therefore, a variety of nanotechnology based drug delivery system have been applied for curcumin to improve its bioavailability and efficient delivery, including nanoparticles, liposomal formulation, micelles, phospholipid complexes, polymeric encapsulation. Of note, Khosropanah et al (2016) reported that both curcumin and nanocurcumin exhibited the anti-proliferative effect on MDA-MB231 cell line, the human breast adenocarcimona cell line, and nanocurcumin had higher efficiency with lower IC50 as compared with curcumin [7]. In the addition, Milano et al (2013) proved that nanocurcumin inhibited proliferation of

esophageal adenocarcinoma cells whereas it did not alter the proliferation of normal esophageal cells. Nanocurcumin also enhanced the sensitivity of esophageal adenocarcimona cells to T cell induced cytotoxicity [8]. These researches indicated that nanocurcumin as promising therapeutic agents for cancer treatment.

Recently, many of functional foods for supporting cancer treatment derived from *Crinum latifolium* and *Curcuma longa*, such as crilin and nanocurcumin, have been introduced into market. However, the synergistic effect of combination of crilin and nanocurcumin on cancer treatment has not been studied yet. In this study, we established the 7, 12 dimethyl benzanthracene (DMBA) induced breast cancer model and investigated the synergistic effect of combination of crilin and nanocurcumin on prevention and treatment of breast cancer.

## 2. Materials & Methods

### *2.1. Chemicals and reagents*

The 7, 12 dimethyl benzanthracene (DMBA), one member of polycyclic aromatic hydrocarbon (PAH) family, was used to induce mammary tumor in mice. DMBA was obtained from Sigma (D2354, Sigma-Aldrich, USA). Crilin capsule, the aqueous extract of *Crinum latifolium*, was provided by Thien Duoc Co. Ltd, Vietnam. Nanocurcumin capsule was purchased from H-LINK Co. Ltd, Vietnam and fucoidan capsule obtained from Kanehide Bio Co. Ltd, Japan, was used as reference drug for breast cancer treatment.

### *2.2. Animals and experimental design*

Six-week old female Swiss albino mice weighting approximately 25-27 g were obtained from Pasteur Institute of Ho Chi Minh City. All of mice have not been mated yet. They were housed under standard husbandry conditions with 12 h light-dark cycle (8:00-20:00) for at least 1 week to acclimate with laboratory environment. They were supplied *ad libitum* with standard chow and distilled water. The experimental procedure was in strictly compliance with Declaration of Helsinki (1964). Briefly, mice were divided into several groups:

+ Control group (Normal group): 5 mice in this group, they were freely access to water and food for 20 weeks.

+ Breast cancer model group (Breast cancer group): 25 mice in this group, they were treated with 0.2 ml DMBA per mouse every week (1 mg/mouse/week) via gastric gauge for 6 weeks [9]. Then, they were maintained for next 14 weeks.

After successfully established breast cancer models (20 weeks), the mice which have mammary tumors were divided into 5 groups with 5 mice/group.

+ Negative control group (Untreat group): they were freely access to water and food for 12 week.
+ Possitive control group (Fucoidan group): they were orally treated with 185 mg fucoidan/kg body weight twice per day for 12 weeks.

+ Crilin treated group (Crilin group): they were orally treated with 500 mg crilin/kg body weight twice per day for 12 weeks.

+ Nanocurcumin treated group (Nanocurcumin group): they were orally treated with 200 mg nanocurcumin/kg body weight twice per day for 12 weeks.

+ Crilin and nanocurcumin combination group (Crilin + Nanocurcumin group): they were orally treated with 200 mg nanocurcumin/kg body weight twice per day and 500 mg crilin/kg body weight three times per day for 12 weeks.

During experimental period, we observed tumor size, the changes of body weight, peripheral erythrocyte and leukocyte concentration, tumor palpation, histological analysis.

### *2.3. Tumor palpation*

Palpation examination was macroscopically performed via observation of the number of tumors and diameter of tumors. The diameters

of tumor were measured using caliper in week 20 and 32, after DMBA induction until the end of treatment. Volume of tumor was calculated using the following formula [10]: V= (L x $W^2$)/2, where V is volume of tumor, L is tumor length, and W is tumor width (L>W). The results were presented as mean and standard deviation ($mm^3$).

### *2.4. Measurement of body weight, peripheral erythrocytes and leukocytes concentration*

In chosen time point, all experimental animals were fasted overnight to reduce the differences of feeding. The body weight were measured by electronic scale, then the change of body weight of mice was recorded. The results were presented as mean and standard deviation.

Then, mice were anesthetized using diethyl ether and then blood were collected from tail veins into the anti-coagulant $K_2$EDTA coated tubes. Blood samples were sent to Department of Hematology, Hoa Hao Hospital Ho Chi Minh city, for determination of peripheral erythrocyte and leukocyte concentration via automated hematology analyzer. The results were presented as mean and standard deviation.

