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In Vitro CYTOTOXIC STUDY FOR PARTIAL PURE LIGNAN EXTRACT FROM FLAX SEED (*Linum usitatissirnurn*) ON RHABDOMYOSARRCOMA (RD) AND MURINE MAMMARY ADENOCARCINOMA (MMA) CELL LINES

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Abstract: *The lignan (Secoisolaricinesinal diglucoside, SDG), was obtained from flaxseed (Linum usitatissirnurn) appear to act as both anti-estrogens and weak estrogens. The anti-estrogenic potential of lignans has been postulated to protect against hormone sensitive cancers such as breast, prostate and colon cancer. The aim of this study was to examine the cytotoxic effect of partial pure lignan on some cell lines including, the Murine Mammary Adenocarcinoma, Rhabdomyosarcoma RD, L20B and normal cell line (Rat Embryo Fibroblast REF). Four concentrations of partial pure SDG which obtained by using alcohol hydrolysis dioxin / ethanol flaxseed extraction and liquid/liquid partition, have been prepared 5, 10, 15, 20 µM with different of exposure periods 24, 48, 72 h. These parameters have been applied on all cancer and normal cell line. The highest percentage of inhibition appears in 5, 10,15 µM at 24 h (60.11, 49.99, 50.28 %) respectively on RD cell line when treated with partial pure. and the with same concentrations this percentage decreases without any significant difference. In case of L20B cell line in 72 h, the inhibitory effect of partial pure at 5,10 µM, 57.67 and 52.11 % respectively. While the cytotoxicity of partial pure SDG on ANM3 cell line appeared in 24h at 15um which was 64.88%. The lignans are growth inhibitors of colon tumor cells and suggest act through mechanisms other than anti-estrogenic activity.*

Key Words*: Lignan (Secoisolaricinesinal diglucoside), matairesinol, Murine mammary adenocarcinoma, Rhabdomyosarcoma RD, L20B cell lines, Inhibitory effect, growth inhibitors*.

I. Introduction

The mammalian lignan production from various raw plant foods has already been determined [1]. However, there is currently a lack of data on the lignan content of processed foods. Further, while flaxseed is the richest source of plant lignan precursors, it is not known if processing will reduce the production of mammalian lignans. Flaxseed has been used in anticarcinogenesis studies because of its high level of mammalian Iignan production [1]. In another study rats fed diets that were supplemented with 5% ground flaxseed for four weeks, showed reductions in epithelial cell proliferation, as measured by labeling index and mitotic index, and decreased nuclear aberrations in the terminal end bud of the mammary gland [2]. Lignans have an important role in breast cancer through some studies, the effects of flaxseed lignans on breast tumor cell biology were investigated in 39 women with newly diagnosed cancerous breast tumors [3]. Lignans could be prevent prostate cancer through their estrogenic activity, these plant compounds can interfere with steroid metabolism and bioavailabity, and also inhibit enzymes, such as tyrosine kinase and topoisomerase, which are crucial to cellular proliferation and hence contribute to lower incidences of prostate cancer. A study involved twenty-five patients with prostate cancer, which were given a lignan-rich flaxseed supplementation. The results show a favorable affect on prostate cancer biology and associated biomarkers [4]. The aim of this study are to determination the optimal cytotoxic concentration(CC50) of partially pure lignan on some cancer cell lines and the period at which the effect will appear on cancer cells growth in vitro.

II. Materials and Methods

2.1.Collections of samples: Flaxseeds were obtained from Al-Rabae center for Agricultural and Food researches/Mnistry of Sciences and Technology and classified under AL- Maha class I. Grinding flaxseeds properly by a grinder machine eventually obtained on a homogenized powder that was ready for extraction.

2.2.Extraction of Crud Lignan: The method which was described by Rickard *et al*. [5]; Mahdi, [6] involves taking (25)g of defatted powder treats with a mixture of Dioxan and Ethanol alcohol (1:1),(v:v), with a ratio (1:8),(w:v),(powder: solvent),sample put on magnetic stir for (4)hrs.

2.3.Separation of Lignan: The process of separation includes dissolving a certain amount of dried material , in 50 ml of hydrolyzing agent solution . The mixing of this sample continued (48) h. To be homogenized properly on a shaker. The mixture was filtered the supernatant was concentrated with a rotary evaporator within (45) ^{0}c. Eventually, a thick sticky texture material, pH was 3.0 then the sample was stored in (4) ^{0}c.

