Farcoderm
TESTED WELLNESS

In collaboration with **UNIVERSITY OF PAVIA**
Prof. FULVIO MARZATICO
PHARMACOBIOCHEMESTRY LAB
PHARMACOLOGY AND TOXICOLOGY Division

IN VITRO EFFICACY STUDY

EVALUATION OF THE MODULATING EFFECT ON BOTH CELL PROLIFERATION AND PROTEIN SYNTHESIS ON CELL CULTURE OF A DIETARY SUPPLEMENT

ROXLOR

CYNATINE® HNS

CYNATINE® HNS PLUS

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STUDY DESIGN

Title

in vitro efficacy study – evaluation of the modulating effect on both cell proliferation and protein synthesis on cell culture of a dietary supplement

Study aim

The study in this report concerns with the in vitro study of the cell proliferation and protein synthesis in human skin keratinocyte culture exposed to treatment with 2 different dietary supplements.
Cell proliferation is used as a marker of the cell growth and turn-over. Protein synthesis (keratin synthesis in this specific case) is used as a marker of trophic and fortifying action of the products.

Test items

The test items are

- Cynatine® HNS, Lot n° FKPD05-107; recommended dosage is 500mg/day
- Cynatine® HNS Plus, Lot n° KERBL20G; recommended dosage is 575.5 mg/day

Preparation of the test items and cell exposure

For each tested product a concentrated solution was prepared in PBS (Dulbecco's Phosphate Buffered Saline) and then it was diluted in cell culture medium to achieve the final concentrations chosen for the test.
3 different concentrations of tested products were chosen for the test, according to indications supplied by the Customer about product dosage: 0.5 dose to mimic a partial product absorption, 1 dose to mimic a single complete product absorption, 2 doses to mimic the repeated product administration.
By considering a blood systemic volume of 5L, in the adopted experimental system a dose is calculated as:

- Cynatine® HNS: 500 mg/5L → 0.1 mg/ml
  Tested concentrations are: 0.5 dose = 0.05 mg/ml
  1 dose = 0.1 mg/ml
  2 doses = 0.2 mg/ml
- Cynatine® HNS Plus: 575.5 mg/5L → 0.115 mg/ml
  Tested concentrations are: 0.5 dose = 0.058 mg/ml
  1 dose = 0.115 mg/ml
  2 doses = 0.23 mg/ml

Cell exposure to tested product was prolonged for 24 and 48 hours. At end of each experimental time cell proliferation and protein synthesis were determined.

All of the tested product concentrations were analyzed for their cytotoxic potential by a preliminary MTT assay. Cell viability for each of them is closed to 100%.

Cell proliferation study

Culture media containing tested samples at chosen concentrations were added to the wells containing cells in the G0 phase of cell cycle. Cells were exposed to each product for 24 and 48 hours (medium was replaced every 24 h). At the end of incubation period, MTT test was performed in order to evaluate cell viability and the increasing proliferating rate compared to untreated control cell culture (CTR-). 6 tests were carried out for each determination.

MTT assay: MTT-medium is prepared by adding 15 mg of MTT to 30 ml of culture medium. After exposure of cells to the test items, they were washed with 200 µl of PBS. After removal of the washing solution, 200 µl of MTT-medium are added to each culture well and then incubated for 4 hours at 37°C and 5% CO_2. At the end of the incubation period, the MTT-medium is removed and 200 µl of MTT Solubilization Solution (10% Triton X-100 plus 0.1 N HCl in

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anhydrous isopropanol) are added.
The plate is shaken on a rotatory plate for 20-30 minutes, in order to ensure that all the crystals in the cells have dissolved and a homogeneous solution has formed. The absorbance is measured at 570 nm on a microplate reader, with background reading at 690 nm.
The results are expressed as % cell proliferation compared to an untreated control cell culture.

Protein synthesis study
Culture medium containing the tested samples at the chosen concentrations were added to the wells containing cells in confluence. Cells were exposed to each solution for 24 and 48 hours (medium was replaced every 24 h). At the end of incubation period, medium was collected in order to determine the concentration of proteins produced and released by the cells. For each determination 10 µl of medium was used. For each determination 6 tests were carried out.

