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Inflammation and neurochemical alterations induced by oral aspartame administration in experimental rats.

KEYWORDS: Aspartame, neurotoxicity, oxidative stress, inflammation

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ABSTRACT *The present study was aimed to investigate the effects of repeated oral administration of aspartame (in a daily acceptable dose 40mg/kg body weight) for 2, 4 and 6 weeks on oxidative stress and inflammation in brain tissue of rats . For this purpose, thirty six healthy adult wistar albino rats were divided into two groups; Group 1 (control group), Group 2 (aspartame group): consisted of 18 animals were subdivided into three subgroups (6 per each) ; subgroup A, subgroup B and subgroupC, orally administrated aspartame (40mg/kg body weight) daily for 2,4 and 6 weeks ,respectively. Results showed that daily aspartame administration in a dose (40mg/kg body weight daily) for 4 and 6 weeks induced oxidative stress in rat's brain which was indicated by the significant increment in protein carbonyl content in association with significant decrease in reduced glutathione concentration ($p<0.05$). Also, there was a significant increase in brain interleukin-1 IL-1β (IL-1β) and tumor necrosis factor-α (TNF-α) production which accompanied with a significant decrease in brain-derived neurotrophic factor (BNDF) and serotonin levels in aspartame –treated rats for 6 weeks compared to 4 and 2 weeks intervals and their corresponding control rats ($p <0.05$). Acetyl choline esterase (AchE) activity was significantly decreased which accompanied by a significant increase in acetyl choline (Ach) concentration in brain homogenates in aspartame administrated groups for 4 and 6 weeks. It was concluded that repeated oral administration of aspartame induce neurotoxicity which progress with increasing cumulative doses of aspartame.*

Introduction:

Aspartame (Asp; L-aspartyl-L-phenylalanine methyl ester) is one of the most widely used artificial sweeteners in over 90 countries worldwide (***Magnuson et al.,2007***). It is found in 6000 products including soft drinks, chewing gum, candy, yoghurt, tabletop sweeteners and some pharmaceuticals such as vitamins and sugar-free cough drops (***Soffritti et al.,2006)***. A recent observation indicated that aspartame is slowly making its way into ordinary products used every day, which do not carry any indication of being for people on diets or diabetics. Thus, aspartame is used not only by the above mentioned group of people, but also by unsuspecting individuals (***Humphries et al.,2008***).

Aspartame is rapidly absorbed and metabolized by intestinal esterase and dipeptidase to aspartic, phenyl alanine and methanol ***(Mourad and Noor,2011).*** Phenylalanine plays an important role in neurotransmitter regulation, whereas aspartic acid is also thought to play a role as an excitatory neurotransmitter in the central nervous system. Glutamate, asparagines and glutamine are formed from their precursor, aspartic acid. Methanol, which forms 10% of the broken down product, is converted in the body to formate, which can either be excreted or can give rise to formaldehyde (***Humphries et al.,2008***). Ingestion of aspartame eventually results in weight gain because formaldehyde stores in the fat cells, particularly in the hips and thighs; Therefore, aspartame is believed to cause problem in diabetic control (***Mehl-Madron, 2005)***. Also, aspartame acts as a chemical stressor because of increased generation of free radicals in serum which may be the reason for variation of cytokine level and results in alteration of immune function. Aspartame metabolites as methanol or formaldehyde may be the causative factors behind the changes observed (***Choudharyand Devi, 2015).***

Moreover, many studies proposed that excessive aspartame ingestion might be involved in the pathogenesis of certain mental disorders and also in compromised learning and emotional functioning (***Humphrise et al., 2008***). In addition, long –term consumption of aspartame alters the balance in the antioxidant/pro-oxidant status in brain ***(Abhilash et al., 2013).*** Therefore, the present study was undertaken to investigate the biochemical changes in brain after oral aspartame administration for a duration of 2, 4and 6 weeks.

Materials and Methods

Animals:

Thirty six adult male Wistar strain albino rats body weight 200g ±5g were purchased from Breeding Unit of Medical Research center (Faculty of Medicine, Ain Shams University). The rats were housed in steel cages under controlled condition of temperature 25°C ± 5°C humidity 50%±10%, and acclimatized to 12 hr light/dark. The experimental period was 6 weeks on which food and water were provide ad libitum. Animal experiment was conducted according to the guidelines of institutional animal ethical committee.

Diet: Balanced diet was based on AIN-93 recommendations as described by ***Reeves et al.(1993)***

Chemicals:

Pure aspartame powder was purchased from ADWIA Co., Cairo, Egypt. Aspartame was dissolved in distilled water immediately before administration. Ach, AchE and BNDF enzyme immunoassay kits were purchased from WKEA Co, USA. Reduced glutathione (GSH), α (TNF-α) and (IL-1β) enzyme immunoassay kits were purchased from IBL Co., Ltd Japan. Protein carbonyl colorimetric assay kits were purchased from CAYMAN chemicals, USA. Serotonin enzyme immunoassay kits were purchased from ABNOVA, USA.

Experimental design:

The animals were divided into: Group 1 (control group): consisted of 18 animals, Group 2 (aspartame group: consisted of 18 animals were subdivided into three subgroups:

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- subgroup A : consisted of 6 animals fed on balanced diet and orally administrated a daily dose of aspartame (40mg/kg body weight) by intragastric tube
- subgroup B: consisted of 6 animals fed on balanced diet and orally administrated a daily dose of aspartame for 4 weeks .
- Subgroup C : consisted of 6 animals fed on balanced diet and orally administrated a daily dose of aspartame for 6 weeks .

