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Anti-Proliferative Effects in Human Breast Cancer MDA. MCF-7 Cells & Human Breast Epithelial MCF-10a Cells and Western Blot Analysis from Adlay (*Coix Lacryma-Jobi* L.) Varieties Phenolic Extracts

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Abstract Analyzed the anti-proliferative activities (EC_{50} values) and cytotoxicities (CC_{50} values) of adlay extracts with adlay (*Coix lacryma-jobi* L.) varieties grains, Guizhou Heigu (hard hull, black), Liaoning 5 (soft hull, black) and Longyi 1 (hard hull, brown) to be the materials, normal human MCF-10a breast epithelial cells, human MDA breast cancer cells and human MCF-7 breast cancer cells to be the research subjects. After being processed by free or bound phenolic extracts of adlay, detected the expression levels of proteins such as PCNA, p21 and CDK4, which were related to cell proliferation and cell cycle in cells, by using western-blot test to preliminary obtain the mechanism of inhibition of tumor cells proliferation of adlay extracts. The results showed that the adlay extracts by different varieties had obvious anti-proliferative effects to tumor cells, and adlay samples themselves had no cytotoxicity in the tested experimental concentration range. Besides, adlay extracts inhibited the proliferation of HepG2 cells by regulating the cell cycle mediated by DNA polymerase adjustment factors such as PCNA and p21.

Keywords: *adlay polyphenol, cytotoxicity, anti-proliferative activity, protein expression*

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1. Introduction

Adlay (*Coix lachryma-jobi* L.) is a kind of traditional medicinal and edible grain resources. In recent years, studies have shown that it has anti-oxidant, anti-allergic, anti-obesity, anti-tumor effects and so on [1,2,3]. Active ingredients of adlay which have been found are mainly CoixanA, B, C [4,5], neutral glucan 1-7, acidic polysaccharides CA-1 and CA-2 [6], Coixenolide [7], Coixol [8], lignans, phenols, unsaturated fatty acids, benzoxazine ketones, triterpenoids open ring compounds such as squalene, sterols, adenosine and amino acids [9]. Currently, few studies have been conducted on the cytotoxicities and anti-proliferative effects of adlay extracts in cells in vitro. On the other hand, studies have mainly focused on the physiological functions of adlay extracts [10,11,12]. Chang et al. [13] reported that adlay, which has anti-tumor properties, can inhibit sarcoma-180 tumors in mice. Methanol extracts of adlay husks have been shown to have anti-proliferative effects in U937 leukemia cells [14] and in A549 lung cancer cells [13]. Even though the total polyphenol content of adlay bran has been assessed, there is no evidence on the content ratio between the free and the bound polyphenols [10,15]. In addition, Hidaka's [16] research has shown that consumption of adlay extracts can significantly enhance the activity of the body's T cells, then form ability of natural killer cells. Besides these, adlay seed extracts can effectively suppress the generation of NO and oxygen free radicals by activating macrophages [17].

Western Blot is a common experimental method in molecular biology, biochemistry and immunogenetic. PCNA has a close relationship with DNA synthesis, plays an important role on starting of cell proliferation; it's a good indicator of reflecting the state of cell proliferation. The expression of PCNA substantial increases in the late G1 phase of the cell cycle, and reaches its peak in S phase. In G2-M phase, the expression will be significantly decreased. Its change is consistent with DNA synthesis. Detecting the expression of PCNA in cells can be used as an evaluation indicator of proliferation state. P21 gene is an important member of the cell family found in recent years whose cell cycle proteins depend on kinase inhibitors. P21 gene is not only closely related to the inhibition of tumor, but also can coordinate the relationship between cell cycle, DNA replication and repair by inhibiting the activity of Cyclin-dependent kinases (CDKs) complex. So that the tumor inhibition and

cell cycle control process was closely linked. Discovery, cloning of p21 gene and its cell cycle control action play an important role in tumorigenesis.

This research mainly studied the cytotoxicities (CC_{50} values) and anti-proliferative effect (EC_{50} values) of adlay free or bound phenolic extracts towards normal cells and different tumor cells. Based on the study on cytotoxicity and anti-proliferative effect on HepG2 cells in vitro, then carried out western blot test to explore the expression of PCNA, CDK4 and p21 protein, in order to know the anti-tumor mechanism of adlay phenolic extracts in cell and protein levels.

2. Materials and Methods

2.1. Materials

Adlay (*Coix lacryma-jobi* L.) varieties grains, Guizhou Heigu (hard hull, black) [18], Liaoning 5 (soft hull, black) [19] and Longyi 1 (hard hull, brown) [20] were obtained from the collection of Jiangsu Key Laboratory of Quality Control and Processing for Cereals and Oils, Nanjing University of Finance and Economics, China.

Samples were dehusked on a Satake Rice Machine (JXFM110, Shanghai Jiading Oils Instrument Co., Ltd., Shanghai, China), milled into flour by passing through a 60-mesh sieve on a Cyclone Sample Mill and were stored at -20°C.

