



Antiedema Effects of Proanthocyanidin on Experimental Traumatic Brain Edema

Deneysel Travmatik Beyin Ödeminde Proantosiyanidin Antiödem Etkileri

Yavuz ERDEM¹, Ayhan TEKINER², Yavuz Selim ERKOC³, Muhammet Bahadır YILMAZ², Haydar CELIK¹, Ali Erdem YILDIRIM⁴, Ayse Selda TEKINER⁵, Mehmet Akif BAYAR¹

¹Ankara Training and Research Hospital, Department of Neurosurgery, Ankara, Turkey

²Kayseri Training and Research Hospital, Department of Neurosurgery, Kayseri, Turkey

³Sanlıurfa Training and Research Hospital, Department of Neurosurgery, Sanlıurfa, Turkey

⁴Ankara Numune Training and Research Hospital, Department of Neurosurgery, Ankara, Turkey

⁵Ankara University, School of Medicine, Department of Family Medicine, Ankara, Turkey

Corresponding Author: Ayhan TEKINER / E-mail: tekinerayhan38@gmail.com

ABSTRACT

AIM: Brain edema developing due to central nervous system trauma is still a significant reason of mortality and morbidity. There is still no definite approach for the medical treatment of brain edema despite many clinical and laboratory studies in recent years. We therefore investigated the effect of proanthocyanidin, an antioxidant agent, on brain edema in this study.

MATERIAL and METHODS: A total of 30 rats were used and divided into three as the control, trauma and treated trauma groups. Subjects were sacrificed after 72 hours. The brain tissue-water ratio was evaluated and biochemical analysis of brain tissue performed.

RESULTS: The difference between the treated trauma and control groups was statistically significant while the trauma and control groups were relatively similar. Rats that had undergone trauma and received proanthocyanidin treatment were statistically significant and different from the trauma group rats regarding the biochemical analysis results, brain tissue water ratio, and the cold damage enzymatic antioxidant defense system of cortical neural tissue.

CONCLUSION: We believe that proanthocyanidin, an antioxidant substance, can be an effective treatment for brain edema.

KEYWORDS: Brain edema, Proanthocyanidin, Head trauma

ÖZ

AMAÇ: Santral sinir sistemi travması sonucu oluşan beyin ödemi, günümüzde halen önemli mortalite ve morbidite nedenidir. Beyin ödeminde medikal tedavi yaklaşımları ise son yıllarda birçok klinik ve laboratuvar çalışma yapılmasına rağmen halen tam çözüme ulaşabilmiş değildir. Bu amaçla çalışmamızda antioksidan bir madde olan proantosiyanidin beyin ödeminde karşı olan etkisi araştırılmak istenmiştir.

YÖNTEM ve GEREÇLER: Bu çalışmada toplam 30 rat kullanıldı. Çalışma grupları; kontrol, travma ve tedavi edilen travma grubu olmak üzere 3 gruba ayrıldı. Deney hayvanları 72 saat sonra sakrifiye edilerek beyin dokusu-su oranı ve beyin dokusunun biyokimyasal analizi yapıldı.

BULGULAR: Çalışma gruplarının sonuçları incelendiğinde; travma ile kontrol grupları arasındaki farkın istatistiksel yönden anlamlı olduğu, tedavi grupları ile bu farkın göreceli olarak kontrole yaklaştığı görülmektedir. Buna göre travma uygulanıp proantosiyanidin tedavisi alan ratlarda beyin dokusu su oranı ve soğuk hasara karşı kortikal nöral dokunun enzimatik antioksidan defans sisteminin biyokimyasal analizlerinin değerlendirilmesinde travma grubundan istatistiksel olarak farklı olduğu ortaya konmuştur.

SONUÇ: Antioksidan bir madde olan proantosiyanidin antiödem tedavisinde etkin olabileceğine dair bulgular elde edilmiştir.

ANAHTAR SÖZCÜKLER: Beyin ödemi, Proantosiyanidin, Kafa travması

INTRODUCTION

Traumatic brain edema can increase the intracranial pressure when not treated effectively and cause high mortality and morbidity (3, 9, 13, 20, 23). Evoked response to injury in tissues is reflected as swelling and leads to spatial growth of one or more tissue parts. The increase in the extravascular tissue fluid is known as edema. Edema may be due to an increase of free extracellular fluid alone or both intracellular and extracel-

lular fluid (23). The term "cerebral edema" refers to increased liquid content within the brain volume.

