

Supplementary Materials

Ficus dubia latex extract induces cell cycle arrest and apoptosis by regulating the NF- κ B pathway in inflammatory human colorectal cancer cell lines

Supplementary Table S1. The evaluation of IC₂₀ and IC₅₀ of FDLE on HCT-116 and HT-29 colorectal cancer cell lines and NIH3T3 normal cells in non-inflammatory state and inflammatory state.

HCT-116 in non-inflammatory state			
	24 hours	48 hours	72 hours
IC ₂₀	578.32±7.63	136.65±5.27	114.58±3.85
IC ₅₀	>1000µg/mL	365.56±10.28	354.68±4.92
HCT-116 in inflammatory state			
	24 hours	48 hours	72 hours
IC ₂₀	512.86±6.12	50.35±5.74***	61.63±6.82**
IC ₅₀	>1000µg/mL	105.81±7.35****	124.95±8.44***
HT-29 in non-inflammatory state			
	24 hours	48 hours	72 hours
IC ₂₀	423.15±8.36	130.43±5.68	116.17±7.42
IC ₅₀	>1000µg/mL	330.72±11.26	370.55±9.81
HT-29 in inflammatory state			
	24 hours	48 hours	72 hours
IC ₂₀	553.64±7.22	64.94±4.12***	68.18±7.35**
IC ₅₀	>1000µg/mL	104.13±6.54****	110.16±12.18****
NIH3T3 in non-inflammatory state			
	24 hours	48 hours	72 hours
IC ₂₀	>600µg/mL	>600µg/mL	>600µg/mL
IC ₅₀	>1000µg/mL	>1000µg/mL	>1000µg/mL
NIH3T3 in inflammatory state			
	24 hours	48 hours	72 hours
IC ₂₀	>600µg/mL	136.85±8.26****	121.67±13.82****
IC ₅₀	>1000µg/mL	246.61±12.65****	225.26±15.33****

* Data are presented as the Mean ± SD of three independent experiments which are similar results, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. non-inflammatory cells in the same time.

Supplementary Table S2. The effect of the same concentration of FDLE on the inhibition ratio of colony formation for HCT-116 and HT-29 colorectal cancer cell lines in non-inflammatory state and inflammatory state.

FDLE($\mu\text{g}/\text{mL}$)	Colony formation inhibition ratio(%)		Colony formation inhibition ratio(%)	
	Non-inflammatory	Inflammatory	Non-inflammatory	Inflammatory
	HCT-116	HCT-116	HT-29	HT-29
50	16.63 \pm 1.22	19.28 \pm 1.85	17.36 \pm 2.12	21.39 \pm 3.54
100	39.81 \pm 1.48	39.40 \pm 6.13	39.50 \pm 5.43	36.34 \pm 6.12
200	47.79 \pm 3.28	58.45 \pm 2.23*	46.57 \pm 3.09	56.85 \pm 4.75*

* The inhibition ratio is relative to untreated group in the non-inflammatory state; The inhibition ratio is relative to cytokines-treated group in the inflammatory state, Data are presented as the Mean \pm SD of three independent experiments which are similar results, * p <0.05 vs. non-inflammatory state in the same cells.

Supplementary Table S3. The effect of the same concentration of FDLE on the increased ratio of cell cycle arrest at G0/G1 phase in HCT-116 and HT-29 colorectal cancer cell lines in non-inflammatory state and inflammatory state.

FDLE($\mu\text{g}/\text{mL}$)	Increased ratio of G0/G1(%)		Increased ratio of G0/G1(%)	
	Non-inflammatory	Inflammatory	Non-inflammatory	Inflammatory
	HCT-116	HCT-116	HT-29	HT-29
50	0.39 \pm 2.00	11.4 \pm 0.40***	1.40 \pm 1.61	7.80 \pm 1.56**
100	4.63 \pm 0.60	15.23 \pm 0.99****	3.30 \pm 2.01	12.10 \pm 1.87**
200	10.33 \pm 2.35	19.33 \pm 0.47**	9.13 \pm 2.07	16.60 \pm 2.01*

* The increased ratio of cell cycle arrest at G0/G1 phase were relative to untreated group in the non-inflammatory state; The increased ratio of cell cycle arrest at G0/G1 phase were relative to cytokines-treated group in the inflammatory state, Data are presented as the Mean \pm SD of three independent experiments which are similar results, * p <0.05, ** p <0.01, *** p <0.001 and **** p <0.0001 vs. non-inflammatory state in the same cells.

