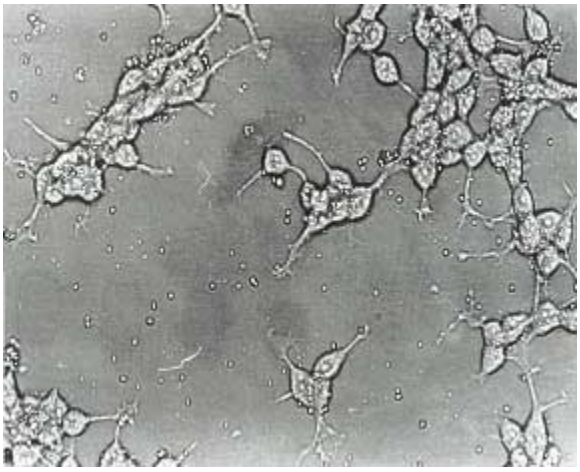
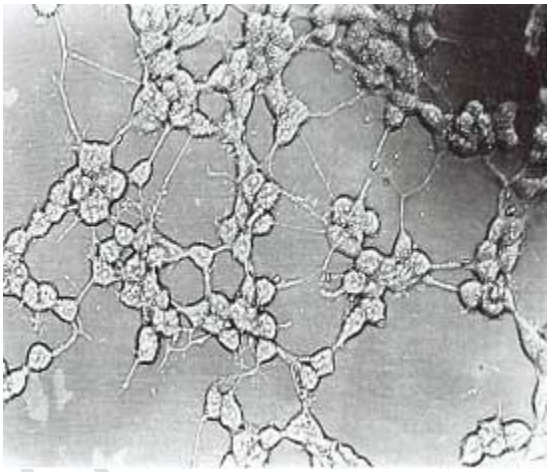
Alfa PXP Forte may Prevent Neuron Vulnerability in Human Neuroblastoma Cells (Preliminary Data) Enzacta International, Minneapolis,MN





Sayan Sawatsri, M.D.(1)
and Wanphen Yankunthong, M.Sc.(2)

(1) Clinical Associate Professor of Department of Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, GA, USA Director of Family Planning Division, Obstetrics and Gynecology Dept., PMK Research Institute, Bangkok, Thailand.

(2) Research assistant in Department of Obstetrics and Gynecology, PMK Research Institute, Bangkok, Thailand.

Abstract (Preliminary data)

Objective: The current study investigated the neurotrophic and neuroprotective action of a unique formulation of Alfa PXP Forte which consists of carbohydrate, crude protein, and essential minerals by using pressure and mechanical hydrolysis to make a complex formulation designated polysaccharidepeptides (PSP – Alfa PXP Forte).

Methods: Using neuronal cell lines prepared from the LA-N-5 (Los Angeles Neuroblastoma) for Alzheimer's disease (AD) in complete media and treated with indicated manner, inverted microscopic evaluated morphological and biochemical analysis were conducted to determine the neurotrophic and neuroprotective properties of Alfa PXP Forte.

Results: Results of these analysis demonstrated that PXP significantly decreased neuronal cell death, a cellular marker of memory formation. Dose response analysis (experiment going on) indicated that the lowest effective concentration of PXP exerted the minimal neurotrophic effect. Result of neuroprotection studies demonstrated the PXP induced highly significant neuroprotection against beta-amyloid, hydrogen peroxide, glutamate-induced toxicity.

Discussion: Abnormality of glucose/energy metabolism shows relation to AD (1, 2, 3, 4). PXP may prevent impairment of glucose/energy metabolism and may improve the ability of neurons to reduce the levels of ("scavenger") free radicals and affecting ATP levels (11, 12).

Conclusion: PXP induced cellular markers of memory function in neurons critical to memory and vulnerable to negative effects of aging, cellular degeneration and Alzheimer's disease. Results of the current study could demonstrate the cellular mechanism of on cognitive function and a possible intervention in Alzheimer's disease.