Cerebral edema has attracted the attention of scientists for a long time. In 1908, Harvey Cushing discussed brain swelling accompanied by severe head trauma and its treatment. In 1919, Weed and McKibben experimentally examined the effects of liquids with various osmolarity values on brain edema. In 1966, Igor Klatzo defined the pathophysiology of va-

sogenic and cytotoxic edema for the first time (16). Cytotoxic edema is defined as cellular swelling and is characterized by the loss of cell membrane ion pump function (2). This type of edema is observed after ischemia and hypoxia.

Brain injuries such as cerebral hypoxia, ischemia and trauma cause arachidonic acid release and this can cause cerebral edema in at least two ways (4). First, this fatty acid enters the phospholipid layer rapidly due to its ampholytic feature and can change the functional properties of these membranes. Secondly, endothelial leukocyte adhesion and penetration increases and leads to endothelial injury. Arachidonic acid metabolites and especially leukotrienes are known to be potential mediators of blood-brain barrier dysfunction (5). Bradykinin also has an effect that is similar to arachidonic acid (19). The current aim in brain edema treatment is to decrease the intracranial pressure by controlling the hydrostatic, osmotic and oncotic effects and providing cerebrovascular permeability (25). Various medical treatments are being investigated for this purpose. We used proanthocyanidin, which has a strong antioxidant action, considering that its antioxidant effect might be effective in preventing the destruction of cell membranes, mitochondria and vascular structures.

MATERIAL and METHODS

A total of 30 male *Rattus norvegicus* type rats weighing 150 \pm 10 g were randomly divided into three groups. Only craniectomy was performed in group I, the control group (n=10); craniectomy and trauma were performed in group II, the trauma group (n=10); craniectomy, trauma and medical treatment were performed in group III, the trauma and treatment group (n=10). Rats in group II and III underwent cold damage after craniectomy (30). The rats were sacrificed at 72 hours. The tissues were examined for water ratio and biochemical analysis of brain tissue was performed.

Surgical Procedure

Anesthesia: We administered 35 mg/kg of ketamine hydrochloride (Ketalar 5% solution, Eczacıbası) and 1.5 mg/kg of Rompun (Xylazine 2% solution, Bayer) intramuscularly before the surgical procedure to all rats.

Craniectomy, cold injury: We followed the method of Tomina-ga and Ohnishi (30). We used a vertical midline incision. The sagittal and left coronal sutures were identified. We peeled the left parietal periosteum away from the midline. The temporal muscle was dissected and the temporal bone exposed. We then opened a small hole in the central part of the parietal bone with a dental drill during irrigation with isotonic solution for cooling purposes. We enlarged the hole with a curved mini hemostat, keeping the dura intact. A 10x15 mm craniectomy was then performed from the left midsagittal plane to the temporoparietal region. We performed standard focal freeze injury with -70°C liquid nitrogen by touching a 4x10 mm metal probe to bare dura for 45 seconds.

Preparation of Tissue Samples

We waited for 72 hours and then provided anesthesia with

ketamine (85 mg/kg) before performing craniotomy. We obtained 5x5x5 mm tissue samples from the hemisphere using a microscope and microsurgical instruments, cleaned the samples with cold nitrogen and wrapped them in aluminum foil. The samples were then immersed in liquid nitrogen immediately and stored at -80°C. At the time of the analyses, they were thawed and weighed. We then used phosphate-buffered saline with a pH of 7.4 for homogenization.

Methods for Evaluation

Cerebral water content: Groups were decapitated under anesthesia after brain samples about 5x5x5 mm were rapidly taken from the damaged hemisphere and the symmetrical area. Samples were placed into numbered aluminum foils with a predetermined weight. We recorded the fresh weight of each specimen, heated them at 105 °C, and then kept them for 48 hours at constant temperature. The fresh and dry weights were used to calculate the results (30).

Thiobarbituric Acid Reactive Substances (TBARS): MDA levels were expressed as Malondialdehyde or Thiobarbituric acid reactive substances (TBARS). We used the method of Wasowicz et al (34) to determine these fluorometrically. We used the method described in our previous studies (29) to determine malondialdehyde (MDA) levels in tissue homogenate samples (29).