Protein determination by Lowry assay: the determination of protein synthesis is carried out by colorimetric method. Like in biuretic method, Cu+ ions complex with proteins in alkaline condition and catalize the oxidation of tyrosine and tryptophan residues. This oxidation causes the reduction of Folin-Ciocalteau reactive that changes its colour from yellow to blue. The colour intensity is proportional to the protein content. Standard keratin is used to calculate a calibration curve.
The results are expressed as % protein synthesis increase compared to an untreated control cell culture (CTR-).

Statistical analysis
Obtained data were subjected to statistical analysis by means of T-test for paired comparison.
For each experimental time the variations vs untreated cell culture (CTR-) are considered significant for *$p<0.05$.
For each experimental time the variations between the two products are considered significant for °$p<0.05$.

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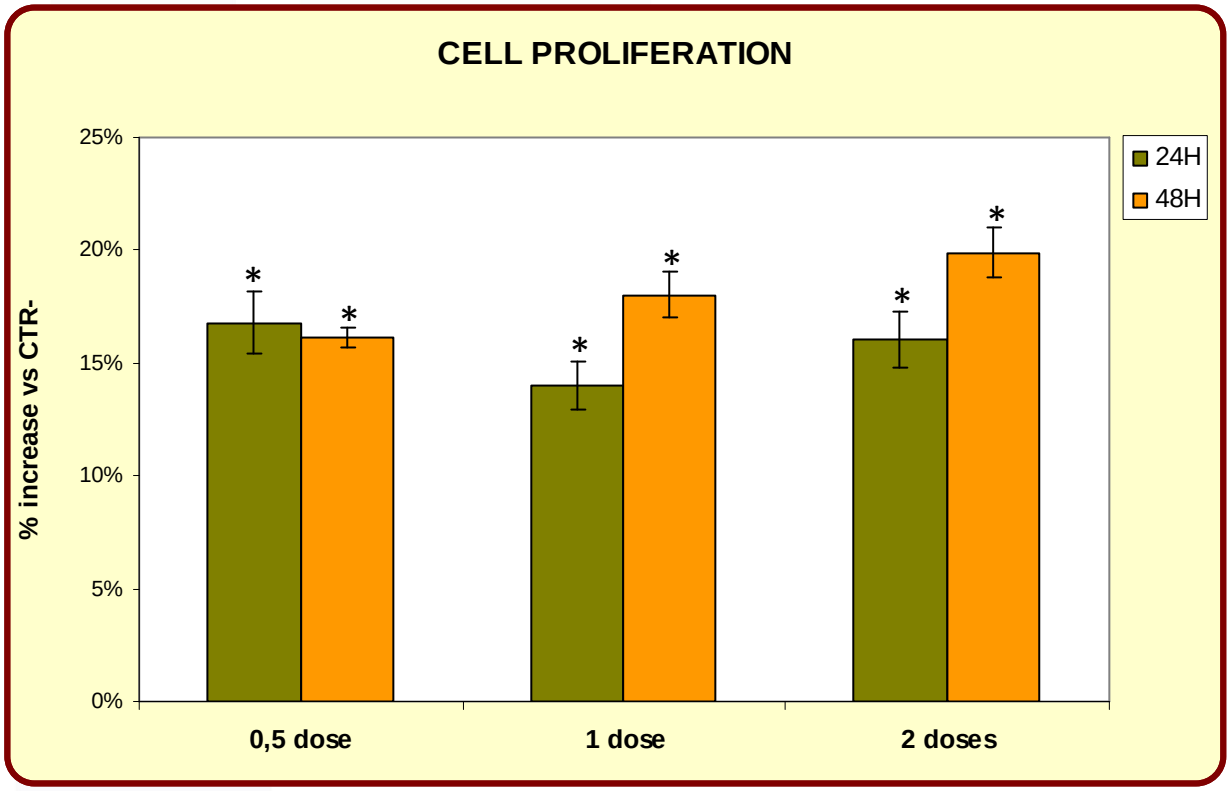
RESULTS AND GRAPHS

In the tables and graphs below the data obtained for each tested series are reported. The data are reported as the percentage increase of cell viability/proliferation and protein synthesis vs CTR- after cells exposure to the test items.

CYNATINE® HNS - CELL PROLIFERATION

Table 1 –Mean increase of cell proliferation in keratinocyte cultures treated with CYNATINE® HNS compared to untreated cells. Data are expressed as mean value ± standard error (SEM).