Key Words: Polysaccharidepeptides (PSP – Alfa PXP Forte), Alzheimer's disease (AD): Cell Death, Neuroprotective.

Introduction

1.1 Alzheimer's disease: A scientific mystery and major impact. Abnormality of glucose/ energy metabolism shows relation to Alzheimer's disease (1, 2, 3, 4). Degenerative and cell death are major causes in AD.

1.2 ALFA PXP FORTE is a complex formulation that consists of carbohydrate, crude protein and essential mineral by using pressure and mechanical hydrolysis to make a complex formulation designated polysaccharidepeptides (PSP – Alfa PXP Forte) (5).

1.3 PXP is very safe because it contains phytochernical that have component of carbohydrate, crude protein and essential mineral by using pressure and mechanical hydrolysis to make a complex formulation designated PXP. Evidence by observation from animal (pig) data showed that PXP can decrease morbidity from Ataxia (PXP may improve cerebral blood flow). In clinical use we found that PXP improves short and long term memory (6).

1.4 LA-N-5 (Neuroblastoma cell lines) have been used for model of Alzheimer's disease in vitro (7, 8, 9, 10).

Objectives

1. To determine if PXP may promote neurotrophic and neuroprotective actions that show decrease cell death (Apoptosis) in the Alzheimer model in vitro.

2. To determine if PXP shows neurotrophic and neuroprotective action in AD model and to determine what the mechanism of PXP is.

For proposed mechanism of PXP induced neuroprotection in AD model in vitro.

Materials and Methods (1)

1. Neuronal culture

Neuronal cell lines were prepared from the LA-N-5; Los Angeles Neuroblastoma derived from bone marrow metastasis of 4-month-old male patients. Cells were propagated in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin, and 1µg/ml fungizone (complete medium).

2. Morphological analysis

By using inverted microscopic evaluated morphological of LA-N-5 in indicated conditions.

3. Neuronal survival

Neuronal viability was determined by inclusion criteria of trypan blue.

4. The neurotrophic and neuroprotective action of PXP

were determined by induced other neurotoxic substrates as indicated conditions.

4.1 Estrogen deprivation exposure: neuronal viability was determined by estrogen deprivation exposure

4.2 Hydrogen peroxide exposure: 1 µM H_2O_2 in HBS for 5 minutes at 37° C. During exposure, E2 or PXP were added concurrently with H202. After 5 min. the culture were rinsed two times with HBS, and fresh medium with E 2 or PXP were replaced.