Nitrite and Nitrate: Total nitrite and nitrate concentrations were determined by a colorimetric assay utilizing Griess reagent (27). Results were expressed as nitrite and nitrate concentrations per gram wet tissue. Nitrate concentrations were calculated by subtracting nitrite concentrations from the total nitrite (nitrate + nitrite) concentrations.

Thiols: We used Habeeb's (10) method to determine SH groups in the homogenates and presented the results as micromoles of SH per grams of wet tissue.

Statistical Analysis: We used the Kruskal-Wallis test to analyze differences among the groups and then used Conover's multiple comparison procedure. Statistical analyses were performed using the SPSS 15.0 statistical software. A p value <0.05 was considered statistically significant.

RESULTS

The values for the water ratio of brain tissue and the biochemical variables (TBARS, Nitrite, Nitrate, Total Nitrite, T-SH) in the three groups were evaluated and compared with the Kruskal-Wallis test. The TBARS level, and Nitrite, Nitrate, Total Nitrite, and T-SH activity were used for oxidative stress status analysis.

All groups differed from the others for all parameters. Figure 1 presents the distribution of the study groups according to brain tissue water content. The water content was significantly higher in the trauma group compared to the control and treatment groups (p<0.05). The treatment group was not statistically different from the control group (p>0.05). Table 1 presents the biochemical analysis of the brain tissue. Group 1

had the lowest and group 2 the highest mean values. There was a significant difference between the treatment group and trauma group ($p < 0.05$).

DISCUSSION

Traumatic brain injury (TBI) continues to be a major public health problem worldwide. Mortality and morbidity of post-traumatic brain injury is mostly determined by the existence and intensity of brain edema. We investigated the anti-edema effects of proanthocyanidin, an antioxidant naturally occurring in grape seed extract, in rats with traumatic brain injury (TBI) and found a considerable effect in the treatment group. The brain tissue water content, TBARS, nitrite, nitrate and total nitrite were increased in the TBI rats as expected and proanthocyanidin treatment reduced these values. Surprisingly, protein thiols (SH groups) were also increased in TBI and decreased with proanthocyanidin treatment.

Expansion of brain volume due to fluid accumulation in the brain parenchyma plays a major role in the morbidity and mortality due to brain edema following TBI with increased intracranial pressure, impaired cerebral oxygenation and perfusion, and with its contribution to ischemic injury (31). How posttraumatic brain edema develops is not well understood but "vasogenic edema" and "cytotoxic/cellular edema", which are the two major types, seem to be linked. The cytotoxic type develops first (1–6 h) after trauma with the astrocytes being affected most. Vasogenic edema develops only once 12–24 h have passed after the trauma (14).

We used the brain water content to evaluate brain edema. Figure 1 shows that brain tissue water content was significantly increased in the trauma group and decreased by proanthocyanidin in the treatment group. The only variation during the study protocol besides time was the proanthocyanidin treatment. Hence the decrease of brain water content in the treatment group seems to be due to a proanthocyanidin effect.

The protective effects in oxidative stress conditions of polyphenols with their antioxidant properties are currently attract significant interest. Phenolic antioxidants such as proanthocyanidin can inhibit the oxidation of lipids, carbohydrates, proteins and nucleic acids and can also protect against free radicals (6, 12, 22, 32). They are polymers of flavonoid molecules that are widely available in vegetables, fruits, nuts, flowers, and seeds (especially grape seeds). The neuroprotective effect of proanthocyanidin is essentially based on its antioxidant properties (1, 8, 28, 33). Our results support this notion as the increased oxidative stress after TBI decreased with proanthocyanidin treatment.

The body constantly forms reactive oxygen/nitrogen species and free radicals and this is necessary for a healthy organism. These are then removed by the antioxidant defense system. The generation and removal of reactive oxygen/nitrogen is normally balanced. An imbalance is called oxidative/nitrosative stress (7, 11).

Traumatic brain injury leads to the generation of reactive oxygen and nitrogen free radicals, resulting in pleiotropic effects on the cell. The lipid peroxidation of fatty acids with two or more double bonds results in the formation of Thiobarbituric acid-reactive substances (TBARS) as an end product. Such oxygen/nitrogen radicals are particularly harmful for brain membranes. TBARS is generally a good measure of lipid peroxidation and increased TBARS concentrations in the brain tissue as in the present study indicate increased oxidative stress.