	24 h		48h	
Concentration	**mean value**	**±** SEM	**mean value**	**±** SEM
0.5 dose	**+ 16.78 %**	1.37%	**+ 16.14 %**	0.44%
1 dose	**+ 14.03 %**	1.08%	**+ 18.04 %**	1.02%
2 doses	**+ 16.02 %**	1.23%	**+ 19.88 %**	1.13%



The cell treatment with **CYNATINE®HNS** is effective in increasing cell viability/proliferation of keratinocytes used in the test compared to untreated cells. Proliferation increase is constant at 24h and shows a slight dose-response at 48h. Recorded increases are statistically significant (*p<0.05) vs cell proliferation of CTR-.

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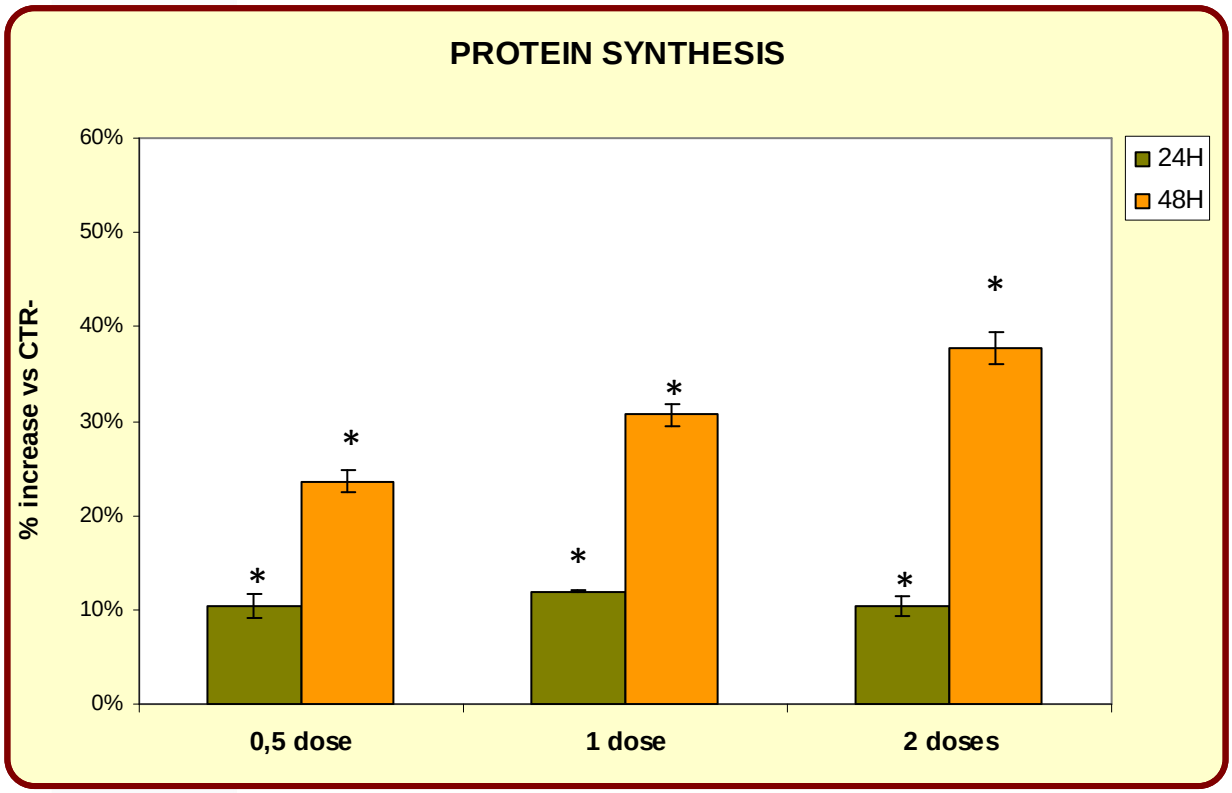
CYNATINE® HNS – PROTEIN SYNTHESIS

Table 2 –Mean increase of protein synthesis in keratinocyte cultures treated with CYNATINE® HNS compared to untreated cells. Data are expressed as mean value ± standard error (SEM).

	24 h		48h	
Concentration	**mean value**	**±** SEM	**mean value**	**±** SEM
0.5 dose	**+ 10.42 %**	1.20%	**+ 23.57 %**	1.15%
1 dose	**+ 11.91%**	0.10%	**+ 30.64 %**	1.11%
2 doses	**+ 10.42 %**	1.00%	**+ 37.71 %**	1.66%



The cell treatment with **CYNATINE®HNS** is effective in increasing protein synthesis of keratinocytes used in the test compared to untreated cells. Protein synthesis increase is constant at 24h and shows a dose-response at 48h. Recorded increases are statistically significant (*p<0.05) vs cell protein synthesis of CTR-.