### *2.5. Histological analysis*

At the end of experiment, all experimental animals were anesthetized using diethyl ether and euthanized by carbon dioxide inhalation. Mammary glands and breast tissue were collected and fixed in 10% formalin. Samples were send to Department Pathological Anatomy, Ho Chi Minh City Oncology Hospital to perform the Hematoxylin and Eosin staining.

### *2.6. Statistical analysis*

Statistical analysis was performed using Statgraphics Centurion XVI software (Statpoint Technologies Inc., Warrenton, Virginia, USA). The data were presented as mean ± standard deviation. Differences between means of different groups were analyzed using ANOVA variance analysis followed with multiple range tests, the criterion of statistical significance was set as $p < 0.05$.

## 3. Results and Discussions

### *3.1. Establishment of breast cancer model*

#### *3.1.1. Changes of body weight, the number of peripheral erythrocytes and leukocytes*

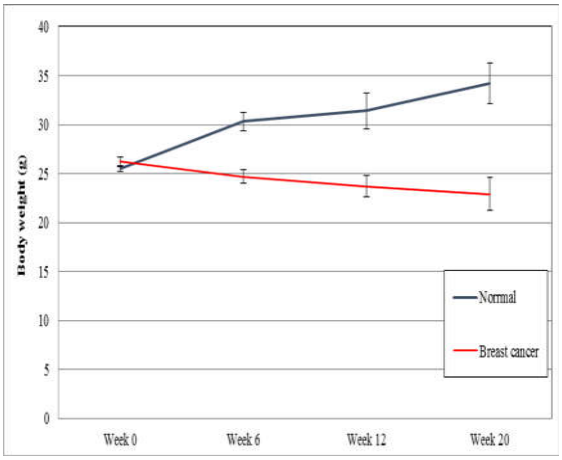


Figure 1. Change of body weights of normal and breast cancer mice.

Body weight of both normal and breast cancer mice was dramatically changed after 20 weeks. As shown in Figure 1, body weight of normal mice was gradually increased from 25.5 to 34.2 g, whereas body weight of breast cancer models was reduced from 26.2 to 22.9 g. Administration of DMBA led to down-regulation of aryl hydrocarbon receptor (AHR) and conversion of proto-oncogenes into oncogenes, which generated cancer cells and decreased cellular metabolism rate, defect normal cellular proliferation. Therefore, DMBA reduced body weights of breast cancer models. These finding was identical with results from Do et al study [9], in which the authors indicated that the weight gain of normal group was higher than DMBA treated group.

Furthermore, the number of erythrocytes of normal mice did not change after 20 weeks. Of note, erythrocytes of breast cancer mice were

significantly decreased to 4.95 x $10^6$ cells/$mm^3$. Erythrocytes exert an important role in oxygen and carbon dioxide transportation, acid-base homeostasis, and blood viscosity. These data proved that DMBA decreased of erythrocytes and resulted in oxygen transportation deficiency. DMBA could form covalent bond with DNA, damaged the duplication and repairmen of DNA and/or destroyed DNA structure, which led to killing of hematopoietic stem cells in bone marrow. Consequently, DMBA administration resulted in the decrease the number of erythrocytes (Table 1). Interestingly, the number of total leukocytes of breast cancer group after 20 weeks treated with DMBA were higher than normal mice (11.15 x $10^3$ versus 6.88 x $10^3$ cells/$mm^3$, respectively). We found that total leukocytes of breast cancer models noticeably increased after 20 weeks, while the number of total leukocytes of normal group were steady during experiment (Table 1). These results were consistent with Chen report [11]. The authors suggested that treatment with DMBA 75 mg/ kg body weight resulted in decrease of body weight and the number of erythrocytes, but elevation of total leukocytes and lymphocytes. Furthermore, Fatemi and Ghandehari (2017) observed a noticeable increase of leukocytes along with decrease of erythrocytes in rat receiving 5 mg DMBA [12]. These findings showed that DMBA did not only reduce body weight but also altered other hematological parameters, such as the number of peripheral erythrocytes and leukocytes.

Table 1. Change of hematological parameters of normal and breast cancer mice

| Time point | Erythrocytes ($10^6$/$mm^3$) | | Leukocytes ($10^3$/$mm^3$) | |
|---|---|---|---|---|
| | Normal | Breast cancer | Normal | Breast cancer |
| Week 0 | $5.42 \pm 0.02^{a}$ | $5.42 \pm 0.02^{a}$ | $6.82 \pm 0.02^{a}$ | $6.85 \pm 0.01^{a}$ |
| Week 6 | $5.45 \pm 0.04^{ab}$ | $5.15 \pm 0.01^{b}$ | $6.85 \pm 0.05^{a}$ | $8.15 \pm 0.08^{b}$ |
| Week 12 | $5.49 \pm 0.03^{b}$ | $5.08 \pm 0.02^{c}$ | $6.86 \pm 0.09^{a}$ | $10.25 \pm 0.05^{c}$ |
| Week 20 | $5.55 \pm 0.04^{b}$ | $4.95 \pm 0.03^{d}$ | $6.88 \pm 0.05^{a}$ | $11.15 \pm 0.04^{d}$ |

$^{a,b,c,d}$ Values with different letters within same column are significantly different ($p < 0.05$).