Increased oxidative stress may contribute to nitrosative stress and further cellular dysfunction in post-traumatic brain tissue. In fact, NO produced by endothelial nitric oxide synthase (eNOS) is considered to be protective against vascular diseases. NO plays a central role in the maintenance of vascular homeostasis, including regulation of the cerebral circulation. NO is also a potent vasodilator and inhibits platelet aggregation (26). In the current study, the increase of nitric oxide metabolites after trauma revealed increased nitrosative stress.

Table I: The Comparison of the Biochemical Parameters for the Three Groups

Variables	Groups			p
	1	2	3	
	Mean±SD, Median (Min-Max)	Mean±SD, Median (Min-Max)	Mean±SD, Median (Min-Max)	
MDA (umol/g) wet tissue	44.07±6.58 [42.37 (38.33-58.62)]	95.89±19.70 [92.41 (72.65-140.94)]	52.66±9.71 [55.30 (38.04-63.22)]	<0.001
Nitrite (ug/g) wet tissue	6.06±1.73 [5.28 (4.97-10.56)]	10.22±1.68 [10.23 (7.65-12.39)]	7.56±1.76 [7.11 (5.03-10.73)]	<0.001
Nitrate (ug/g) wet tissue	31.70±2.63 [32.56 (27.69-35.29)]	55.70±4.30 [55.82 (48.78-61.03)]	38.94±7.09 [38.86 (30.57-51.01)]	<0.001
Total Nitrite (ug/g) wet tissue	37.76±3.45 [38.98 (32.67-43.55)]	65.92±3.93 [65.62 (60.89-71.14)]	46.44±8.59 [46.97 (37.31-60.89)]	<0.001
T-SH (umol/g) wet tissue	107.98±6.05 [109.52 (97.80-115.02)]	175.46±29.98 [184.93 (112.04-204.70)]	121.97±27.38 [114.92 (102.06-198.37)]	<0.001

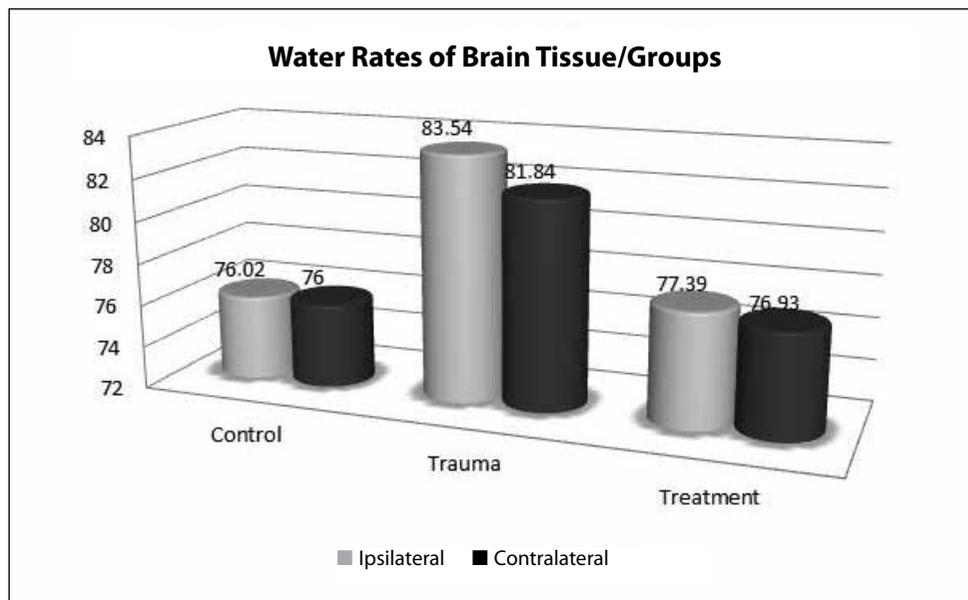


Figure 1: The brain tissue water ratios of the three groups.