2.4.Liquid/Liquid Partition: This method involves (liquid/ liquid) separation according to Westcott and Muir,[7].There were two separating solvent systems which were differed in their polarity these systems include: Ethyl acetate: distilled water (1:7).

2.5.Cytotoxic Effects of Lignan on Cell Lines *in vitro:* The *in vitro* method was used to investigate the effect of pure lignan on two types of tumor cell lines REF Rat Embryo Fibroblast and AMN3 Mouse Mammary Adenocarcinoma were obtained from Iraqi Center for Cancer and Medical Genetics research, passage (119) and passage (54) respectively. These lines were cultured on RPMI 1640 with (10%) fetal calf serum (FCS). Another Two lines RD Human Rhabdomyosarcoma and L20B were obtained from Central health laboratory, passage (13) and passage (21). These lines were cultured on MEM media that supplemented with (10%) fetal calf serum (FCS).

2.6.Cytotoxicity assay: It is also called a cell growth inhibition assay. In this assay, the three types of cell lines were treated with pure lignan extract and standard concentrations ranging from 50 µg/ml to 100 µg/ml, at the same time the lines were exposed to methotrexate drug in concentrations ranging from 0.05 µg/ml to 0.4 µg/ml using a microtiteration plate (96 wells) cell culture technique according to Freshney [8]. The plates of different cell culture at the end of the assay were examined by ELISA reader at 492 nm transmitting wave length .Only viable cells were able to take the stain , the dead cells were not. The proliferation rate was measured according to Freshney [8] as follows:

roliferation rate % =

$$\frac{\text{Absorbance at 492 nm of control}}{\text{Absorbance at 492 nm of test}} \times 100$$

While the inhibitor rate was measured according to Thompson *et al*.,[9] as follows:

Inhibitor rate % =

$$\frac{\text{Abs. at 492 nm of control} - \text{Abs. at 492 nm of test}}{\text{Abs. at 492 nm of control}} \times 100$$

Abs = Absorbance

The –ve results referred to the inhibition rate %

While the +ve results referred to proliferation rate %.

Data were analyzed statistically by using the Complete Random Design (CRD) in factorial an experiment which was include four factors (multifactorial) (cell lines, type of purification concentrations and durations of exposure). The statistical programme that was used for analysis of the data is SPSS version 10 [10]. And for examination of the specific significant differences among factors by using the multi limits Duncan test [11].

III. Results

The lignan spots from silica gel (60F 254) were scratched according to their affinity to the mobile phase (ethyl acetate : ethyl alcohol)(1:9) only apots that gave positive molish's test and benedict's test and fehling' were collected. Pure lignan gave violet fluorescence spots on silica gel with Rf value=0.45 [6]. These results were compared with other researchers on the same compounds like [12;13] and Westcott *et al*., [14] which they used the same technique. All cell lines were treated with different four concentrations and with different exposure times 24, 48, 72 hours these concentrations are measured together with (5,10,15,20 µM) respectively.

Cytotoxic Effect of Lignan on RD Cell line: The cells were treated with partial pure SDG under the same conditions as shown in table (1) which shows that the direction of lignan effect is similar to pure SDG. The

inhibitory effect of compound decreases with the increase of the concentrations and with a difference of the interval period in exposure. The apparent significant (P<0.05) differences were observed within different concentrations. The highest ratio of inhibition was reported in 5 µM for 24 h. This ratio was started to decrease with the elevation of concentration. On the other hand there is no difference between 5, 10 and 20 µM at all times except a difference in 20 µM that was started at this concentration at 72 h (P<0.01). In this table data indicate that the partial pure lignan act properly against cells proliferation or metastases. The ratio of inhibition

was observed in 15 and 20 µM at 24, 48 h respectively between both, where at 24 h there was a good effect of the partial pure. These results were analyzed statistically with high significant differences among concentrations that were used, as demonstrated in tables (1)**.** The cytotoxic effect of partial pure is at 5 µM after 24 h of exposure at this time of treatment the severity of cytotoxicity was very high compared with 48 and 72 h respectively, this effect was decreased with increasing the time of exposure. So, the concentration of high significance was started towards the lowest concentration when duration of exposure was decreased for partial pure lignan i.e. the severity of effect depends on time and dose (time-dose-dependent), more potent in the inhibition of cancer cells growth or act as anti-proliferative compound. Hongyan *et al*., [15] when they used lignans on SW480 cell line (colon cancer), They observed that the lignans have no effect on cancer cells at a high concentrations i.e. no cytotoxic effect on cells, but at the same time they reported that the lignans metabolites effect on cell cycle and through DNA flow cytometric analysis.