4.3 Glutamate exposure: 0.2 gM Glutamate 20 min. at room temperature

4.4 Beta amyliod 25-35 exposure: 8 gg/ml beta-amyloid 25-35 24 hrs. at 37C

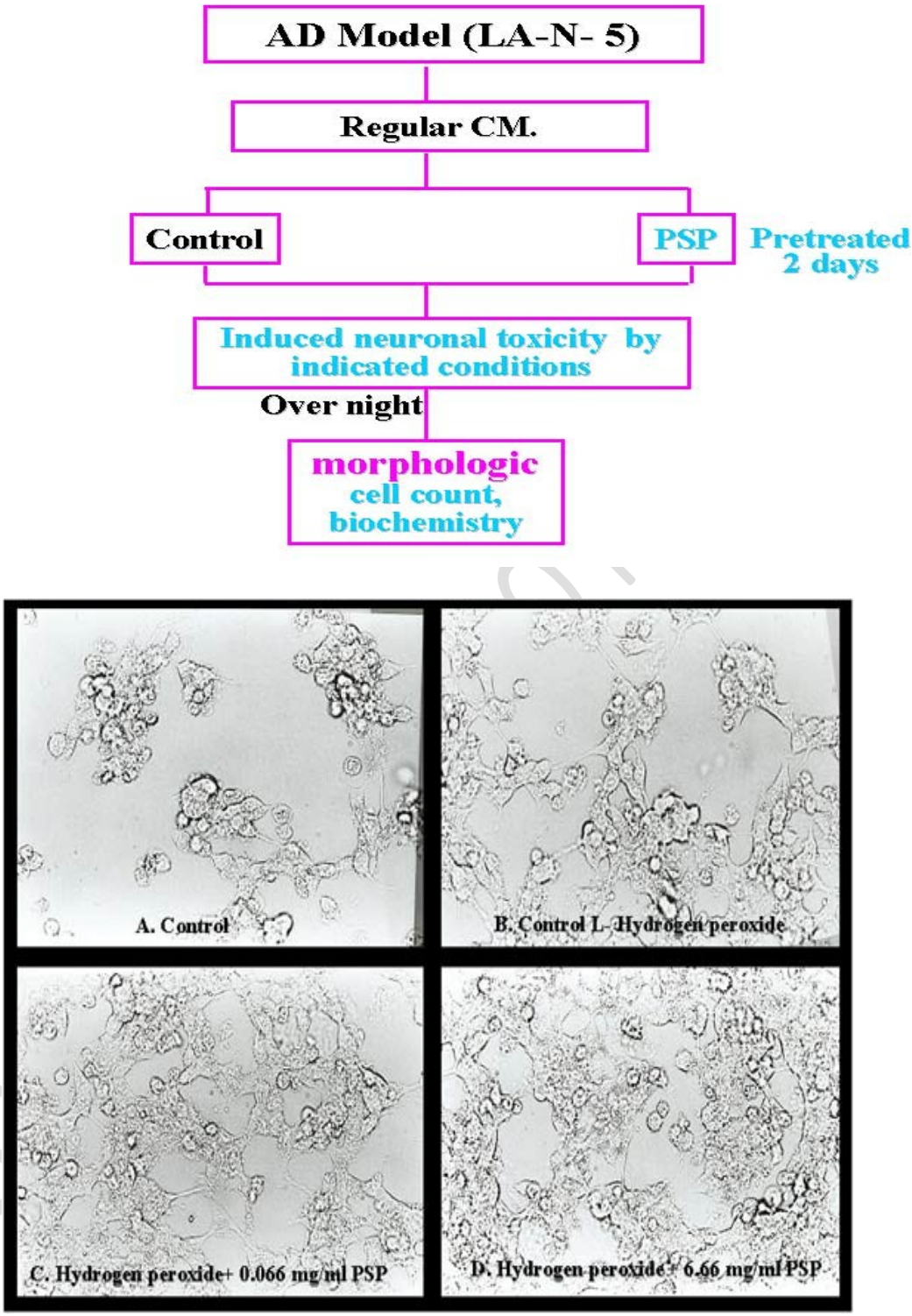


Fig. Dose dependent show neuroprotective effect of PXP against H2O2-induced. toxicity in neuroblastoma (LA-N-5). After pretreated with 6.66 mg/ml PXP for 2 days and treated with 1 μM H2O2 for 5 min exhibit after 1 day neuronal cells death, A. Control LA-

N-5 exposed to 1 µM H202 after 24 h display numerous cell death, degeneration of neuronal process. B. LA-N-5 grown in the presence of 0.26 mg/ml PXP for 2 days prior to exposure to 1 µM H202. C. similar B but when dosed with PXP at 1.33 mg/ml, showed decrease in neuronal cell death compared with control D. similar C but if increased dose PXP to 6.66 mg/ml, showed decrease in neuronal cell death compared with control. (X 400).