Cell lines, MDA-MB-231 breast cancer cells, MCF-7 breast cancer cells and MCF-10a human breast epithelial cells were purchased from American Type Culture collection Stock (ATCC) (Maryland, USA); MEM medium, DMEM medium and HBSS were purchased from Gibco Biotechnology company (Maryland, USA); FBS was purchased from Atlanta Biotechnology company (Atalanta, USA); Cholera toxin, hydrocortisone, Penicillin, streptomycin and gentamicin were purchased from Sigma company (USA); Tris (Acid) and Tris (Alkali) were purchased from American J. T. Baker company (USA); Mouse antibody CDK4 were purchased from American Santa Cruz company (USA); and mouse polyclonal antibody anti-PCNA were purchased from American Oncogene company (USA); Other reagents were of analytical grade.

2.2. Extraction of Phenolic Compounds

Free and bound phenolic compounds of adlay samples were extracted using the modified method reported previously in our laboratory [21,22], which is acetone extraction method and NaOH digestion method. Briefly, 4 g of adlay flour was blended with 30 ml 80% chilled acetone. The mixture was then centrifuged at 2500 g for 10 min. The supernatant was removed and the remaining pellet was extracted again with 80% chilled acetone and repeated it three times. The supernatant were pooled and evaporated using a rotary evaporator at 45°C to dryness. The solution was then reconstituted in 10 ml of 70% methanol. This free extracts were stored at -20°C until analysis. Bound phenolics were extracted from the residue from the free phenolic extraction. The residue was first digested with 20 ml of 2 $mol \cdot l^{-1}$ sodium hydroxide at room temperature for one hour while shaking under nitrogen. The mixture was the then neutralized with concentrated hydrochloric acid. Hexanes were used to extract lipids in the mixture. The remaining mixture was then extracted five times with ethyl acetate. The ethyl acetate fractions were pooled and evaporated using a rotary evaporator at 45°C to dryness. The bound phenolics were then reconstituted in 10 ml of 70% methanol. The extracts were stored at -20°C until analysis. Each sample was extracted at least three times.

2.3. Cell Culture

Cell lines, MCF-7 and MDA-MB-231 grown in growth medium (i.e., MEM supplemented with 10% FBS, 10 $mmol \cdot l^{-1}$ Hepes, 0.01 $mg \cdot ml^{-1}$ insulin, 50 $units \cdot ml^{-1}$ penicillin, 50 $\mu g \cdot ml^{-1}$ streptomycin and 100 $\mu g \cdot ml^{-1}$ gentamicin) [23,24,25] and MCF-10a grown in growth medium (i.e., DMEM supplemented with 5% FBS, 10 $\mu g \cdot ml^{-1}$ insulin, 20 $ng \cdot ml^{-1}$ epidermal growth factor, 100 $ng \cdot ml^{-1}$ cholera toxin, 0.5 $\mu g \cdot ml^{-1}$ hydrocortisone, 100 $U \cdot ml^{-1}$ penicillin and 0.1 $g \cdot l^{-1}$ streptomycin) [26,27], were maintained at 37°C and 5% CO_2.

2.4. Cytotoxicity Test

A cytotoxicity test was performed using the modified methylene blue assay [28]. Briefly, MCF-7, MDA-MB-231 and MCF-10a cells were seeded at a density of 4×10^4 $cells \cdot well^{-1}$ on a 96-well microplate in 100 µl of growth $medium \cdot well^{-1}$. The cells were incubated for 24 h at 37°C. After the cells had attached to the wells, the growth medium was removed and the cells were washed with PBS. Then 100 µl of medium with different concentrations (30, 60, 90, 120, 150, 180, 210, 240, 280, 320 $mg \cdot ml^{-1}$) of adlay extracts was added to each well; wells that received medium without adlay extract served as the control. After 24 h of incubation at 37°C, the medium was removed and the wells were washed with PBS. The cells were then incubated for 1 h at 37°C after adding 50 µl methylene blue colored liquid (98% HBSS, 0.7% glutaraldehyde and 0.6% methylene blue) to each well. After the incubation, the staining solution was removed and the cells were washed six times in deionized water until the water was clear. Subsequently, 100 µl of elution buffer (49% PBS, 50% ethanol, 1% acetic acid) was added to each well. The plates were placed on a table oscillator for 20 min and absorbance was measured at 570 nm in a microplate reader (Dynex Company, Canada). The different concentrations of adlay extract were compared to the control; if a certain concentration of adlay extract reduced cell viability by compared to the control, then that concentration was considered to be cytotoxic [29].

2.5. Cell Proliferation Inhibiting Test

The anti-proliferative effects of adlay extracts were assessed in a modified MCF-7, MDA-MB-231 and MCF-10 a cells methylene blue colorimetric method [30]. Briefly, MCF-7, MDA-MB-231 and MCF-10a cells were seeded at a density of 2.5×10^4 $cells \cdot well^{-1}$ on a 96-well microplate. Only 100 µl of cell-free medium was added to the peripheral wells of 96-well microplate. In the central wells of the 96-well microplate, 100 µl of the cell suspension was added. The 96-well microplate was incubated for certain time (MCF-7 and MDA-MB-231 cells generally 8 h, MCF-10a generally 6 h) at 37°C. The

medium was then removed and 100 µl of fresh medium containing different concentrations of adlay extracts (30, 60, 90, 120, 150, 180, 210, 240, 280, 320 mg·ml^{-1}) was added. The wells receiving cell suspension without adlay extract served as the control. The plates were incubated for 96 h at 37°C. Following the incubation, the staining solution was removed and the 96-well microplates were washed six times in deionized water until the water was clear. Then 100 µl of elution buffer (49% PBS, 50% ethanol, 1% acetic acid) was added to each well. The 96-well microplates were transferred to a tablet oscillator for 20 min. Absorbance was measured at 570 nm using a microplate reader (Dynex Company, Canada). Each sample was measured at least three times. The anti-proliferative effects were assessed by the EC_{50} values, which were expressed as mg of adlay extracts·ml^{-1}.