Table (1): Inhibitory rate (IR%) of partial pure SDG on RD cell line

CONC.		Durations of exposure			SIG.
		24h	**48h**	**72h**	
pure Partial SDG	5µM	60.11± 5.55a(A)	57.55 ±2.7a(A)	52.47 ±7.34 a(A)	0.633
	10µM	49.99 ±5.30a(A)	50.65±1.23 a(A)	42.15±2.33a(A)	0.227
	15µM	50.28±5.20a(A)	34.08±3.30b(A)	15.84±6.64 b(B)	0.01
	20µM	19.25±3.50b(A)	12.43±3.84c(A)	12.51±2.62b(A)	0.327
SIG.		0.002	0.001	0.002	

Small same letters mean there are no significant differences within same column among different concentrations at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG. Capital same letters mean there are no significant differences within the same row among different periods at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG.

Cytotoxic Effect of Lignan on L20B Cell line: Table (2) where shows an apparent significant difference among concentrations and times of exposure observed for 24 h in 5 µM the percentage of inhibition 39.10 % . It was very high compared with other concentrations. So, an apparent difference was reported at this period.This result changes in 48 h. the ratio was increased into 50.68% but in 10 µM the inhibition rate was increased 53.82% at the same time. For 72 h the highest ratio recorded is 57.67% within 5 µM. This indicates that the pure was reduced with respect to other concentrations during the same period of treatment. So, a high significant (P<0.05) difference was observed for 24 and 72 h among concentrations, while for the 48 h there was no differences within concentrations. According to the data listed in table (2). There is no difference in the concentrations of partial pure SDG at the first 24 h, but the highest inhibition rate at this period was observed within 20 µM, so the viable cell was reduced into half of its number 53.30 %. In the other periods 48 and 72 h apparent significant (P< 0.001) differences was reported. The differences were observed within all concentrations at all times except within 15 µM when no difference was reported. Partial pure has in the concentrations at the same periods. Significant (P<0.05) differences were observed for 24 and 48 and 72 h.However for 24 h within 5 µM,48 h within 20 µM and for 72 h within 10 and 15 µM no differences were observed. Results indicate that the partial pure SDG have anti proliferative effect on L20Bcell line according the data documented from this experiment. The highest inhibition rate was observed at 72 h within 5 µM. At the same concentration, no significant differences were observed at 24, 48 h. So, these cells are sensitive to lignan. This pattern was observed when these cells are treated with partial pure .At 72 h there was an effect on cells growth. The effect of partial pure was depends on dose and not on time (dose-dependent manner). There were no studies examining the effects of partial pure lignan on cell lines generally. These data are similar to the previous results observed on RD and AMN3. So, pure can inhibit the growth of MCF-7 breast cancer cell line

(dose-dependent manner) [16] and LS174T, Caco-2, HCT-15, and T-84 human colon tumor cell lines [17], and more effectively displaced both estradiol and testosterone binding by sex hormone-binding globulin [18]. So, low dose was played an important role in the inhibition of cells growth while the duration of exposure 24,48,72 h was not important in the change of inhibition rate with simple differences between these ratios ,this agrees with all studies mentioned above. These results were agreement with a study carried out by Waldschager *et al*., [19] which showed an inhibitory influence of some of the isolated flaxseed fractions on the Jeg3 tumor cells.

Proliferation of the Jeg3 cells was decreased by flaxseed fractions I, V, VI and VII in a dose-dependent manner. Some extract fractions showed a stimulating effect on hormone production and cell proliferation.

Table (2): Inhibitory rate (IR %) of partial pure SDG on L20B cell line

Conc.		Durations of exposure			SIG.
		24h	48h	72h	
Partial pure SDG	5 µM	39.10±7.67 a(B)	50.68±1.66 a(AB)	57.67±1.26a(A)	0.073
	10 µM	39.32±1.79a(B)	53.82±0.73a(A)	52.11±2.33ab(A)	0.005
	15 µM	46.80±2.55a(A)	41.91±0.96b(A)	43.67±2.75b(A)	0.357
	20 µM	53.30±3.50a(A)	33.77±1.36c(B)	31.77±3.58c(B)	0.001
SIG.		0.118	0.001	0.001	

Small same letters mean there are no significant differences within same column among different concentrations at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG.
Capital same letters mean there are no significant differences within the same row among different periods at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG.