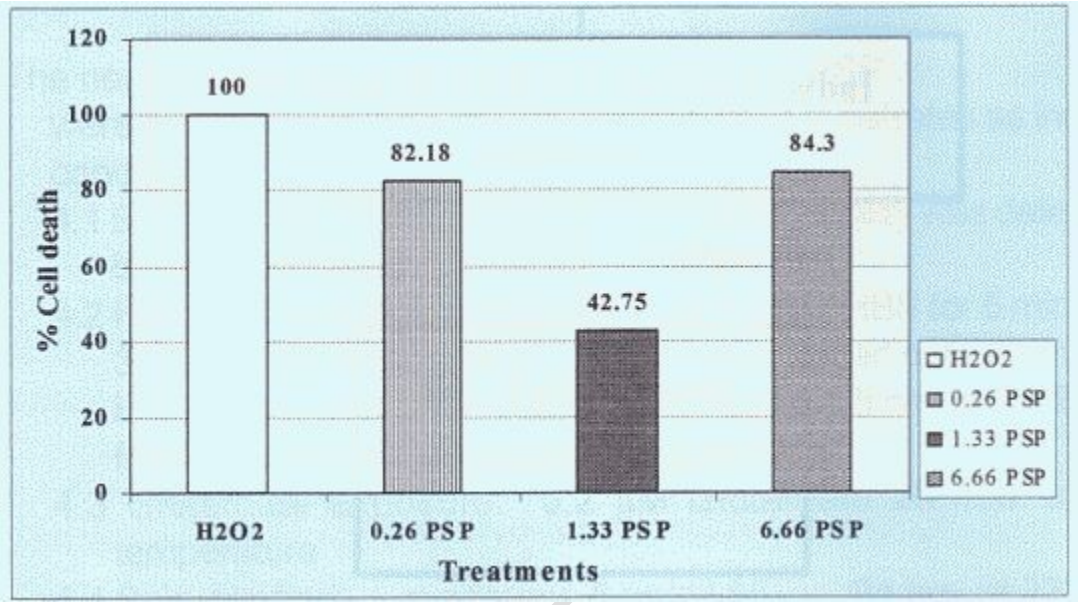


Fig. Dose dependent shows neuroprotective effect of PXP against H2O2 Induced toxicity in neuroblastoma (LA-N-5). If increased dose PXP to 6.66 mg/ml, showed decrease in neuronal cell death compared with control.