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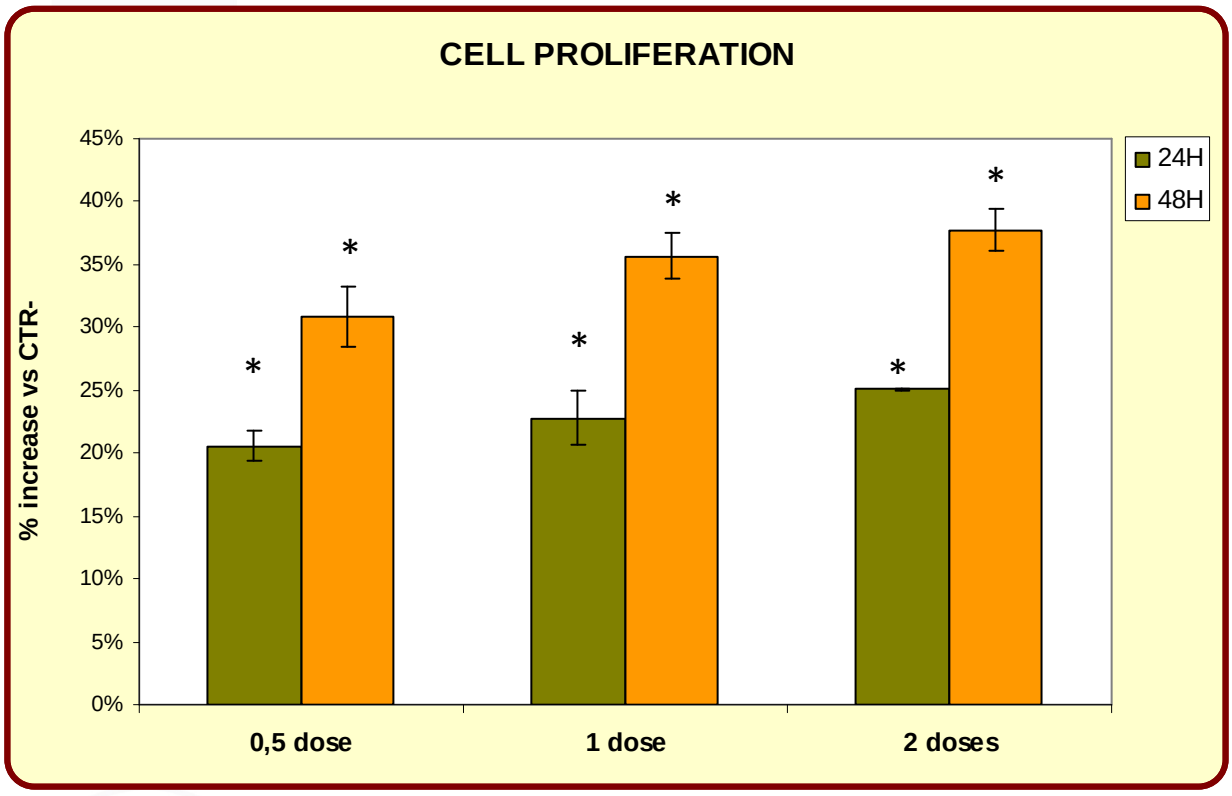
CYNATINE® HNS PLUS- CELL PROLIFERATION

Table 3 –Mean increase of cell proliferation in keratinocyte cultures treated with CYNATINE® HNS PLUS compared to untreated cells. Data are expressed as mean value ± standard error (SEM).

	24 h		48h	
Concentration	**mean value**	**±** SEM	**mean value**	**±** SEM
0.5 dose	**+ 20.57 %**	1.21%	**+ 30.87 %**	2.36%
1 dose	**+ 22.78 %**	2.14%	**+ 35.68 %**	1.82%
2 doses	**+ 25.05 %**	0.12%	**+ 37.73 %**	1.69%



The cell treatment with **CYNATINE®HNS PLUS** is effective in increasing cell viability/proliferation of keratinocytes used in the test compared to untreated cells. Proliferation increase is quite constant at 24h and shows a slight dose-response at 48h. Recorded increases are statistically significant (*p<0.05) vs untreated cell proliferation.