Cytotoxic Effect of Lignan on REF Cell line (Normal cells): According to the data listed in table (3). There are no significant differences for all exposure times 24, 48 and 72 h within four concentrations except at 48 h within 10 µM. When the normal cell line was treated with pure lignan, no effect was observed, i.e. the cells continue in growth and lignan not prevent or stopped the cells proliferation according to its viable number (CC 50). However, when cells were treated with partial pure, differences were reported in the concentrations for 24 h and 72 h but without effect, but within 5 µM for 24, 48 h no differences were found, but for 72 h which was differed from both. Within 15 µM, a significant difference was observed for all three periods, as shown in table (3). The degrees of significance partial pure were listed in table (3). No significant differences within four concentrations and all times of exposure except for 72 h a difference observed within 15 µM a significant (P<0.03)difference is observed, where the percentage in case of pure for 72 h within 15 µM is 4.09 %. Although there is no effect on normal cells growth of partial pure lignan this difference within 15 µM has an apparent effect .there are no differences among other concentrations at all times of exposure. According to the data obtained ,when normal cell line Rat Embryo Fibroblast (REF), these results indicate that the pure and partially pure lignan have no effect on normal cells growth, hence cells were continued in their proliferation at all durations of exposure and within all concentrations although some significance (P<0.05) differences appeared among concentrations. So, the results of this study agree with other studies and also the recommendations related to the consumption of flaxseed that were contained on lignans or their derivatives or unsaturated fatty acids aLA, which play an important role as antitumors or take part in the prevention against cancer by certain mechanisms that were mentioned previously. Many studies indicate that the lignans or their metabolites have no effect on normal cells growth. The results of this study do not differ from these studies or recommendations, which related to this field. Thompson *et al*.[9] , achieved a study. They found that the insulin-like growth factor (IGF) could develop breast cancer when exist in a high level. IGF mediates the actions of estrogen in breast cancer cell lines, and can stimulate the estrogen-signaling pathway in the absence of estrogen. Insulin-like growth factor (IGF) increases cell proliferation by protecting tumor and normal cells from apoptosis; so lignan can reduce the level of (IGF) through a study that was applied *in vivo* on rats that were either fed a 5% flaxseed (i.e.high-lignan) diet or given 1.5 mg/day of solariciresinol diglycoside (a plant lignan precursor of mammalian lignans) had IGF-1 levels significantly lower than those in rats fed a control diet.

Table (3): Inhibitory rate (IR %) of partial pure SDG on REF cell line

Conc.		Durations of exposure			SIG.
		24h	48h	72h	
Partial pure SDG	5 µM	3.27±1.71b(B)	7.35±3.19a(B)	37.05±9.66a(A)	0.014
	10 µM	18.41±5.56a(A)	8.55±3.96a(A)	24.61±8.18ab(A)	0.255
	15 µM	9.32±0.89ab(A)	3.31±0.38a(B)	4.09±2.54b(AB)	0.068
	20 µM	4.65±2.49b(A)	10.58±6.70a(A)	11.16±6.07b(A)	0.662
SIG.		0.038	0.675	0.047	

Small same letters mean there are no significant differences within same column among different concentrations at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG.
Capital same letters mean there are no significant differences within the same row among different periods at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG.

Compression between four types of cell lines within the concentrations at all Durations of Exposure for partial pure lignan