2.6. Western-blot Test

Extraction method of cytoplasmic protein was reported by Yoon et al. [28]. Briefly, HepG2 cells were seeded at a density of 5×10^5·well^{-1} on a 6-well microplate. Removed the supernatant after culturing them for 6 hours under 37°C, then added fresh WME medium with different contents of adlay extracts (0, 120, 150 and 180 mg·ml^{-1}) and cultured them for 16h. Removed the supernatant and washed them with precooled (4°C) PBS for twice, then scraped and collected the cells, centrifuged for 5 min under 12000 g, 4°C. Removed the supernatant and put the cells in ice for the subsequent western-blot test.

Added cell lysate into the above cells and put in ice for 20 min, vibrated time to time during this period to make sure the cells were lysated plenty. Cell lysate contains 50 mmol·l^{-1} Tris (pH 7.4), 1% Igepal, 150 mmol·l^{-1} NaCl, 1 mmol·l^{-1} EDTA and some protein inhibitors (1 µg·ml^{-1} leupeptin, 1 µg·ml^{-1} aprotinin, 1 µg·ml^{-1} Pepsin, 1 mmol·l^{-1} phenylmethylsulfonyl fluoride, 1 mmol·l^{-1} sodium vanadate, 1 mmol·l^{-1} NaF) and it was centrifuged under 12000 g, 4°C for 5 min, then carefully removed the supernatant. Protein content in lysate was determined by Lowry method which was specifically shown by Yoon et al. [30].

Western-blot test was carried out by modified Liu's method. In this experiment, both used 10% SDS-PAGE gel to separate PCNA and CDK4. After electrophoresis, the proteins were transferred to Immobilon-P PVDF transfer membrane by wet transfer method. The membranes were closed for half an hour in TBST solution (Tris buffer containing 0.1% Tween-20) with 5% non-fat dry milk at room temperature, then stayed overnight in TBST solution containing the first antibody under 4°C. Collected the first antibody, and washed the membranes by TBST solution for 15 min three times. Then the membranes stayed in TBST solution containing the second antibody for an hour at room temperature. Washed the membranes by TBST solution for 15 min three times and then by deionized water twice. After developing the film with the enhanced chemiluminescence kit (Cell signaling), analyzed the area and optical density of the corresponding bands to determine the strength of protein bands on the membrane by using ABWORKS gel imaging and analysis software.

2.7. Statistical Analyses

Data, which were expressed as mean ± standard deviation (SD), included at least three replicates per sample. ANOVA and Tukey's test were performed using SPSS (Statistics Programe for Social Science) version 17.0 (Chicago, USA). EC_{25}, EC_{50} and CC_{50} values were calculated by CalcuSynl 2.1.

3. Results and Discussion

3.1. Anti-proliferative Activities and Cytotoxicities of Adlay Extracts Towards MCF-10a cells

MCF-10a is a kind of non-tumorigenic normal human breast epithelial cells [31]. Generally, MCF-10a cells are sialomucins positive, cytokeratin-positive, milk fat globule antigen positive [32], and they were positive through MFA-Breast and MC-5 monoclonal antibodies, which indicated that MCF-10a cells express breast specific antigen [33,34].

The inhibition of human MCF-10a breast epithelial cell proliferation by the adlay extracts and the cytotoxic effects are shown in Figure 1. From Figure 1, for human MCF-10a breast epithelial cells, there were few differences among the three varieties of adlay polyphenol extracts in the dose range of 0-320 mg·ml^{-1}. As shown in Figure 1a, compared with the control, at 320 mg·ml^{-1} dose, free Guizhou Heigu adlay extracts has about 10% inhibition. All varieties of adlay, the calculated CC_{50} values were greater than 320 mg·ml^{-1}, which indicated that in the experimental concentration conditions, adlay extract samples did not reach 50% of cytotoxicity. From Figure 1 (a~f), adlay extracts has substantially no inhibition on human MCF-10a breast epithelial cells. Overall, free Guizhou Heigu adlay has slight inhibition on human MCF-10a breast epithelial cells, however, still amounted to less than 25% inhibition (Figure 1a and Table 1). While free Longyi 1 adlay has some promoting effect on human MCF-10a breast epithelial cells (Figure 1e and Table 1). Under 320 mg·ml^{-1} adlay extracts concentration condition, all adlay samples were not detected toxicity on human MCF-10a breast epithelial cells (Table 1). All these showed that the cytotoxicities and anti-proliferation of adlay extracts samples on normal human breast epithelial cells were in the normal range.