Bioavailable NO is depleted and local oxidative/nitrosative stress increased when the levels of reactive oxygen species and especially superoxide anion increase and combine with NO, forming peroxynitrite (ONOO⁻). This damages the endothelium as it leads to nitric oxide uncoupling and initiates or accelerates stress. Decreased NO also adheres to the endothelial surface and disrupts the vascular endothelium.

Ischemia secondary to brain trauma can also damage brain tissue with mechanisms such as superoxide anion by xanthine oxidase, NOS, and NADP(H) oxidases and mitochondrial electron transport chain leakage (17). The brain injury itself increases neuronal, glial and endothelial NOS activity. Later on, NOS activity is increased in the infiltrating neutrophils and macrophages, activated microglia and astrocytes (21). The post-ischemia reperfusion can also lead to excessive oxygen/nitrogen free radical formation. These mainly consist of superoxide anion and NO, both strongly reactive themselves, that can also combine to form a stronger and more stable oxidant, peroxynitrite anion (18).

It has previously been reported by experimental studies that oxidative/nitrosative stress is increased in TBI. Such stress plays an important role in TBI pathogenesis. Panickar et al reported that free radical production increased when cultured astrocytes were exposed to trauma (24). Furthermore, Jayakumar et al. recently reported that traumatized astrocytes showed increased protein oxidation/nitration of Na-K-Cl cotransporter-1, and that antioxidant or NOS inhibitor (L-NAME) treatment of cultures led to decreased trauma-induced NKCC1 activity and its phosphorylation (14). Jayakumar et al. reported in another study that cultured astrocytes showed peak NO levels peaked at 1 h after trauma and the levels decreased after 2 and 3 h (15). Increased oxidation/nitration are therefore primarily responsible for increased Na-K-Cl cotransporter-1 activity and consequently cellular swelling in traumatized cultured astrocytes.

CONCLUSION

Proanthocyanidin attenuated oxidative and nitrosative stress and decreased brain edema in this study. This effect of proanthocyanidin seems to be based on its antioxidant effects.

REFERENCES

1. Asha Devi S, Sagar Chandrasekar BK, Manjula KR, Ishii N: Grape seed proanthocyanidin lowers brain oxidative stress in adult and middle-aged rats. *Exp Gerontol* 46(11): 958-964, 2011
2. Asttrups J: Energy requiring cell functions in the ischemic brain. *J Neurosurg* 56: 482-497, 1982
3. Berger S, Schürer L, Hartl R, Messmer K, Baethmann A: Reduction of post-traumatic intracranial hypertension by hypertonic/hyperoncotic saline/dextran and hypertonic mannitol. *Neurosurgery* 37:98-108, 1995
4. Black KL, Hoff JT: Leukotrienes increase blood-brain barrier permeability following intraparenchymal injection in rats. *Ann Neurol*, 6: 632-640, 1987
5. Colden-Stanfield M, Schilling WP, Ritchie AK, Eskin SG, Navarro LT, Kunze DL: Bradykinin induced increased in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. *Circ Res* 632-640, 1987
6. Dajas F, Rivera-Megret F, Blasina F, Arredondo F, Abin-Carriquiry JA, Costa G, Echeverry C, Lafon L, Heizen H, Ferreira M, Morquio A: Neuroprotection by flavonoids. *Braz J Med Biol Res* 36(12): 1613-1620, 2003
7. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A: Biomarkers of oxidative damage in human disease. *Clin Chem* 52(4): 601-623, 2006
8. Devi A, Jolitha AB, Ishii N: Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. *Med Sci Monit* 12(4): 124-129, 2006