Supplementary Table S4. To compare the relative change ratio of FDLE at high dose of 200 $\mu\text{g}/\text{mL}$ on proteins related to cell proliferation in non-inflammatory and inflammatory HCT-116 and HT-29 human colorectal cancer cell lines

Proteins	Relative change ration in HCT-116 cells(%)		Relative change ration in HT-29 cells(%)	
	Non-inflammation	Inflammation	Non-inflammation	Inflammation
	(200 $\mu\text{g}/\text{mL}$ FDLE)	(cytokines+200 $\mu\text{g}/\text{mL}$ FDLE)	(200 $\mu\text{g}/\text{mL}$ FDLE)	(cytokines+200 $\mu\text{g}/\text{mL}$ FDLE)
NF- κ B(p65)	42.52 \pm 1.51 ↓	54.02 \pm 2.66** ↓	33.87 \pm 5.50 ↓	52.40 \pm 5.62* ↓
Cyclin D1	23.16 \pm 1.81 ↓	34.97 \pm 2.72** ↓	26.72 \pm 3.23 ↓	39.76 \pm 4.21* ↓
CDK4	17.90 \pm 4.90 ↓	23.34 \pm 4.50 ↓	41.04 \pm 3.84 ↓	54.89 \pm 4.00* ↓
P21	14.43 \pm 6.64 ↑	98.37 \pm 22.12** ↑	139.36 \pm 9.97 ↑	175.29 \pm 6.86* ↑
PCNA	4.69 \pm 9.09 ↓	6.74 \pm 16.40 ↓	0.01 \pm 8.20 ↓	8.99 \pm 5.73 ↓

The relative change ration in non-inflammation (200 $\mu\text{g}/\text{mL}$ FDLE) was compared to the untreated group. The relative change ration in inflammation (cytokines+200 $\mu\text{g}/\text{mL}$ FDLE) was compared to the cytokines-treated group. ↓: down-regulation, ↑: up-regulation. The results were represented as mean \pm SD of three independent experiments, * p <0.05 and ** p <0.01 vs. non-inflammation (200 $\mu\text{g}/\text{mL}$ FDLE) in the same cells.

Supplementary Table S5. The effect of the same concentration of FDLE on the increased ratio of apoptosis in HCT-116 and HT-29 colorectal cancer cell lines in non-inflammatory state and inflammatory state.

FDLE($\mu\text{g}/\text{mL}$)	Increased ratio of apoptosis(%)		Increased ratio of apoptosis (%)	
	Non-inflammatory	Inflammatory	Non-inflammatory	Inflammatory
	HCT-116	HCT-116	HT-29	HT-29
50	0.18 \pm 0.46	2.39 \pm 0.70*	0.11 \pm 0.16	0.06 \pm 0.09
100	0.11 \pm 0.02	6.32 \pm 1.03***	0.21 \pm 0.24	0.15 \pm 0.16
200	0.11 \pm 0.17	8.08 \pm 1.35***	0.05 \pm 0.34	0.03 \pm 0.12

* The increased ratio of apoptosis was relative to untreated group in the non-inflammatory state; The increased ratio of apoptosis was relative to cytokines-treated group in the inflammatory state, Data are presented as the Mean \pm SD of three independent experiments which are similar results, * p <0.05 and *** p <0.05 vs. non-inflammatory state in the same cells.

Supplementary Table S6. To compare the relative change ratio of FDLE at high dose of 200 $\mu\text{g}/\text{ml}$ on proteins related to apoptosis in non-inflammatory and inflammatory HCT-116 and HT-29 human colorectal cancer cell lines

Proteins	Relative change ration in HCT-116 cells(%)		Relative change ration in HT-29 cells(%)	
	Non-inflammation	Inflammation	Non-inflammation	Inflammation
	(200 $\mu\text{g}/\text{ml}$ FDLE)	(cytokines+200 $\mu\text{g}/\text{ml}$ FDLE)	(200 $\mu\text{g}/\text{ml}$ FDLE)	(cytokines+200 $\mu\text{g}/\text{ml}$ FDLE)
Bid	13.64 \pm 11.34 \uparrow	70.08 \pm 6.10*** \uparrow	15.63 \pm 7.71 \uparrow	67.92 \pm 11.99** \uparrow
Bak	5.41 \pm 5.54 \uparrow	123.12 \pm 40.20** \uparrow	11.25 \pm 4.66 \uparrow	43.78 \pm 10.57* \uparrow
Bcl-xL	25.50 \pm 8.30 \downarrow	9.34 \pm 5.24 \downarrow	6.41 \pm 6.71 \downarrow	3.61 \pm 5.44 \downarrow
Clv. caspase-7	6.78 \pm 7.63 \uparrow	108.43 \pm 19.40** \uparrow	4.34 \pm 4.48 \uparrow	5.36 \pm 6.09 \uparrow
Int. caspase-7	0.24 \pm 6.20 \uparrow	67.17 \pm 7.46*** \uparrow	5.83 \pm 7.32 \uparrow	15.45 \pm 13.04 \uparrow
Clv. caspase-3	2.87 \pm 4.97 \uparrow	39.49 \pm 15.12** \uparrow	12.82 \pm 6.35 \uparrow	7.94 \pm 1.39 \uparrow

The relative change ration in non-inflammation (200 $\mu\text{g}/\text{ml}$ FDLE) was compared to the untreated group. The relative change ration in inflammation (cytokines+200 $\mu\text{g}/\text{ml}$ FDLE) was compared to the cytokines-treated group. \downarrow : down-regulation, \uparrow : up-regulation. The results were represented as mean \pm SD of three independent experiments, * p <0.05, ** p <0.01 and *** p <0.001 vs. non-inflammation (200 $\mu\text{g}/\text{ml}$ FDLE) in the same cells.