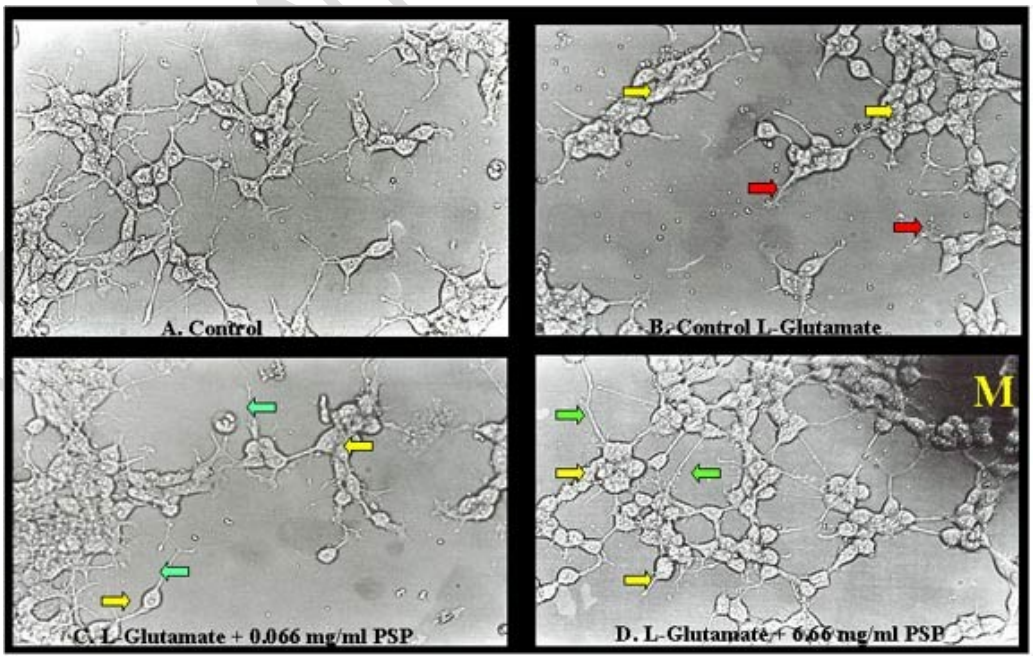


Fig. Dose dependent of PXP against glutamate-induced toxicity to cells and dendrites, A. LA-N-5 under control conditions appear healthy (cytoplasm and neuronal processes). B. LA-N-5 exposed to 0.2 µM Glutamate after 24hrs display shrunken cell bodies and degeneration of neuronal process. C. LA-N-5 grow in the presence of 0.066 mg/ml PXP for 2 days prior to exposure to 0.2 µM Glutamate after 24hrs display exhibit clear features of neuronal viability for cell bodies and clearly defined neuronal process similar to those of control neurons not treated with 0.2 µM Glutamate. D. similar C but if increased dose PXP to 6.66 mg/ml, showed neuronal viability and neuronal process obviously similar with control (C compare B, D compare B). (X 400)

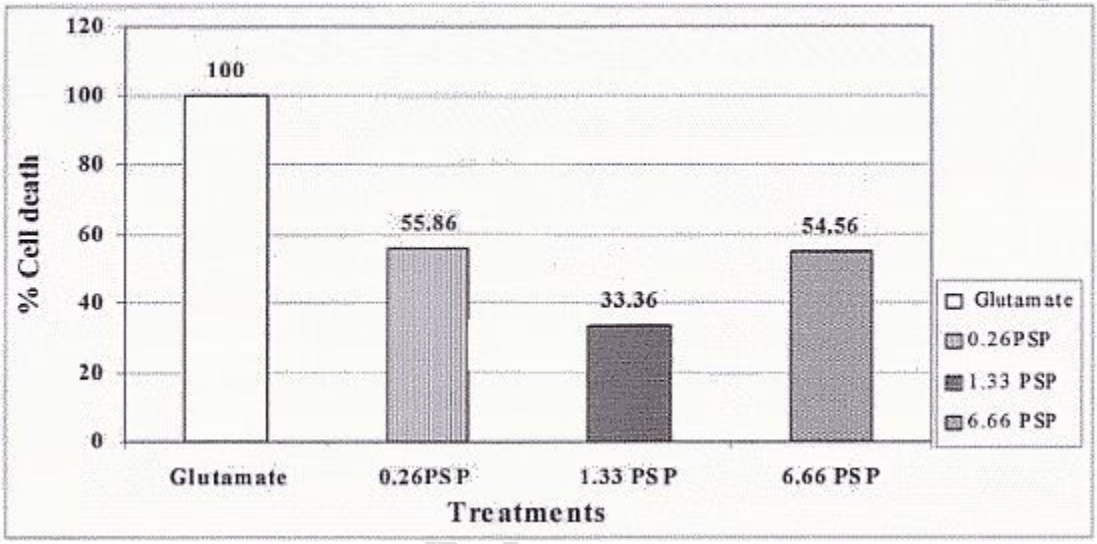


Fig. Dose dependent of PXP against glutamate-induced toxicity to cells and dendrites. If increased dose PXP to 6.66 mg/ml, showed neuronal viability and neuronal process obviously similar with control.