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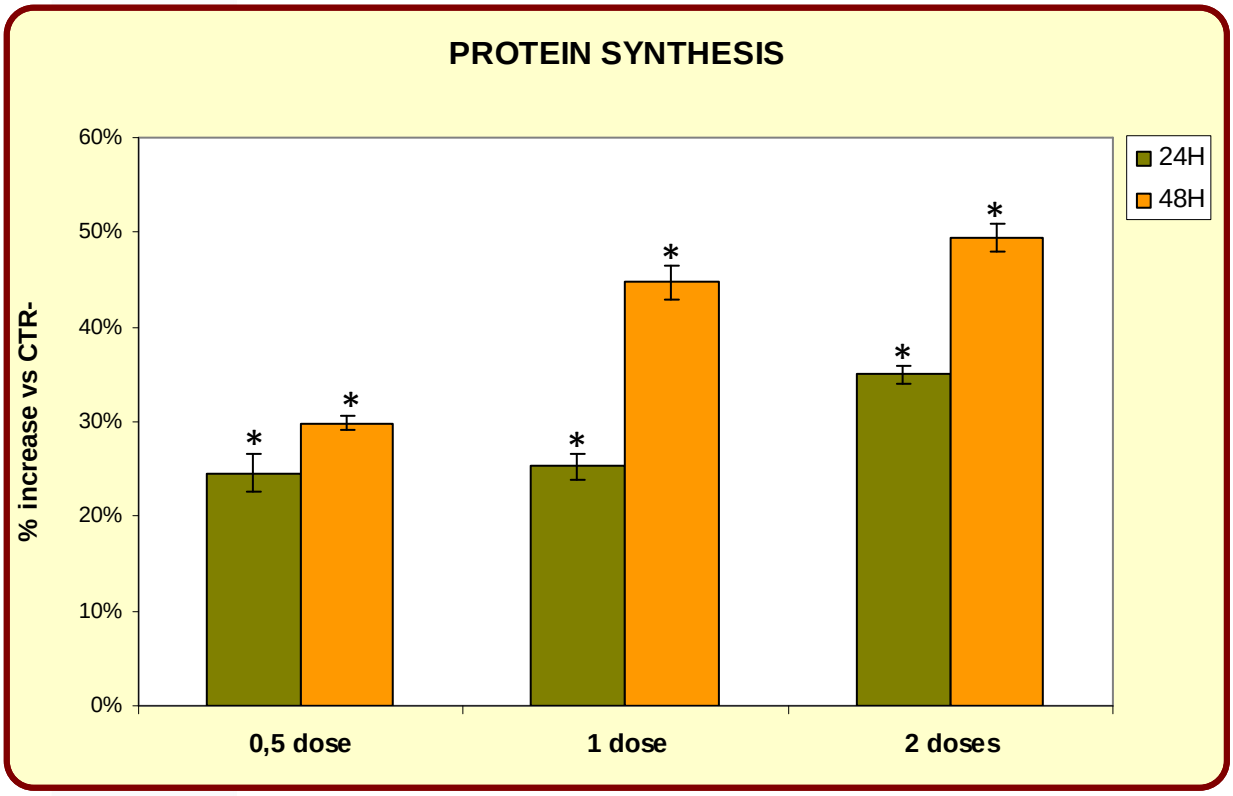
CYNATINE® HNS PLUS– PROTEIN SYNTHESIS

Table 4 –Mean increase of protein synthesis in keratinocyte cultures treated with CYNATINE® HNS PLUS compared to untreated cells. Data are expressed as mean value ± standard error (SEM).

	24 h		48h	
Concentration	**mean value**	**±** SEM	**mean value**	**±** SEM
0.5 dose	**+ 24.57 %**	2.05%	**+ 29.85 %**	0.76%
1 dose	**+ 25.32%**	1.40%	**+ 44.78 %**	1.81%
2 doses	**+ 35.00 %**	0.96%	**+ 49.49 %**	1.51%



The cell treatment with **CYNATINE®HNS PLUS** is effective in increasing protein synthesis of keratinocytes used in the test compared to untreated cells. Protein synthesis increase shows a dose-response Recorded increases are statistically significant (*p<0.05) vs cell protein synthesis of CTR-.