*3.1.2. Histological changes of breast cancer model*

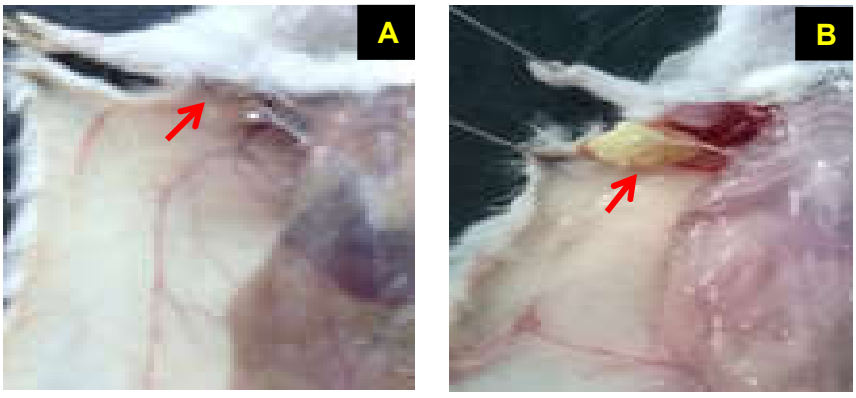


Figure 2. Anatomical analysis of breast cancer mice induced by DMBA treatment after 20 weeks. Control mice showed the normal structure of mammary gland, red arrow indicated the mammary gland (A). Mammary gland of DMBA treated mice developed a tumor, red arrow indicated the tumor site (B).

After 20 weeks treated with DMBA, breast macroscopic morphologies of breast cancer models were noticeably changed. All of DMBA treated mice developed mammary tumors with tumor size approximately 213.80 ± 45.60 $mm^3$.

Furthermore, the data from histological analysis also supported the change of mammary morphologies. In DMBA treat mice, carcinoma cells spread into surrounding stromal tissue, which resulted that stromal cells disorganized and loosely connected. Immune cells infiltrated into stromal tissue and several empty spaces occurred in stromal section (Figure 3A, E). In adipose tissue, carcinoma cell widely invaded into nearby adipocytes, resulting deformation of their structure and loose connection of adipocytes (Figure 3B, F). In mammary ductal section, ductal carcinoma in situ micropapillary type (DCIS-micropapillary type) was observed. Mammary ducts were thicken, myoepithelial layer changed its structure and morphology, mammary ductal epithelial cells poorly organized and un-tightly bound together (Figure 3C, G). The mammary central lobular region was necrotized, and some regions exhibited atrophy phenomenon. Furthermore, tumor cells formed excess fibrous connective tissue enriched with collagen fibers in neighboring region (Figure 3D, H).