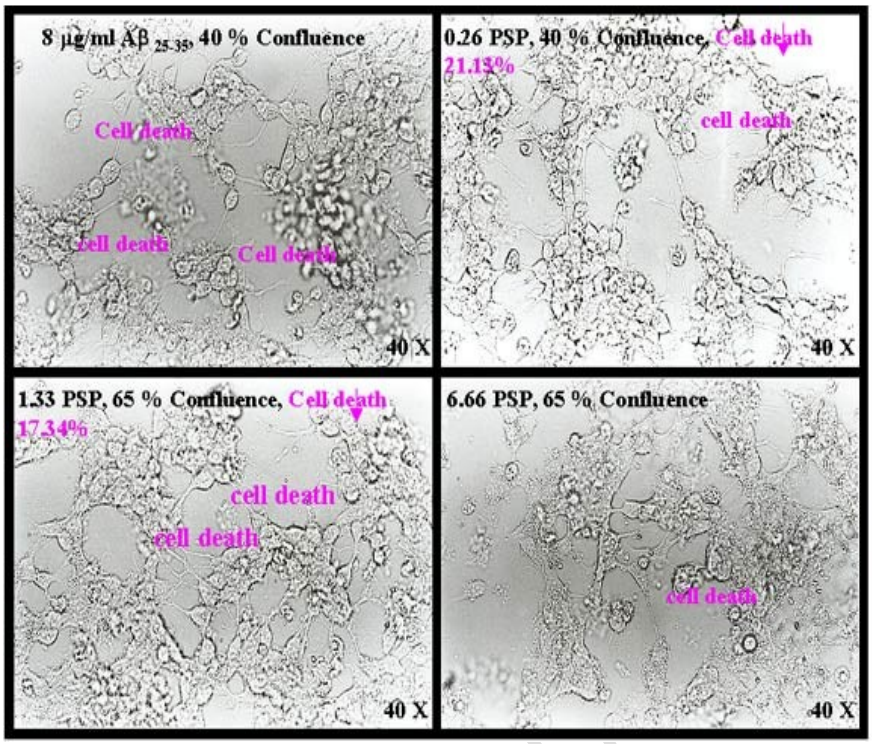


Fig. Dose dependent show neuroprotective effect of PXP against Aß 25-35 induced toxicity in neuroblastoma (LA-N 5). After pretreated with 6.66 mg/ml, PXP for 2 days and treated with Aß 25-35 for 24 h exhibit after 1 day neuronal cell death, A. Control LA-N-5 exposed to Aß 25-30 after 24 h display numerous cells death, degeneration of neuronal process. B. LA-N-5 grow in the presence of 0.26 mg/ml PXP for 2 days prior to exposure to Aß 25-35 C. similar B but when dosed with PXP at 1.33 mg/ml, showed decrease in neuronal cell death compared with control D. similar C but if increased dose PXP to 6.66 mg/ml, showed decrease in neuronal cell death compared with control. (X 400)

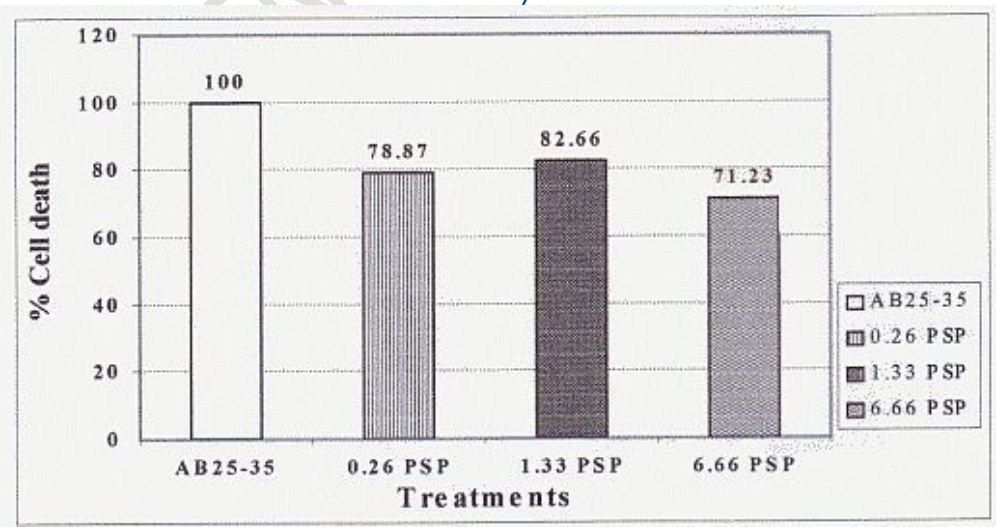


Fig. Dose dependent show neuroprotective effect of PXP against Aß25-35-induced toxicity in neuroblastoma (LA-N 5). If increased dose PXP to 6.66 mg/ml, showed decrease in neuronal cell death compared with control.