9. Feldman Z, Zachari S, Reichenthal E, Artru AA, Shapira Y: Brain edema and neurological status with rapid infusion of lactated Ringer's or 5% dextrose solution following head trauma. *J Neurosurg* 83: 1060-1066, 1995
10. Habeeb AF: Reaction of protein sulfhydryl groups with Ellman's reagent. *Methods Enzymol* 25: 457-464, 1972
11. Halliwell B: The role of oxygen radicals in human disease, with particular reference to the vascular system. *Haemostasis Suppl* 1: 118-126, 1993
12. Halliwell B: Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Arch Biochem Biophys* 476(2): 107-112, 2008
13. Hariri RJ: Cerebral edema. *Neurosurg Clin N Am* 5(4): 687-706, 1994
14. Jayakumar AR, Panickar KS, Curtis KM, Tong XY, Moriyama M, Norenberg MD: Na-K-Cl cotransporter-1 in the mechanism of cell swelling in cultured astrocytes after fluid percussion injury. *J Neurochem* 117(3): 437-448, 2011
15. Jayakumar AR, Rao KV, Panickar KS, Moriyama M, Reddy PV, Norenberg MD: Trauma-induced cell swelling in cultured astrocytes. *J Neuropathol Exp Neurol* 67(5): 417-427, 2008
16. Klatzo I: Neuropathological aspects of brain edema. *J Neuropathol Exp Neurol* 26(1): 1-14, 1967
17. Kontos HA: Oxygen radicals in cerebral ischemia: The 2001 Willis lecture. *Stroke* 32(11): 2712-2716, 2001
18. Kuzkaya N, Weissmann N, Harrison DG, Dikalov S: Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: Implications for uncoupling endothelial nitric-oxide synthase. *J Biol Chem* 278(25): 22546-22554, 2003
19. Lanfitt TW: Increased intracranial pressure and the cerebral circulation. In: Youmans (ed), *Neurological Surgery*, Vol: 1, Philadelphia: WB Saunders, 1996: 482-490,
20. Long DM: Traumatic brain edema. *Clin Neurosurg* 29: 174-202, 1982
21. Love S: Oxidative stress in brain ischemia. *Brain Pathol* 9(1): 119-131, 1999
22. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA: Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr* 74(4): 418-425, 2001
23. Onal C, Unal F, Turantan MI: The effect of intraventricular albumin in experimental brain oedema. *Acta Neurochir (Wien)* 139: 661-669, 1997
24. Panickar KS, Jayakumar AR, Norenberg MD: Differential response of neural cells to trauma-induced free radical production in vitro. *Neurochem Res* 27(1-2): 161-166, 2002
25. Pollay M: Blood-Brain Barrier: Cerebral Edema. In: Wilkins RH, Rengachary SS (eds), *Neurosurgery*, 2nd ed. Vol: 1, New York: McGraw Hill, 1996: 335-340
26. Senes M, Kazan N, Coskun O, Zengi O, Inan L, Yucel D: Oxidative and nitrosative stress in acute ischaemic stroke. *Ann Clin Biochem* 44: 43-47, 2007
27. Smárason AK, Allman KG, Young D, Redman CW: Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with pre-eclampsia. *Br J Obstet Gynaecol* 104(5): 538-543, 1997
28. Strathearn KE, Yusef GG, Grace MH, Roy SL, Tambe MA, Ferruzzi MG, Wu QL, Simon JE, Lila MA, Rochet JC: Neuroprotective effects of anthocyanin- and proanthocyanidin-rich extracts in cellular models of Parkinson's disease. *Brain Res* 1555:60-77, 2014
29. Tekiner A, Yucel D, Bayar MA, Yucel O, Erdem Y, Karakaya J: The effect of Nitrogen Mustard on the enzymatic antioxidant defense of rat brain tissue and the therapeutic value of proanthocyanidin. *Turk Neurosurg* 21(4): 461-466, 2011
30. Tominaga T, Ohnishi ST: Ion movements and edema formation in CNS injury. In: Ohnishi ST, Ohnishi T, *Central Nervous System Trauma and Research Techniques*. NY: CRC Boca Raton, 1995: 85-94
31. Unterberg AW, Stover J, Kress B, Kiening KL: Edema and brain trauma. *Neuroscience*. 129(4): 1021-1029, 2004
32. Urquiaga I, Leighton F: Plant polyphenol antioxidants and oxidative stress. *Biol Res* 33(2): 55-64, 2000
33. Wang J, Ferruzzi MG, Ho L, Blount J, Janle EM, Gong B, Pan Y, Gowda GA, Raftery D, Arrieta-Cruz I, Sharma V, Cooper B, Lobo J, Simon JE, Zhang C, Cheng A, Qian X, Ono K, Teplow DB, Pavlides C, Dixon RA, Pasinetti GM: Brain-targeted proanthocyanidin metabolites for Alzheimer's disease treatment. *J Neurosci* 32(15): 5144-5150, 2012
34. Wasowicz W, Nève J, Peretz A: Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: Importance of extraction pH and influence of sample preservation and storage. *Clin Chem* 39(12):2522-2526, 1993