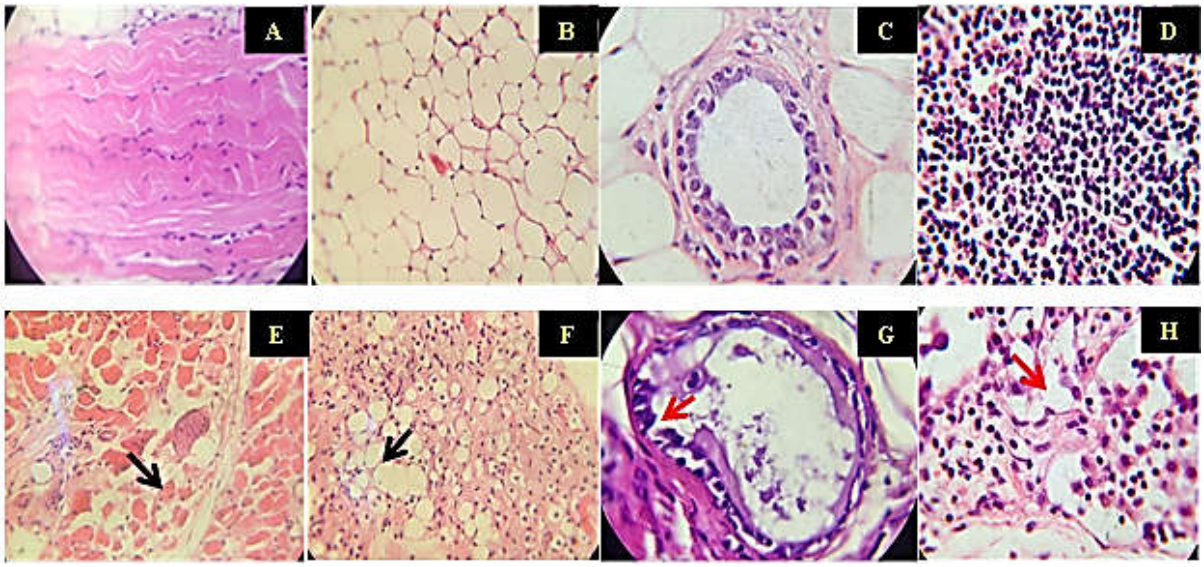


Figure 3. Histological analysis of mammary glands of breast cancer mice induced by DMBA after 20 weeks. Microscopic appearance of mammary glands of normal mice (A. Stromal tissue; B. Adipose tissue; C. Mammary duct; D. Mammary lobule). Microscopic appearance of mammary glands of breast cancer mice treated with DMBA after 20 weeks (E. Stromal tissue; F. Adipose tissue; G. Mammary duct; H. Mammary lobule).

### *3.2. Synergistic effect of crilin and nanocurcumin on treatment of breast cancer*

#### *3.2.1. The change of body weights of experimental mice during different treatment regimens*

Body weights of all mice received the treatment with crilin, nanocurcumin, crilin and nanocurcumin, fuicodan were significant increase whereas untreated mice showed a decrease in body weight during experiment (Figure 4). Briefly, the mice treated with crilin were increased body weight from 23.3 into 25.2 g, and the body weights of mice treated with nanocurcumin were recovered from 23.3 into 26.0 g. Of note, the increase of body weight of the mice which co-treated with nanocurcumin and crilin (23.3→26.4g) was higher than either crilin treated or nanocurcumin groups ($p$<0.05),

and it was similar with the increase of body weight of fucoidan treated mice (reference drug). The extract from *C. latifolium* had cellular toxicity on cancer cells through activation of macrophages and hindered the cancer cell proliferation [4, 5]. Curcumin also inhibited the tumor growth and angiogenesis [13]. Consequently, cancer cells could not compete the oxygen and nutrient with normal cells, which leads to recovery of cellular metabolism and energy balance, body weight of either nanocurcumin or crilin as well as combination of crilin and nanocurcumin treated mice.

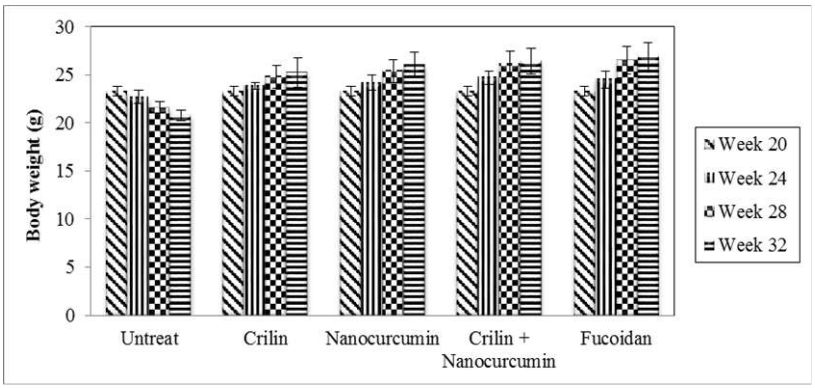


Figure 4. Beneficial effect of different functional foods on the body weight of mice during treatment.

*3.2.2. The change of hematological parameters of experimental mice during different treatment regimens*

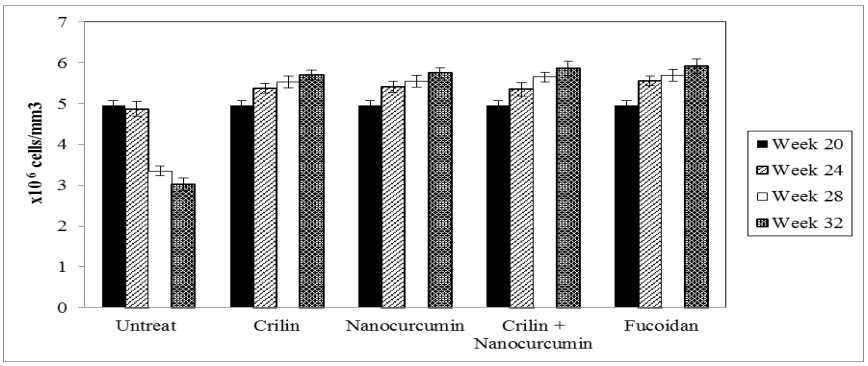


Figure 5. Beneficial effect of different functional foods on the number of peripheral erythrocyte during treatment.