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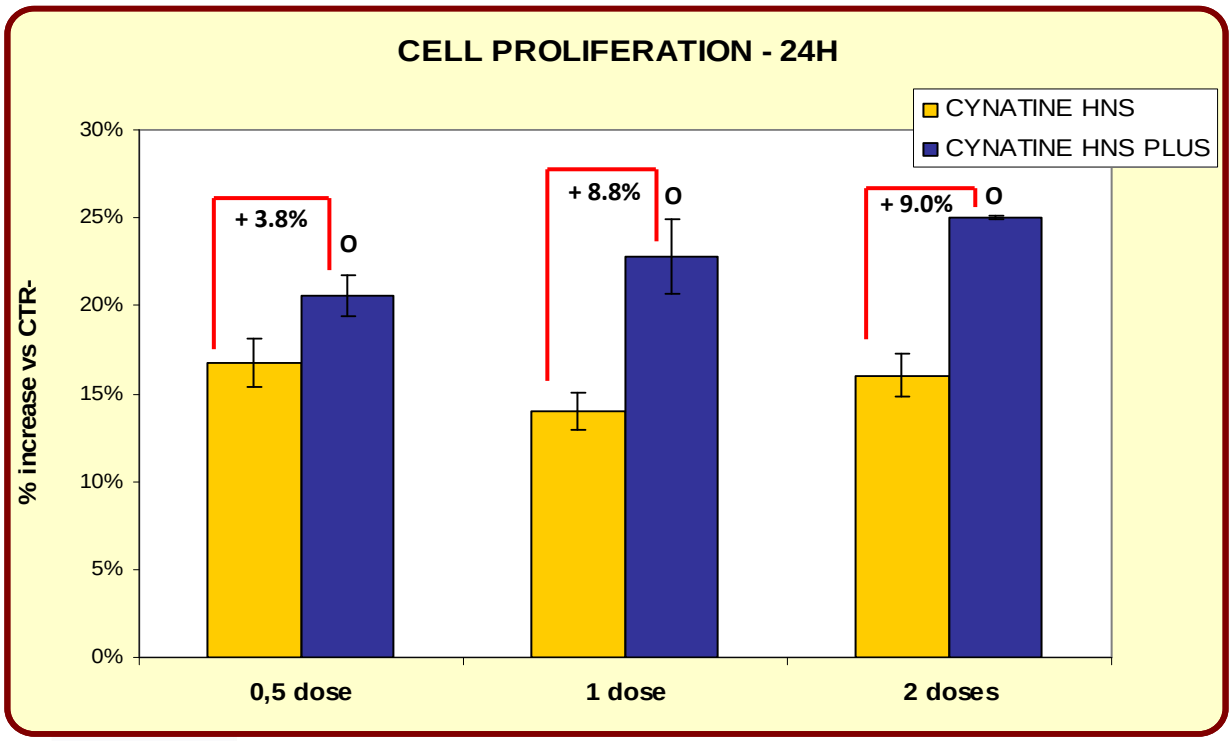
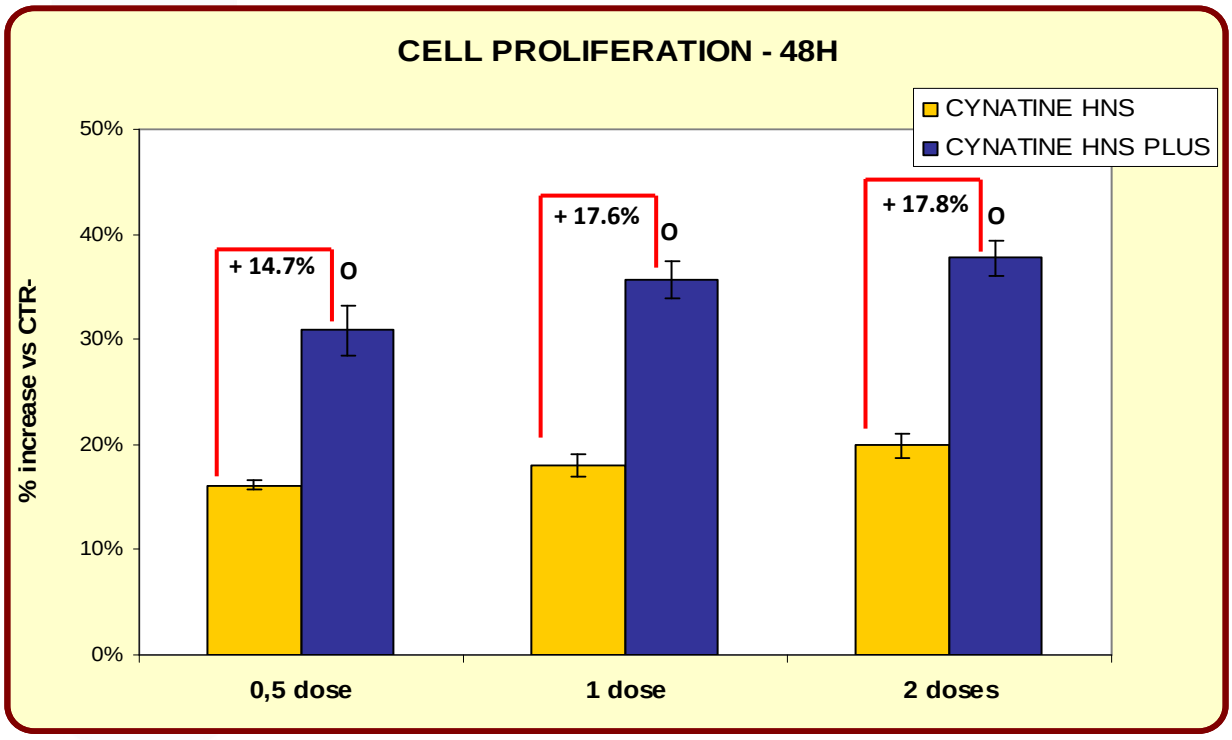
CYNATINE® HNS vs CYNATINE® HNS PLUS
CELL PROLIFERATION COMPARISON