Results

- Neuroprotection against other neurotoxic results by PXP

*Estrogen deprivation induced neurotoxicity
*Hydrogen peroxide-induced neurotoxicity
*Glutamate- induced neurotoxicity
*Beta amyloid 25-35-induced neurotoxicity

- Dose dependent of PXP shows neuroprotection against other neurotoxins (experiment going on).

Discussion

Abnormality of glucose/energy metabolism shows relation to AD (1, 2, 3, 4). PXP may prevent impairment of glucose/energy metabolism and may improve the ability of neurons to reduce the levels of ("scavenger") free radicals and affecting ATP levels (11, 12).

Conclusions

ALFA PXP FORTE contains a unique formulation consisting of a complex form of carbohydrate, crude protein and essential minerals by using high pressure and mechanical hydrolysis to make a complex formulation designated polysaccharide peptides (PSP – Alfa PXP Forte). PSI", in vitro, has consistently demonstrated that it prevent cells death from other neurotoxicity agents, significantly in Alzheimer's disease model.

PXP induced cellular markers of memory function in neurons critical to memory and vulnerable to negative effects of aging, cellular degeneration and Alzheimer's disease. Results of the current study could demonstrate the cellular mechanism of PXP on cognitive function and a possible intervention in Alzheimer's disease.

In clinical application, PXP may promote cellular mechanism in memory and neuronal survival and may be used as a nutritional supplement in aging, cellular degenerative process and a possible use for preventing Alzheimer's disease.

References

1. Blass, J.P., Gibson, GE., Shimada, M., Kihara, T., WatanabeM., and KuriniotoK. (1980) Brain carbohydrate metabolism and dementia, in Biochemistry of Dementia (Burman, D. and Pennock, C.A., eds.), Wiley, London, pp, 121-134.

2. Blass, J.P., Sheu, K.-F.R., and Cederbaum, J.M. (1988) Energy metabolism in disorders of the nervous system, Rev.Neurol. (Paris) 144, 543-563.

3. Beal, M.F. (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative diseases -Ann. Neurol. 31, 119-123.

4. Blass, J.P., Sheu, K.-F.R., and Tanzi, R. (1996) a-Ketoglutarate dehydrogenase in Alzheimer,s disease, in Energy Metabolism in Neurodegenerative Diseases (Fiskum, G., ed.), Plenum, NewYork, pp. 185-192.

5. Laboratory report from Pacific Lab Services, Report No: /396-3971/LS/ 2001 Date: February 19th, 2001.

6. Interview with Medical Doctors Testimonial No.007, 018, 058 and 068.

7. Preuss U, Mandelhow EM. Mitotic phosphorylation of tau protein in neuronal cell lines resembles phosphorylation in Alzheimer's disease. Eur J Cell Biol 1998; 76 (3): 176-84.

8. Mesco ER, Timiras PS. Tau-ubiquitin protein conjugates I a human cell Hine. Mech Ageig Dev 199 1; 61(l): 1-9.

9. Davis PK, Johnson GV. Monoclonal antibody Alz-50 reacts with bovine and human ser albumin. J Neurosci Res 1994; 39(5): 589-94.

10. Fabrizi C, BusinaroR, Lauro GM, Starace G, Fumagalli L. Activated alpha2macroglobulin increase beta-amyloid (25-35)- induced toxicity in LA-N5 human neuroblastoma cells. Exp Neurol 1999; 155(2): 252-9.

11. Beal, M.F. (1995) Aging, energy, and oxidative stress in neurodegenative diseases, Ann. Neurol. 38,357-366.

12. Mattson, M.P. (1994) Mechanism of neuronal degeneration and preventive approaches: Quickening the pace of AD research, Neurobiol, Aging 15(Suppl.2), S121-S125