Table 2. Alteration of functional foods on total peripheral leukocyte numbers in breast cancer model

| Time point | Total peripheral leukocytes ( $x10^3$ cells/$mm^3$) | | | | |
|---|---|---|---|---|---|
| | Untreat | Crilin | Nanocurcumin | Crilin + Nanocurcumin | Fucoidan |
| Week 20 | $11.15 \pm 0.04^a$ | $11.15 \pm 0.04^a$ | $11.15 \pm 0.04^a$ | $11.15 \pm 0.04^a$ | $11.15 \pm 0.04^a$ |
| Week 24 | $12.11 \pm 0.03^a$ | $9.59 \pm 0.02^b$ | $9.65 \pm 0.05^b$ | $9.88 \pm 0.07^c$ | $10.21 \pm 0.05^d$ |
| Week 28 | $12.34 \pm 0.03^a$ | $9.22 \pm 0.03^b$ | $9.30 \pm 0.03^c$ | $9.54 \pm 0.04^d$ | $9.72 \pm 0.06^e$ |
| Week 32 | $12.67 \pm 0.05^a$ | $8.64 \pm 0.01^b$ | $8.51 \pm 0.02^c$ | $8.62 \pm 0.05^b$ | $9.22 \pm 0.03^d$ |

$^{a,b,c,d,e}$ Values with different letters within same row are significantly different ($p < 0.05$).

As shown in Figure 5, peripheral erythrocytes of treated groups were increased during the treatment period. On the contrary, the number of erythrocytes of untreated group was decreased significantly ($p$<0.05). After 12 weeks administered to crilin and nanocurcumin, the number of peripheral erythrocytes of treated mice were remarkably increased from 4.95 x $10^6$ cells/mm$^3$ to 5.86 x $10^6$ cells/mm$^3$. Noted that the increase of erythrocytes of crilin and nanocurcumin treated mice was identical to fucoidan treated group, reference drug (4.95 x $10^6$ cells/mm$^3$ to 5.92 x $10^6$ cells/mm$^3$). This finding implied that the treatment of crilin and nanocucurmin could improve the erythrocyte regeneration in breast cancer model. Furthermore, the increase of the number of total peripheral leukocytes of breast cancer mice was observed during treatment from 11.15 x $10^3$/mm$^3$ to 12.67 x $10^3$/mm$^3$. In contrast, all of crilin, nanocurcumin, crilin nanocurcumin, and fucoidan treatment reduced the numbers of total peripheral leukocytes (8.64 x$10^3$, 8.51 x$10^3$, 8.62 x$10^3$, and 9.22 x$10^3$/ mm$^3$, respectively). These results proved that crilin and nanocurcumin could inversed the alteration of DMBA on total leukocytes number into the number of normal mice (Table 2).

*3.2.3. The change of tumor volume of experimental mice during different treatment regimens*

The change of tumor morphology and volume were presented in Figure 6. Briefly, The tumor volume of untreated mice was significantly increase during experiment, from 213.80 ± 45.60 mm$^3$ at begin of experiment to 386.07 ± 72.46 mm$^3$ at the end of experiment ($p$<0.05). In contrast, all tumors of treated mice with functional foods, such as crilin, nanocurcumin, crilin and nanocurcumin, and fucoidan, dramatically reduced their volumes (135.80 ± 9.74, 126.82 ± 11.66, 87.80 ± 8.45 and 78.42 ± 3.38 mm$^3$, respectively, $p$<0.05). Fucoidan treatment downregulates expression of Bcl-2, Survivin, ERKs, and VEGF and enhances activation of caspase-3, which results activation of apoptosis and inhibition of angiogenesis. Therefore, the tumor volume of fucoidan treated mice was reduced [14]. The anti-tumor effect of curcumin was well-described in Lv work, in which the authors proved that curcumin could induce apoptosis of human breast cancer cell lines, such as MCF-7 and MDA-MB-231 cells, via augmentation of Bax/Bcl-2 ratio and inhibited tumor growth in MDA-MB-231 xenograft mice [15]. Furthermore, nanotechnology based drug delivery systems of curcumin improve the water solubility and bioavailability of curcumin, which in turn enhances the anti-proliferative activity of curcumin [7]. As a consequence, nanocurcumin administrated mice exhibited a decline of tumor volume during treatment regime. Additionally, Pizzorno et al (2016) suggested that *Crinum latifolium* treatment could reduce the tumor size and inhibited the tumor growth in 79.5% of female patients suffering from fibroid tumors, and decreased the tumor growth rate (20.5%) [16]. In this study, crilin treated tumors were reduced their volume from 213.80 ± 45.60 mm$^3$ to 135.80 ± 9.74 mm$^3$ after treatment period, which was consistent with that report. Note that, we found that the decrease of tumor volume in crilin and nanocurcumin treated mice (87.80 ± 8.45 mm$^3$) was higher than individually treated by crilin or nanocurcumin treated mice (135.80 ± 9.74 and 126.82 ± 11.66 mm$^3$, respectively, $p$<0.05), and it was similar with tumor volume of reference drug, fucoidan, treated mice (78.42 ± 3.38 mm$^3$). These data implied that the combination of crilin and nanocurcumin had the synergistic effect on the decrease of mammary tumor volume and its reducing tumor size efficiency was equivalent to reference drug efficiency.