Table 1. Anti-proliferative activities (EC_{25} and EC_{50}) and cytotoxicities (CC_{50}) of adlay extracts towards human MCF-10a breast epithelial cells

Variety		Anti-proliferative activities		Cytotoxicities
		EC_{25} [mg·ml^{-1}]	EC_{50} [mg·ml^{-1}]	CC_{50} [mg·ml^{-1}]
Guizhou Heigu	Free	ND	ND	>320
	Bound	ND	ND	>320
Liaoning 5	Free	ND	ND	>320
	Bound	ND	ND	>320
Longyi 1	Free	ND	ND	>320
	Bound	ND	ND	>320

*ND (not detected)

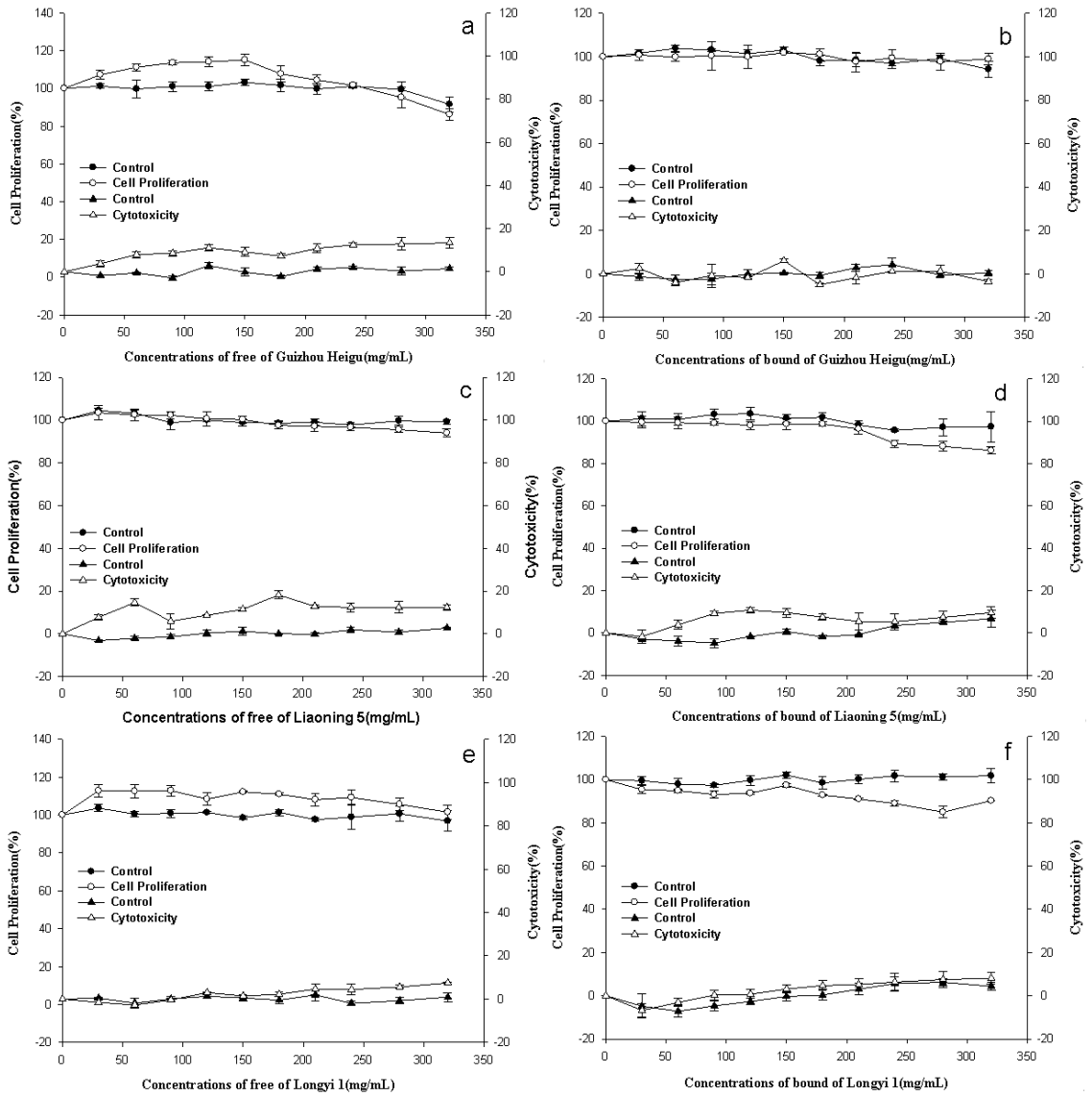

Figure 1. Percent inhibition of human MCF-10a breast epithelial cells proliferation and cytotoxicity by 3 adlay extracts (a~f)

3.2. Anti-proliferative Activities and Cytotoxicities of Adlay Extracts towards MDA-MB-231 Cells

Table 2. Anti-proliferative activities (EC_{25} and EC_{50}) and cytotoxicities (CC_{50}) of adlay extracts towards human MDA-MB-231 breast cancer cells

Variety		Anti-proliferative activities		Cytotoxicities
		EC_{25} (mg.ml^{-1})	EC_{50}(mg.ml^{-1})	CC_{50},(mg·ml^{-1})
Guizhou Heigu	Free	ND	ND	>320
	Bound	105.64±8.36	234.47±13.76	>320
Liaoning 5	Free	60.39±6.54	124.52±10.17	>320
	Bound	111.56±3.30	170.84±6.53	>320
Longyi 1	Free	324.51±13.14	ND	>320
	Bound	189.73±6.99	223.08±11.73	>320