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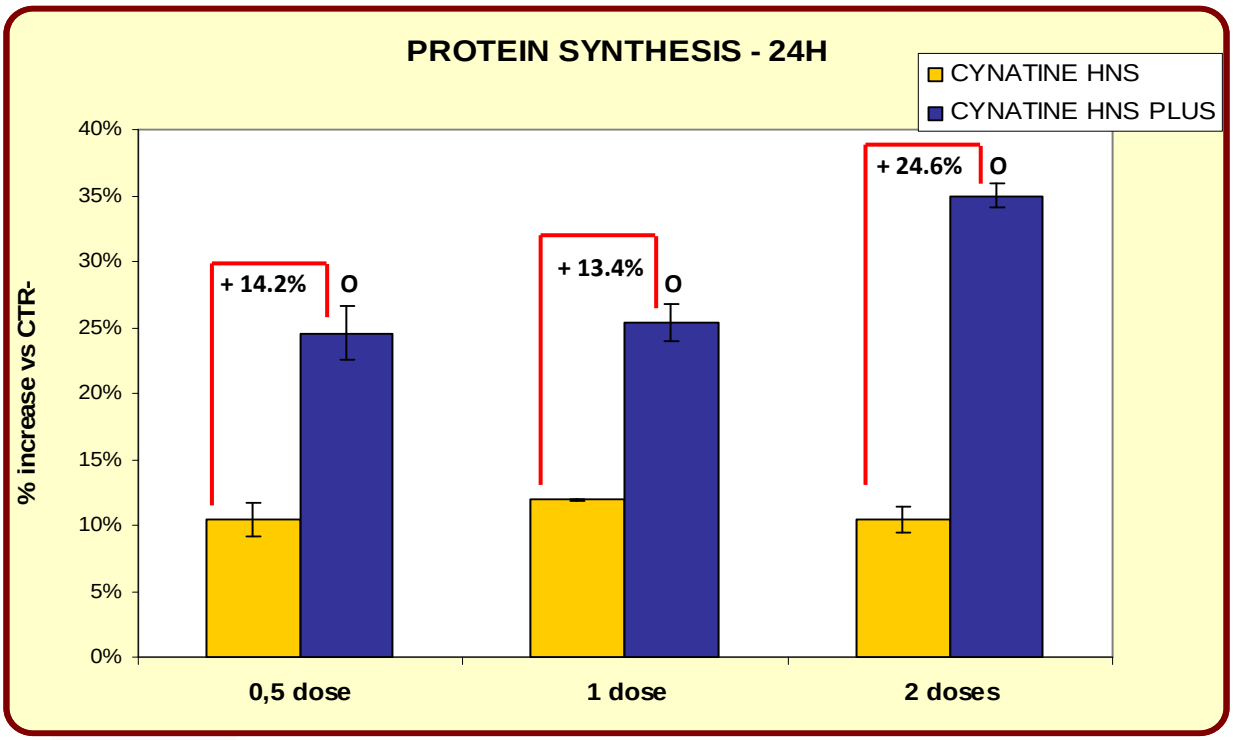
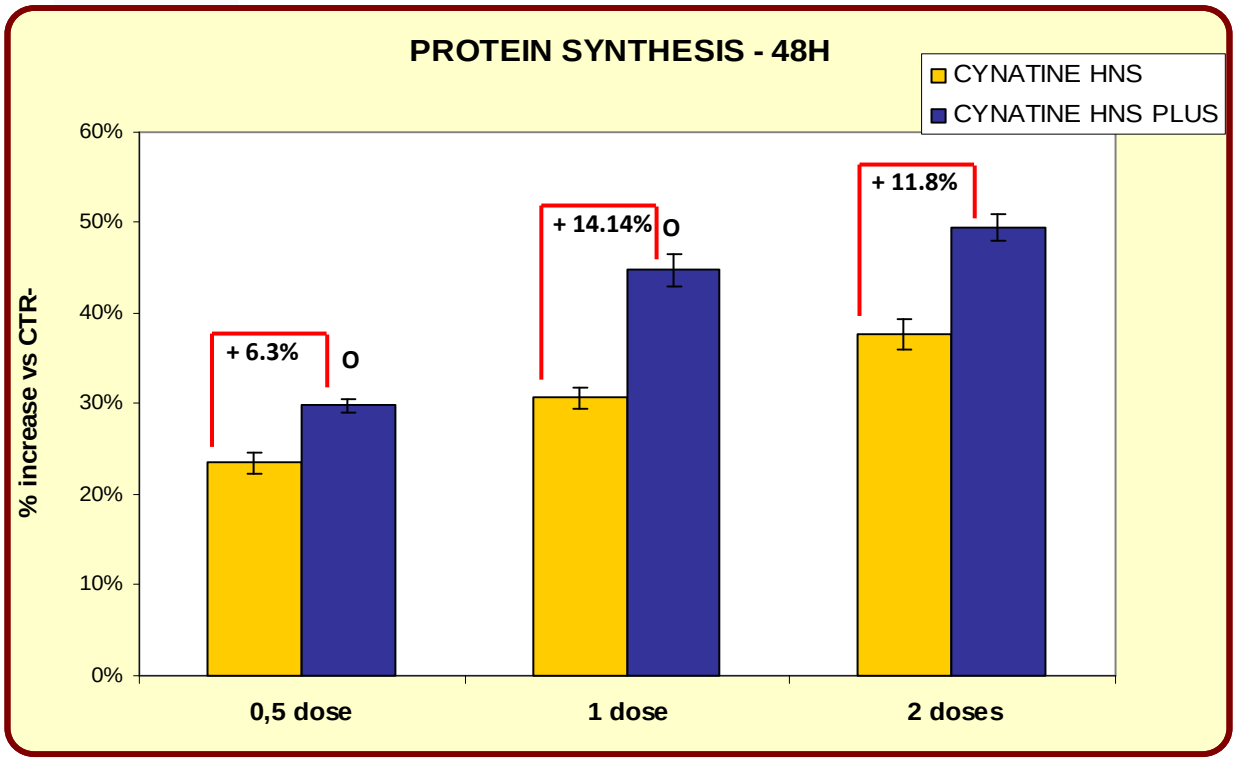
CYNATINE® HNS vs CYNATINE® HNS PLUS
PROTEIN SYNTHESIS COMPARISON





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Data reported in previous tables and graphs show that the treatment of cell culture with **CYNATINE® HNS and CYANTINE®HNS PLUS are capable to significantly stimulate cell proliferation and protein synthesis. Evaluated increases have a general dose-dependent trend.**

Data comparison highlights that **CYANTINE®HNS PLUS enhances cell trophism more effectively than CYNATINE® HNS. This effect is demonstrated by a higher growth of cell population and a more efficient metabolic activation.**

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CONCLUSION

According to the data obtained and reported in this report, we can assess that

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and

CYNATINE® HNS PLUS

have increased the cell growth rate and the protein synthesis in the considered experimental model, highlighting **modulating effect in the cell proliferation and turn-over** and a **prostimolatory effect in the production of the protein**.

Cynatine® HNS Plus shows a more potent and faster capability to **enhance the trophic activity of cells** than Cynatine®HNS

San Martino Siccomario, 5 Novembre 2010
San Martino Siccomario, 5th November 2010

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