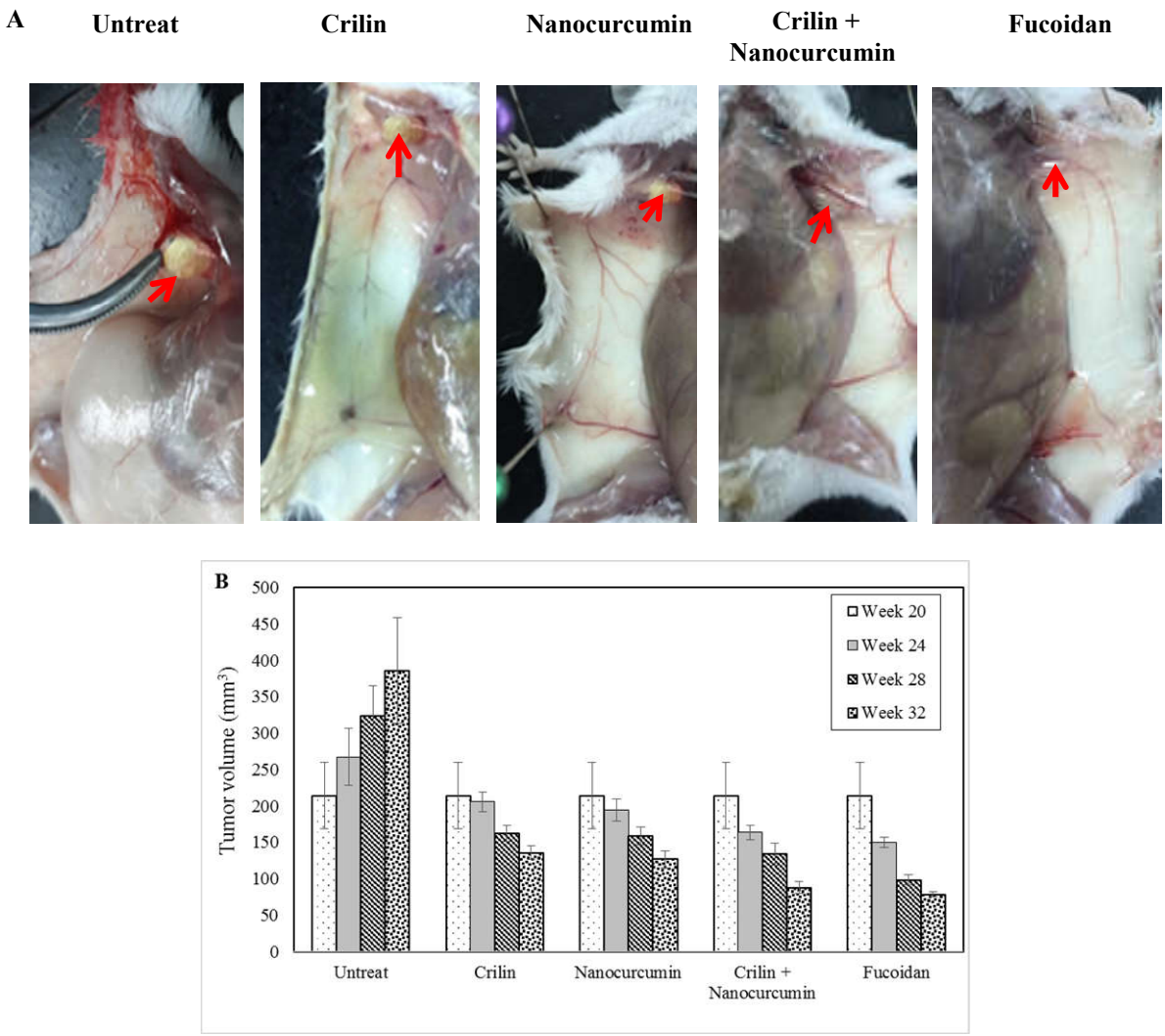


Figure 6. Morphological changes of mammary glands of experimental mice. Anatomical analysis of mammary glands (A) and alteration of tumor volume (B) of breast cancer mice with different treatment regimens were presented. Red arrows indicated the tumor site.