*ND (not detected)

Human MDA-MB-231 breast cancer cells is a kind of breast epithelial cancer cells, which is classic one of the model cells to study breast cancer metastasisl. The inhibition of human MDA-MB-231 breast epithelial cell proliferation by the adlay extracts and the cytotoxic effects are shown in Figure 2. From Figure 2, adlay extracts have effectively anti-proliferative activity to human MDA-MB-231 breast cancer cells in the dose range that they can grow. At an adlay extract concentration of 320 mg·ml^{-1}, the inhibitory effect ranged from 12% (for free Guizhou Heigu adlay, Figure 2a) to 85% (for free Liaoning 5 adlay, Figure 2c). Free Liaoning 5 adlay, bound Guizhou Heigu adlay, bound Liaoning 5 adlay and bound Longyi 1 adlay have a relatively high anti-proliferative effect capability on human MDA-MB-231 breast cancer cells (Figure 2 and Table 2). Anti-proliferative activities (EC_{25} and EC_{50}) of different varieties of adlay polyphenol extracts towards human MDA-MB-231 breast cancer cells were shown in Table 2. The lower the EC_{25} and EC_{50} values are, the higher the ability of its anti-proliferative effect is. In these three varieties of adlay extracts, the anti-proliferative abilities towards human MDA-MB-231 breast cancer cells were obviously different. Overall, the anti-proliferative capacity of bound adlay extracts to MDA-MB-231 cells is greater than free one. Meanwhile, the anti-proliferative capacity of Liaoning 5 adlay extracts to human MDA-MB-231 breast cancer cells is the strongest. The 320 mg·ml^{-1} adlay concentration had no cytotoxic effects towards MDA-MB-231 cells (Table 2), which indicated that inhibitory effect was not attributed to a cytotoxic effect but to the anti-tumor effects of the extracts. Chang et al. [35] have studied that feed 30% adlay powder to the experimental animals can reduced approximately 50% of the incidence of lung cancer, which was close to this

experimental results. As can be seen from Figure 2, the anti-proliferative capacities of the extracts of bound Guizhou Heigu adlay, Liaoning 5 adlay, Longyi 1 adlay and free Liaoning 5 adlay towards human breast cancer cell were more than 50%. And free Liaoning 5 adlay was the strongest, and its inhibition rate reached 85%. Different from lung cancer cells studied in Chang et al' experiment, this experiment was focused on breast cancer and liver cancer cells. The results showed that the adlay extracts all have relatively good anti-proliferative effect to tumor cells. Moreover, studies have reported that, coix ester also has a certain anti-cancer effect [32], and its anti-tumor effect has been proved in vitro and test tube conditions currently.