Effect of Partial Pure within 24 hours within concentrations: According to the results demonstrated in table (4), significant differences were observed for 24 h within all concentrations used, for all types of cell lines, the highest percentage of inhibition rate was appeared on AMN3 cell line within 15 µM and reached to 76.48%. This ratio differed in another cell lines. So, apparent significant (P<0.001) differences were observed within this concentration. On RD cell line the highest inhibitory rate was reported within 5 µM when reached 69.85% and there are differences between RD, AMN3, L20B and REF within this concentration. The lowest effect of lignan which was appeared on REF cell line within 5 µM is 9.67% i.e. pure lignan was played a role in an inhibition of three types of cancer cell lines and at the same time has no effect on normal cell line within the same concentration and the same time. Significant differences were appeared too with in the 10 µM among cell lines that are similar to that appeared last. While in 10 µM, the inhibitory rate on RD, L20B, AMN3 and REF cell lines 63.18,46.21,66.42 and26.31% respectively at (P<0.001).This pattern was continued with the other concentrations. On the level of the same cell line, there was a high significant difference (P<0.001) was reported in RD cell line. Within four serial concentrations. This difference was appeared on L20B (P<0.03) and AMN3 (P<0.001). Pure lignan has an effective role in stopping cancer cell proliferation within some concentrations and has no effect within the four concentrations on normal cell line. So, no significant difference (P<0.05) was observed. According to the data in table (4), significant differences were observed among concentrations on four types of cell lines. Within four

concentrations a high significant (P<0.001) differences.The highest inhibition rate was observed within 15 µM which was reached to 64.88%on AMN3 but the lowest ratio was appeared on REF cell line which was reduced into 3.27% within 5 µM This pattern was appeared on the other concentration when treated with partial pure lignan, but on the level of the same cell line no apparent significant difference was observed on all cell lines except L20B cell line where no difference appeared within the four concentrations as shown in table (4).

Table (4), Compression between types of cell lines (dose-time dependent) for 24 h.

CONC.		Cell lines				SIG.
		REF	AMN	L20B	RD	
PARTIAL PURE SDG	5 µM	3.27±1.71b(C)	63.15±2.14a(A)	39.10±7.67a(B)	60.11±5.55a(A)	**0.001**
	10µM	18.41±5.56a(B)	50.73±1.16b(A)	39.32±1.79a(A)	49.99±5.30a(A)	**0.001**
	15µM	9.32±0.89ab(C)	64.88±1.61a(A)	46.80±2.55a(B)	50.28±5.20a(B)	**0.001**
	20µM	4.65±2.49b(C)	50.77±0.74b(A)	53.30±3.50a(A)	19.25±3.50b(B)	**0.001**
SIG.		**0.038**	**0.001**	**0.118**	**0.002**	

Small same letters mean there are no significant differences within the same column among different concentrations at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG. Capital same letters mean there are no significant differences within the same row among different periods at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG.

Effect of Partial Pure for 48 hours within concentrations:

According to the data listed in table (5), the effect of pure is apparent with high significant (P<0.001) d ifferences within all concentrations at all times of exposure, except within 20 µM (P<0.06). So, the inhibitory effect of pure lignan is clear on RD cell line which was started within 5 µM with a percentage of 51.68% and this effect was continued in its elevation when within 10 µM was reached to 54.47% and decline slightly within 15 µM until reaching into 42.45% within 20 µM with a significant (P<0.08).This type of effect was observed on the other difference types of cancer cell lines AMN3 and L20B,with differences in the proportions of inhibition.

Returning to the table (5) the data which show partial pure was effected the growth of all cancer cell lines except REF cell line with a high significant differences (P<0.001) with all concentrations. The data listed in table (5) demonstrate a significant effect on cancer cells proliferation. Therefore, the inhibition rates were appeared within 5 and10 µM on cancer cells but there was no inhibitory effect on REF normal cell line within these concentrations. This rate was reduced by increasing the concentrations observed in cancer cells. Thereby, these results prove that the partial pure lignan was played an important role in inhibition of cancer cells growth and that turn, proves that it has no inhibitory effect on REF normal cells too.

Table (5): Compression between types of cell lines (dose-time dependent) for 48 h.

Small same letters mean there are no significant differences within same column among

CONC.		Cell lines				SIG.
		REF	AMN	L20B	RD	
PARTIAL PURE SDG	5µM	7.35+3.19a(B)	52.44+2.28a(A)	50.68+1.66a(A)	57.55+2.70a(A)	0.001
	10µM	8.55±3.96a(B)	55.29±2.26a(A)	53.82±0.73a(A)	50.65±1.23a(A)	0.001
	15µM	3.31+0.38a(C)	47.56+4.15a(A)	41.91+0.96b(AB)	34.08+3.30b(B)	0.001
	20µM	10.58+6.70a(B)	48.45+4.45a(A)	33.77+1.36c(A)	12.43+3.84c(B)	0.001
SIG.		0.675	0.407	0.001	0.001	

different concentrations at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure

Table (6): Compression between types of cell lines (dose-time dependent) for 72 h.