*3.2.4. The histological change of mammary gland of experimental mice during different treatment regimens*

In untreated mice, invasion region of mammary carcinoma was significantly expanded into mammary stromal tissue along with severe impairment of stromal tissue structure. Moreover, most adipocytes were compressed by carcimona cells leading to the complete deformation of mammary adipose tissue. Ductal carcimona in situ solid type was observed in mammary gland, cancer cells were highly proliferated and completely filled ductal lumen along with lacking the define myoepithelium. In mammary lobular tissue, cancer cells were uncontrollably proliferated and overlapped together along with abnormal shape of cancer cell nuclei with hyperchromasia and leukocyte composition (Figure 7A, B, C, D). Histological analysis revealed that mammary tumor developed toward advantage stage of cancer with poor prognosis during the treatment period.

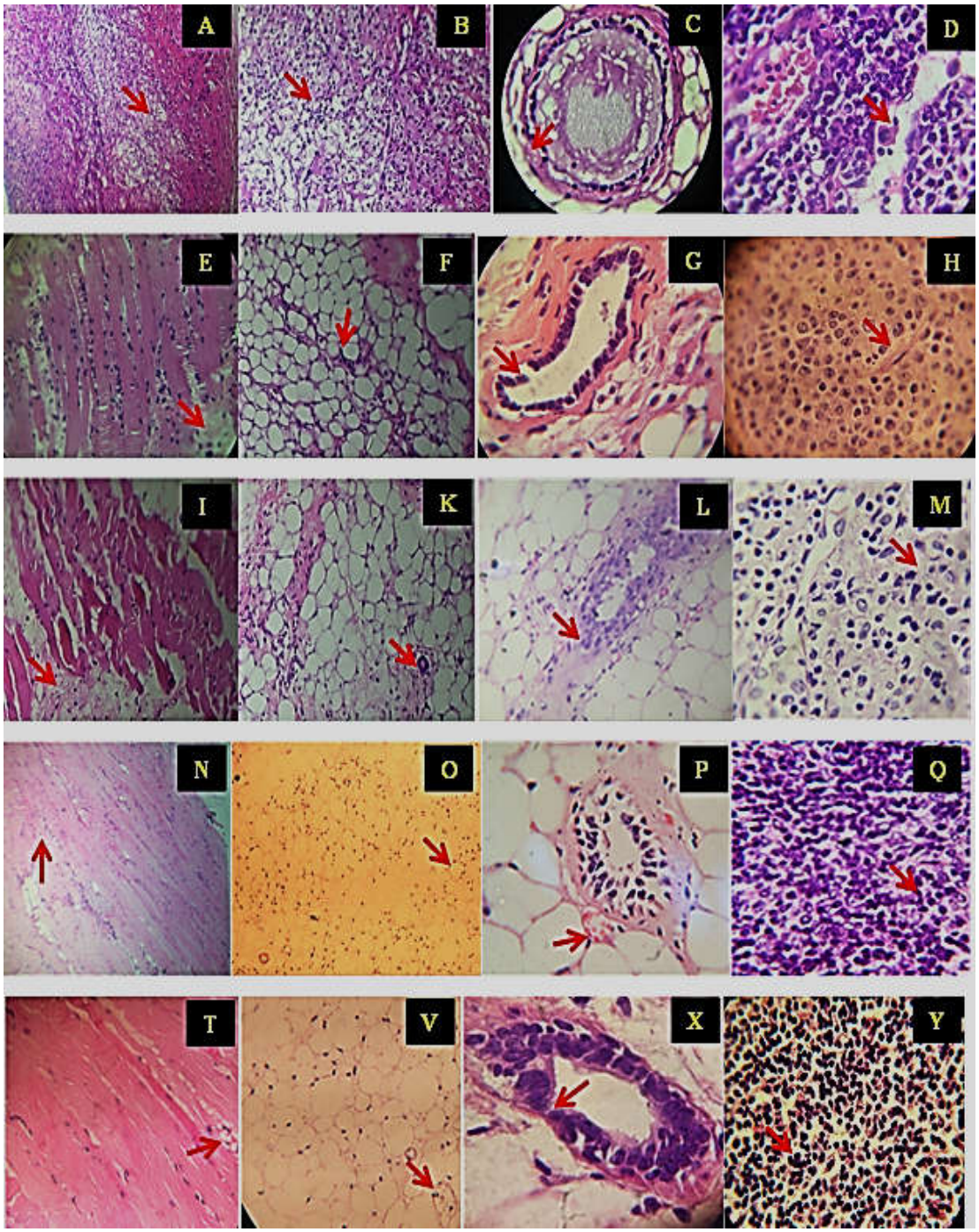


Figure 7. Histological analysis of mammary glands of breast cancer mice exposed to different treatment regimes. Untreated mice (A. Stromal tissue; B. Adipose tissue; C. Mammary duct; D. Mammary lobule), crilin treated mice (E. Stromal tissue; F. Adipose tissue; G. Mammary duct; H. Mammary lobule), nanocurcumin treated mice (I. Stromal tissue; K. Adipose tissue; L. Mammary duct; M. Mammary lobule), crilin and nanocurcumin treated mice (N. Stromal tissue; O. Adipose tissue; P. Mammary duct; Q. Mammary lobule), fucoidan treated mice (T. Stromal tissue; V. Adipose tissue; X. Mammary duct; Y. Mammary lobule).