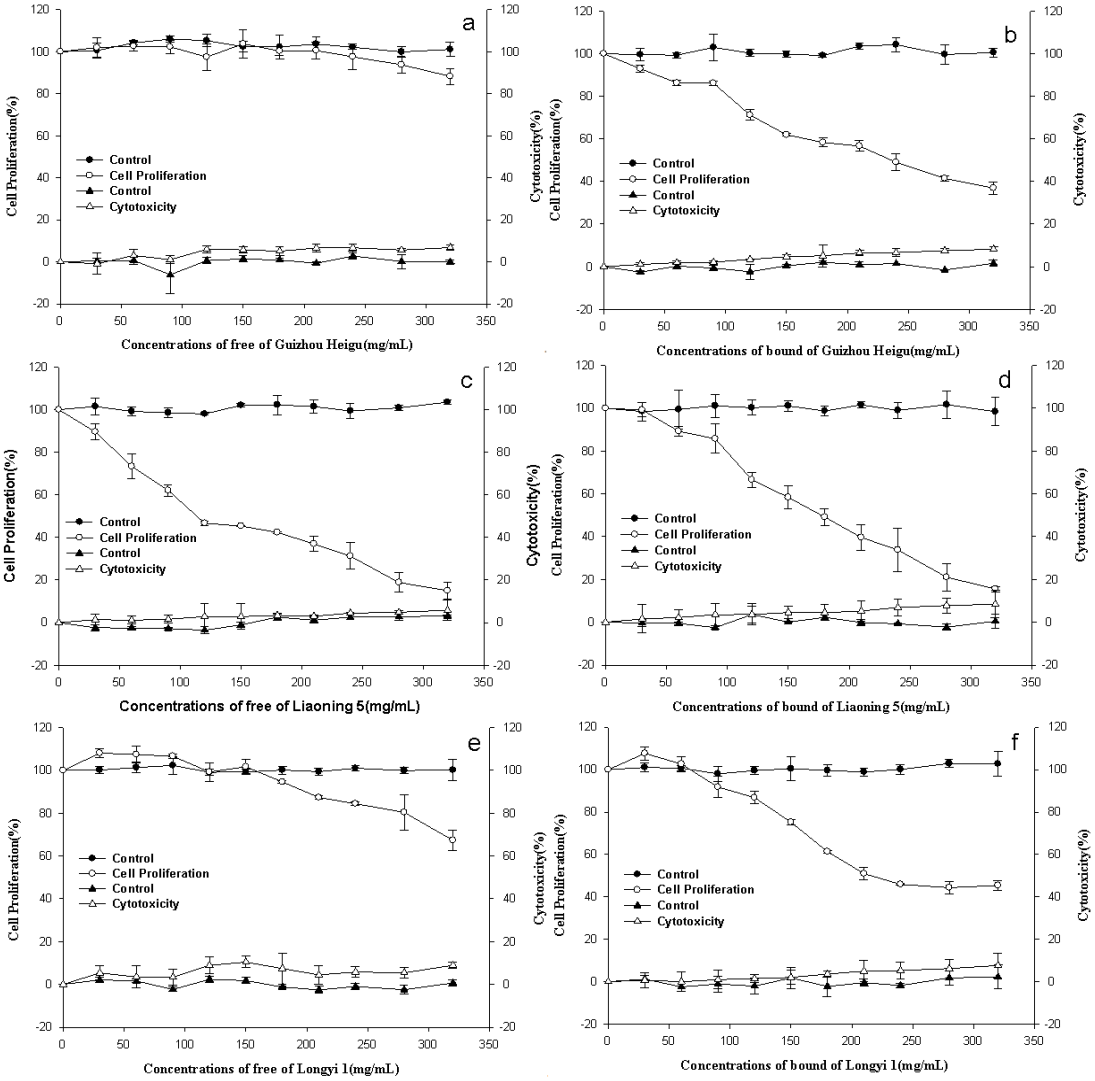

Figure 2. Percent inhibition of human MDA-MB-231 breast cancer cells proliferation and cytotoxicity by 3 adlay extracts (a~f)

3.3. Anti-proliferative Activities and Cytotoxicities of Adlay Extracts towards MCF-7 cells

Human MCF-7 breast cancer cells, compared with MDA-MB-231 cells, is more commonly used as a model of breast cancer metastasis. MCF-7 cells are epithelial cells, exhibit some characteristics of differentiation breast epithelial cells [36], and produce insulin-like growth factor binding protein [37]. The inhibition of human MCF-7 breast epithelial cell proliferation by the adlay extracts and the cytotoxic effects are shown in Figure 3. From Figure 3, anti-proliferative activities of different adlay extract samples towards human breast cancer MCF-7 cells were significantly different ($P<0.05$), and the difference of its cytotoxicity compared to the cells of the above studies is relatively large. EC_{50} values can be no measured for the anti-proliferative activities of free Guizhou Heigu adlay and Longyi 1 adlay (including free and bound) (Table 3). Compared with human MDA-MB-231 breast cancer cells, the results were consistency that anti-proliferative activities of the free Guizhou Heigu adlay and Longyi 1 adlay (including free and bound) were relatively weak. All these indicated that there were significant differences among antitumor effect of different adlay extract samples towards human breast cancer cells. At an adlay extract concentration of 320 $mg \cdot ml^{-1}$, the inhibitory effect ranged from 28% (for free Longyi 1 adlay, Figure 3e) to 85% (for free Liaoning 5 adlay, Figure 3c), and the CC_{50} value of Liaoning 5 adlay was 288.85 ± 7.83 $mg \cdot ml^{-1}$, while CC_{50} values of the remaining samples can not be calculated (Table 3). This showed that while bound Liaoning 5 adlay had very strong anti-proliferative activity, its cytotoxicity also increased with increasing concentration and reached more than 50%. So it was not sure while the anti-proliferative effect of bound Liaoning 5 adlay was due to its anti-tumor capability or cytotoxicity which caused the existence of its anti

proliferation effect. For bound Liaoning 5 adlay, the EC_{50} and CC_{50} values intersected at 289 mg·ml^{-1} (Figure 3d). At this concentration, we believe that the anti proliferation effect of bound Liaoning 5 adlay is caused by the sample's anti-tumor effect, rather than the cytotoxicity. And at this time, inhibition of cell proliferation of bound Liaoning 5 adlay was 70%.

Kim [38] studied the anti-proliferative activity of lemon extracts on MDA-MB-231 and MCF-7 cells. The results showed that the inhibitory effect of lemon extracts on MDA-MB-231 cells was very weak, while for MCF-7 cells, the extracts had obvious anti-proliferation capability. This experiment also compared the proliferation effect of two breast cancer cells, and the effects of these two kinds of cells were basically the same. This may indicate different from lemon, anti-proliferative effect of adlay extracts samples towards breast cancer cells was essentially at the same level.

