

## Study on the Functionality of Mare's Milk

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Mare's milk is mainly consumed in the raw state for dietetic therapy by nomads. The effect of mare's milk proteins was assessed in normal mouse fibroblast cells BALB/3T3 clone A31, SV40 transformed mouse fibroblast cells SV-T2 and human hepatoma cell line HepG2. Cellular viability was determined by MTT assay. Mare's milk and whey protein were able to stimulate cell growth by dose-dependent manner. Even mare's milk and whey protein could serve as a serum and growth factor for certain cells cultured under conditions of serum deprivation (i.e. without serum). The data showed that high milk concentration increased the cell viability by 300-660% on A31 cells and 120-210% on SV-T2 cells, which were cultured serum free medium. The protective effect of mare's milk protein against oxidative stress caused by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in different cell lines was tested by treating cells with 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> and various dilution of whey protein. H<sub>2</sub>O<sub>2</sub> is one of the main reactive oxygen species that known to cause lipid peroxidation and DNA damage in cell. The results showed that H<sub>2</sub>O<sub>2</sub> suppressed the growth of the cells and the addition of whey protein significantly reduced the suppression in HepG2 and marginally in A31 cells, whereas there was not any protective effect in SV-T2. The mare's whey protein was hydrolysed with pepsin and trypsin in combination. The total hydrolysates were filtered by a membrane (cut-off molecular weight 10 kDa). Protective effect of whey hydrolysates against hydrogen peroxide in HepG2 cells was analyzed by MTT assay. Cell viability was significantly increased by 50% and 70% in response to addition of hydrolysed whey protein at 30  $\mu$ g/ml and 300  $\mu$ g/ml, respectively. Whey-derived peptides with protective effect against hydrogen peroxide were isolated by reversed-phase chromatography and identified by sequencing analysis. These observations suggest that several peptides derived from mare's whey protein by the action of pepsin-trypsin may have a protective effect against the cytotoxicity caused by reactive oxygen species.