After co-treatment with crilin and nanocurcumin for 12 weeks, histological analysis of mammary gland of mice showed the good prognosis of disease. Stromal tissue recovered its normal structure, collagen fibers clustered together into bundles, nuclei of stromal cells were clearly stained with no hyperchomasia, and mammary stromal cells were well-organized and recovered their normal structure (Figure 7N). The number of invasive carcinoma cells was noticeably decrease, adipose tissue recovered the normal structure, and adipocytes were well organized. Nuclei of adipocytes were homologous and even stained, cell proliferation was reduced (Figure 7O). Ductal carcinoma in situ micropapillary type (DCIS-micropapillary type) was disappeared, normal structure of mammary ductal cells were observed. The level of hyperplasia of myoepithelial layer was reduced along with no leukocyte composition. Myoepithelial cells were homologous and well-stained, but their connection was looser than normal mice (Figure 7P). Mammary lobule structure was remarkably different with untreated mice, mammary lobular cells were closely connected with each other, leukocyte composition was reduced (Figure 7Q). Note that, all of functional food treated mice were showed the similarly histological pattern of stromal tissue, adipose tissue, mammary duct and lobule (Figure 7). Therefore, treatment of breast cancer model with functional foods, such as crilin, nanocurcumin, combination of crilin and nanocurcumin, and fucoidan, was recovered the normal structure of mammary glands.

## 4. Conclusion

This study was successfully established the breast cancer model using DMBA. All of pathological mice were developed tumor with $213.80 \pm 45.60$ mm$^3$. The breast cancer model showed a decline of body weight as well as peripheral erythrocyte number, and an increase of peripheral leukocyte number. Furthermore, breast cancer mice showed abnormal structure of stromal tissue, adipose tissue, mammary duct and lobule. Treatment with functional foods, such as, crilin, nanocurcumin, combination of crlin and nanocurcumin, and fucoidan inversed the decline of body weight as well as alteration of hematological parameters of breast cancer mice. Furthermore, all of functional foods reduced the tumor volume and recovered mammary normal gland morphology. This study also demonstrated the synergistic effect of combination crilin and nanocurcumin on DMBA induced alteration of mammary morphology and body weight, and hematological parameters.

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# Tác động phối hợp của crilin và nanocurcumin đến quá trình chữa trị trên mô hình chuột ung thư vú cảm ứng bởi 7, 12 dimethylbenz[a]athracene (DMBA)

Trần Gia Bửu, Trần Thị Phương Nhung, Nguyễn Thị Trang

*Viện Công Nghệ Sinh Học-Thực Phẩm, Trường Đại học Công nghiệp TP. HCM,*
*12 Nguyễn Văn Bảo, Gò Vấp, TP. Hồ Chí Minh, Việt Nam*

**Tóm tắt:** Ung thư vú là một dạng bệnh tân sản đặc trưng bởi tăng sản quá mức của tế bào ống và thùy tuyến vú. Một số dược liệu đã được nghiên cứu nhằm hạn chế ung thư như: crilin, chiết xuất từ cây trinh nữ hoàng cung; curcumin, chiết xuất từ cây nghệ. Tuy nhiên tác động phối hợp khi sử dụng chung hai loại thuốc này chưa được làm rõ. Trong nghiên cứu này, chúng tôi đã xây dựng mô hình

chuột nhắt trắng bị bệnh ung thư vú bằng DMBA và chữa bệnh nhờ tác động phối hợp của Crilin với Nanocurcumin. Sau 3 tháng uống kết hợp nanocurcumin và crilin, sự thay đổi về các chỉ số về trọng lượng, số lượng hồng cầu, bạch cầu tổng trong máu chuột ngoại vi cảm ứng bởi DMBA bị đảo ngược. Đồng thời, kết quả phân tích mô học cho thấy sử dụng đồng thời crilin và nanocurcumin giúp kìm hãm sự xâm lấn lên mô đệm xung quanh của các tế bào carcinoma ống tuyến vú lên và hồi phục cấu trúc tế bào tiểu thùy, làm giảm ổ bạch cầu khu trú. Tóm lại, sự phối hợp crilin và nanocurcumin giúp hồi phục hệ thống miễn dịch, ngăn chặn sự phát triển ung thư vú.

*Từ khóa*: Ung thư vú, DMBA, *Cirnum latifolum,* nanocucumin, tác động phối hợp.