Table 3. Anti-proliferative activities (EC_{25} and EC_{50}) and cytotoxicities (CC_{50}) of adlay extracts towards human MCF-7 breast cancer cells

Variety		Anti-proliferative activities		Cytotoxicities
		EC_{25} (mg.ml^{-1})	EC_{50} (mg.ml^{-1})	CC_{50},(mg·ml^{-1})
Guizhou Heigu	Free	213.68±13.58	ND	>320
	Bound	162.45±6.57	260.56±16.77	>320
Liaoning 5	Free	125.45±1.51	210.65±0.81	>320
	Bound	174.44±3.16	288.85±4.02	288.85±7.83
Longyi 1	Free	273.25±16.23	ND	>320
	Bound	234.08±12.51	ND	>320

*ND (not detected)

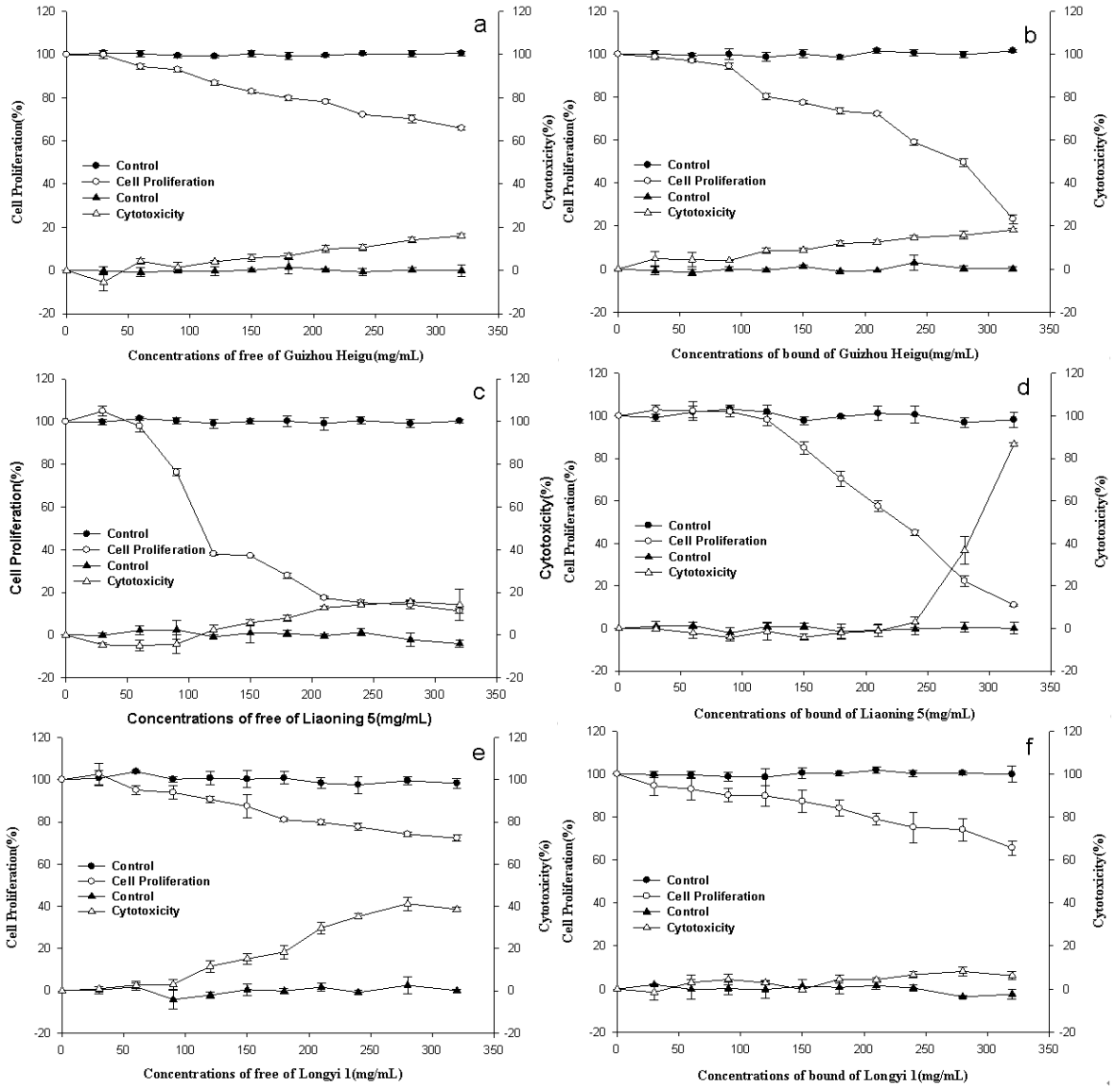

Figure 3. Percent inhibition of human MCF-7 breast cancer cells proliferation and cytotoxicity by 3 adlay extracts (a~f)

3.4. Effects of Adlay Extracts on Expression of Cyclin in HepG2 Cells

Previous study has showed that adlay extracts have obvious anti-proliferation effect on human hepatoma HepG2 cells [39]. Further to study the mechanism of inhibition of tumor cell proliferation of adlay extracts, detected the expression levels of proteins which were related to cell proliferation and cell cycle in HepG2 cells after being processed by adlay extracts by using western-blot test. The results were shown in Figure 4.

From Figure 4, the expression of PCNA in HepG2 cells significantly decreased with adlay extracts concentration increased and showed a significant dose-effect dependent relationship. When the concentration of adlay extracts was 180 mg·ml^{-1}, PCNA in HepG2 cells was inhibited 50%, which indicated that adlay extracts played an important role during S phase (DNA synthesis phase) of cell cycle of

HepG2 cells. On this basis, the study has further analyzed the effect of p21 and CDK4 in HepG2 cells to cells cycle. When the concentration of adlay extracts was 150 mg·ml^{-1}, the expression of p21 increased. And when the concentration of adlay extracts was 180 mg·ml^{-1}, the expression of p21 increased 100% compared with the control. Besides these, the experiment also has studied the effect of CDK4 to protein expression. As shown in the figure, CDK4 in HepG2 cells was not measured apparently in the experiment, which may be due to significant decreased expression. All these showed that adlay extracts inhibit the proliferation of HepG2 cells by regulating the cell cycle mediated by DNA polymerase adjustment factors such as PCNA and p21.