Capital same letters mean there are no significant differences within the same row among different periods at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for partial pure SDG.

CONC.		Cell lines				SIG.
		REF	AMN	L20B	RD	
PARTIAL PURE SDG	5µM	37.05±9.66a(A)	52.90±3.81a(A)	57.67±1.26a(A)	52.47±7.34a(A)	0.197
	10µM	24.61±8.18ab(B)	51.74±1.84a(A)	52.11±2.33ab(A)	42.15±2.33a(A)	0.009
	15µM	4.09±2.54b(B)	43.50+4.29ab(A)	43.67+2.75b(A)	15.84+6.64b(B)	0.001
	20µM	11.16±6.07b(B)	37.18±2.24b(A)	31.77±3.58c(A)	12.51±2.62b(B)	0.003
SIG.		0.047	0.027	0.001	0.002	

Effect of Partial Pure within 72 hours within concentrations: Table (6) demonstrates that the partial pure has an effect on cancer cells proliferation with an apparent significant (P<0.05) difference within all concentrations, except within 5 µM when no difference among cells was reported. Therefore, the highest effect of partial pure was started within low concentrations and the inhibition effect was reduced with high concentrations in case of caner cells. This pattern was observed in pure lignan. This proves that the low concentrations have more effects on cancer cells than high concentrations for 72 h. At the same time, partial pure has no effect on normal cell line REF although a significant (P<0.04) difference was observed among the concentrations used. However, the cells go on in growing partial pure have no effects on normal cells. Small same letters mean there are no significant differences within same column among different concentrations at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for both pure and partial pure SDG. Capital same letters mean there are no significant differences within the same row among different periods at (P<0.05) according to Duncan's multiple ways test (Duncan, 1955) for both pure and partial pure SDG.

IV. Discussion:

The statistical results demonstrate the cytotoxic effect of partial pure lignan on cancer cell line and has no effect on normal cell line of Rat Embryo Fibroblast, during the interval periods of exposure which were involved (24, 48 and 72 h) respectively, and by the results which were analyzed statistically by observing significant differences among concentrations for both when cell lines were treated with these certain serial concentrations. Secoisolariciresinol (SDG) is the lignan found in high levels in flaxseed [20,21]. When consumed in the diet, SDG converted by intestinal bacteria to the important phytoestrogen, enterodiol and enterolactone (mammalian lignans) [22]. An abundance of animal and human research suggests that the flaxseed lignan SDG exerts effects similar to the anti-estrogen drug tamoxifen with an apparently much greater degree of tolerabilsity. Indeed, SDG is chemically similar to tamoxifen. Lignans are a promising class of compounds for use in breast cancer prevention and may be considered as natural Selective Estrogen Receptor Modulators SERMs [23,24]. That elucidated the mechanisms by which the mammary gland differentiation was enhanced and determined whether exposure to flaxseed or SDG during suckling could protect against dimethylbenzanthracene (DMBA)-induced mammary tumorigenesis at adulthood without adverse effects on selected reproductive indexes. In conclusion, exposure to flaxseed or SDG during suckling enhanced mammary gland

morphogenesis through modulation of epidermal growth factor receptor, and estrogen receptor- α and -ß, which resulted in more differentiated mammary glands [25].Lignans induced apoptosis in colorectal tumor cells through changing mitochondrial membrane potential and down regulation of the antiapoptotic protein, bcl (xl) [26].Another research was performed which involved the mammalian lignans enterolacton*e* (EL) and enterodiol (ED), which are derived from SDG. In this research, four human colon tumor cell lines were incubated with various levels of EL, ED, or 17 betal-estradiol for 8 to 10 days. At 100-microM concentration, both lignans significantly reduced cell proliferation of all cell lines. Enterolacton was more than twice as effective as ED was at this concentration. The growth was not affective by the presence of 17 beta-estradiol, implying that these cells are not estrogen-sensitive [26].

V. Conclusion:

These results led to the conclusion that the lignan has high influence on AMN3 cancer cell and has no influence on the growth of REF normal cell line. Tumor in our country has increased and distributed among many people, with different ratio in its type and stage. Therefore lignan, was selected in this project.*Linum usitatissimum* which extracted and purified from flaxseed, which already available locally and considered as a strong therapeutic agent against many disease as well as antitumor.

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