

Effects of herbal medicines on proliferation of cow Mammary Epithelial Cells *in vitro*

Ding Yueyun , Bao Hongduo , Jiang Fang , Zhang Lili , Wang Tian

College of Animal Science and Technology , Nanjing Agricultural University , Nanjing 210095 china , E-mail : tianwang@njau.edu.cn

Key words : MEC , *Astragalus membranaceus* , seed of cowheeb , p27 , Cyclin D1

Introduction Medical plants have been used as herb feed additives in animals for many years .Recent studies have demonstrated that the crude aqueous extracts of some herbs have the ability of increasing milk yield ,altering synthesis and secretion of milk proteins in cows (Fan ,2001 ; Zhou ,2005) .The present study was to detect the direct cellular level effect of seven aqueous extracts of medicinal plants on the bovine mammary epithelial cells (MEC) cultured *in vitro* .The hypothesis was to test whether these herbs have the ability to increase the number of MEC by increasing growth rate or longevity ,and to find out the potential modulators which influence the proliferative effect of herbal medicine and the underlying mechanism .

Materials and Methods The MEC line used in this study was established by our laboratory .MEC was incubated with herbal extracts and their proliferation was assessed by the MTT assay .MEC was seeded in 96-well plates ,incubated with herbal extracts in different concentrations (0 ,100 ,200 ,300 ,400 ,500 μ g/ml and 10 ,20 ,30 ,40 ,50mg/ml respectively) for 48h before the absorbance was recorded at 492nm .The changes of cell cycle were analyzed by flow cytometry .Immunohistochemical staining techniques was used to detect the expression of cyclinD1 and p27 at protein level .Data were expressed as means \pm SD ,statistical analysis was performed using one-way ANOVA .

Results The herbs of *Seed of cowheeb* (DSC) ,*Astragalus membranaceus* (AM) and the commixture of DSC and AM (DSC+AM) demonstrated proliferative activities in MEC .The highest potency was detected in DSC (10mg/ml) ,AM (20mg/ml) and DSC+AM (10mg/ml+20mg/ml) with OD 0 .76 ,0 .75 ,0 .78 respectively ,compared with the control OD 0 .55 .Flow cytometry analysis showed an increase number of cells in S phase and accumulation of cells in the sub-G₂/M phase ,the positive expression rates (PER) of cyclinD1 were increased and p27 expression were decreased in the groups treated with DSC ,AM ,and the commixture of DSC and AM ,compared with the control group .

Table 1 The maximal proliferation ,changes of cell cycle and cyclinD1 ,p27 expression in MEC after incubated with herbal medicines .

Group	n	Dose(mg/ml)	OD	G0-G1(%)	S(%)	G2-M(%)	PER of cyclinD1	PER of p27
Control	6	/	0 .65 \pm 0 .11 ^b	82 .32 \pm 0 .68 ^A	14 .87 \pm 0 .25 ^B	3 .29 \pm 0 .81 ^B	73% \pm 0 .03 ^B	84% \pm 0 .03 ^B
DSC	6	10	0 .76 \pm 0 .12 ^b	60 .77 \pm 0 .44 ^B	32 .62 \pm 0 .46 ^B	6 .61 \pm 0 .70 ^B	88% \pm 0 .03 ^B	55% \pm 0 .03 ^B
AM	6	20	0 .75 \pm 0 .13 ^b	62 .02 \pm 0 .53 ^B	30 .41 \pm 0 .32 ^B	6 .98 \pm 0 .35 ^B	85% \pm 0 .02 ^B	59% \pm 0 .04 ^B
DSC+AM	6	10+20	0 .78 \pm 0 .22 ^a	57 .63 \pm 0 .27 ^B	35 .34 \pm 0 .43 ^A	7 .03 \pm 0 .69 ^A	92% \pm 0 .02 ^A	52% \pm 0 .03 ^A

Note : Differing superscripts (A ,B) indicate most significant differences ($P < 0 .01$) ,and (a ,b) indicate significant differences ($P < 0 .05$) .

Conclusions *Seed of cowheeb* (DSC) ,*Astragalus membranaceus* (AM) could stimulate obviously the proliferation of cow MEC in primary culture .Moreover ,the commixture of DSC in 10mg/ml concentration and AM in 20mg/ml had the best role .Physiological concentrations of aqueous extracts of DSC ,AM promoted MEC proliferation by increasing the expression of cyclinD1 and decreasing the expression of p27 ,thereby changed the cell proliferation cycle ,stimulated the growth and development of MEC .

References

- Fan Z .H . , Sun ,R .f . ,Liu ,Q (2001) .Feeding effect of adding Chinese herbal medicines to ration of lactating cows .*China Dairy Cattle* , 4 ,20-24 .
- Zhou ,Q . ,Mo ,C .K . ,Li ,X (2005) .Effect of Chinese herb additives on mastitis curing and milk output and quality of lactating cows .*Hubei Agricultural Sciences* , 1 ,32-35 .