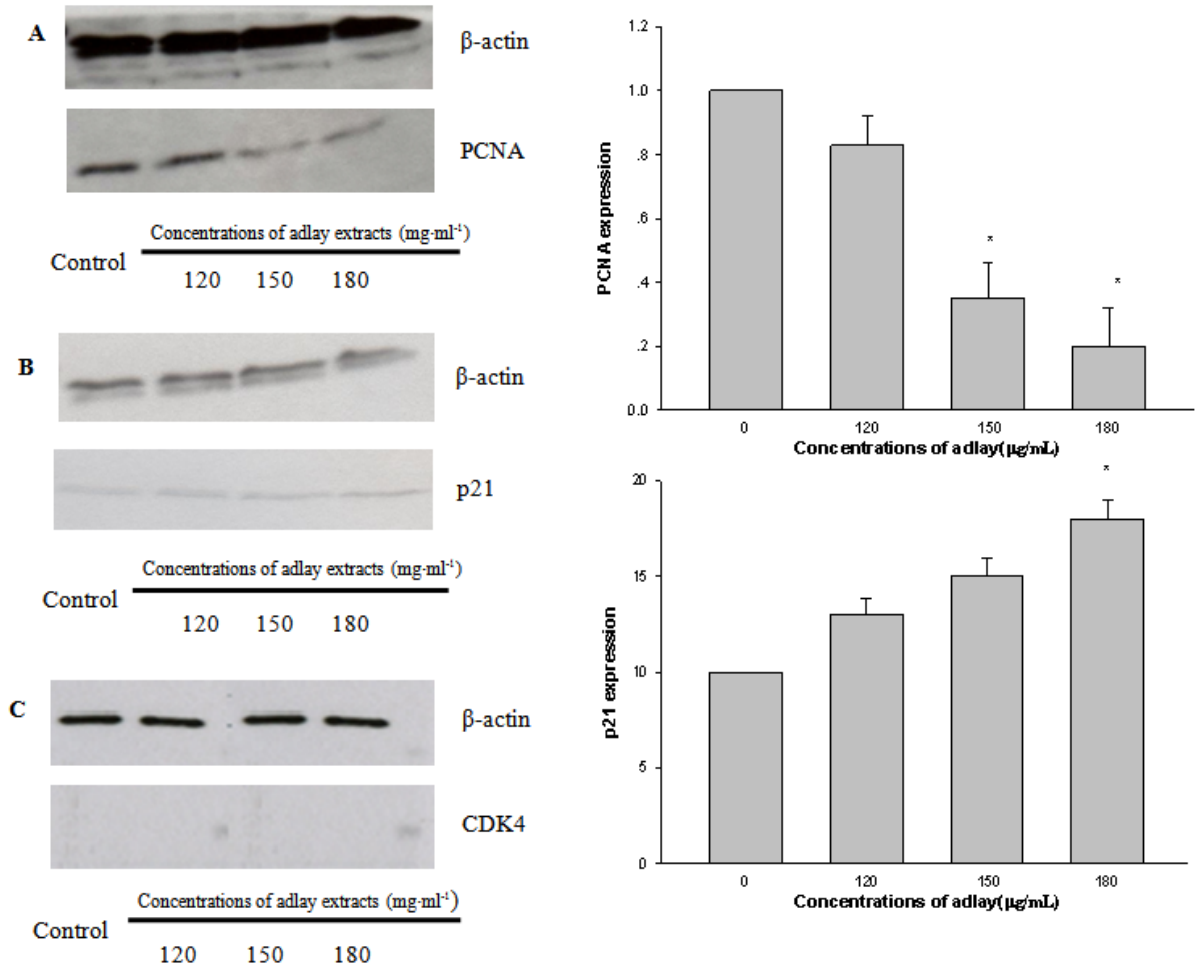

Figure 4. Effects of adlay extracts on expression of PCNA (A), p21 (B), and CDK4 (C) in HepG2 cells. An asterisk (*) indicate $p < 0.05$ when compared to the control

4. Conclusion

This research mainly focus on the anti-proliferative activities and cytotoxicities of adlay phenolic extracts towards normal cells and tumor cells in vitro. The results showed that different varieties of adlay extracts had obvious different anti-proliferative effect to tumor cells, and adlay samples themselves had no cytotoxicity in the experimental concentration range, which proved that the anti-proliferative activity was not caused by the toxicity of the sample but its anti-tumor effects.which proved that the anti-proliferative activity was not caused by the toxicity of the sample itself. Moreover, to HepG2, adlay extracts can obviously reduce the expression of PCNA, and increase that of p21 at the same time, which showed that the extracts inhibit the proliferation of HepG2 cells by regulating the cell cycle mediated by DNA polymerase adjustment factors such as PCNA and p21.

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