6 animals of each experimental subgroup and 6 animals of control group were scarified by cervical dislocation after 2, 4 and 6 weeks successively. Brain tissue were removed, then washed with cold saline (0.9% Na Cl), and stored at −80°C for biochemical analyses.

Tissue preparation:
For the determination of BNDF, (TNF-α) , and (IL-1β), the brain samples were homogenized with 0.1M phosphate buffer saline at pH 7.4, to give a final concentration of 10% W/V. Brain homogenate samples were centrifuged at 4000 rpm for 15 min at 4°C. Supernatant was collected and stored at −80°C until assay of biomarkers. For the determination of monoamine neurotransmitters, frozen samples were homogenized in cold 0.1N perchloric acid. For determination of protein carbonyl and GSH, brain samples were homogenized with in ice-cold 50 mM Tris (pH 7.4)

Biochemical assays:
Protein carbonyl content was analyzed in brain homogenate by 2,4-dinitrophenylhydrazine (DNPH) method as described by ***Levine et al.(1990).*** 5-hydroxytrypatmine concentrations were analyzed by ultra-sensitive immunoassay according to method described by ***Curzon and Green (1970).*** The brain homogenate was then evaluated for AchE activity and Ach level using the methods of ***Den Blaauwen et al. (1983),*** and ***Oswald et al. (2008)***, respectively. Also, BDNF, , TNF-α and IL-1β were measured by various commercially available rat enzyme-linked immunosorbent assay kits according to ***Rios et al. (2001), Beutler et al. (1985) and Grassi et al. (1991),*** respectively. Brain GSH concentration was determined according to ***Pastore et al. (2001)***

Statistical Analysis:
The data were presented as means ±SD. One way analysis of variance (ANOVA) followed by post- hoc least tukey for multiple comparisons. Significant difference analysis (LSD) at ($p< 0.05$) was performed using the statistical package for social science (SPSS) version 9 to compare all treated groups. Differences were considered to be significant when ($p<0.05$).

Results:
The results of the current study demonstrated that oral aspartame administration in a daily dose of 40mg/kg body weight daily induced a pathological changes in brain chemistry. These changes were gradual and progressive throughout three experimental intervals 2, 4 and 6 weeks.

Table (1): Effect of oral aspartame administration on protein carbonyl and reduced glutathione in the experimental groups.

Parameters	Duration	Control group	Aspartame group
Protein carbonyl (µg/ mg protein)	2 weeks	1.65 ±0.2 a,1	1.74±0.56 a,1
	4 weeks	1.72 ±0.16 a,1	2.4±0.15 b,2
	6 weeks	1.68 ±0.33 a,1	2.7±0.11 b,2
GSH (µg/mg protein)	2 weeks	42.20±3.2 a,1	43.18±0.44 a,1
	4 weeks	42.21±5.1 a,1	33.55±1.1 b,2
	6 weeks	43.69±3.3 a,1	30.89±1.9 b,2

The values are expressed as mean ± SD. The same letters means that there is no significant difference between groups in the same column. The same numbers means that there is no significant difference between groups in the same raw.

From the results recorded in table (1) it is clear that aspartame administration induce oxidative stress in rat's brain which indicated by the significant increase in protein carbonyl content and the significant decrease in reduced glutathione concentration ($p <0.05$). Protein carbonyl content increased by 37 % and 55% in brain homogenate of aspartame treated group for 4 and 6 weeks as compared to control rats. On the other hand, reduced glutathione concentration decreased by 22% and 28 % in brain homogenate of aspartame treated group for 4 and 6 weeksas compared to control rats.

Table (2): Effect of oral aspartame administration on acetylcholine esterase activity, acetyl choline and serotonin concentration in different experimental groups

Parameters	Duration	Control group	Aspartame group
Acetyl choline esterase (Umol/mg protein)	2 weeks	615.13±7.00 a,1	681.21±8.4 a,1
	4 weeks	618.73±7.00 a,1	655.6±9.15 b,2
	6 weeks	613.23±13 a,1	617.13±0.65 b,2
Acetyl choline (µ mol/mg protein)	2 weeks	94.67±4.1 a,1	85.73±1.93 a,1
	4 weeks	94.09 ±5.00 a,1	94.24±1.34
	6 weeks	95.09±10 a,1	93.79±0.61 b,2
Serotonin (ng/ mg tissue)	2 weeks	25.23±5.01 a,1	25.7 3±0.31 a,1
	4 weeks	27.06±2.00 a,1	23.52±0.68 a,1
	6 weeks	25.23±5.01 a,1	15±0. 8 b,2

As legend in table(1)
As shown in table (2) acetyl choline esterase activity significantly decreased in aspartame administrated group after 4 and 6 weeks in comparison with 2 weeks interval and their corresponding controls. This decrease in acetyl choline esterase activity resulted in a subsequent increase in acetyl choline concentration in brain tissue. In this context, acetyl choline concentration significantly increased after aspartame administration for after 4 and 6 weeks. Concerning serotonin levels, there were a significant decrease observed only after aspartame administration for 6 weeks.

Table (3): Effect of oral aspartame administration (40mg/kg body weight) on interleukin-1B, TNF-α and BNDF in different experimental groups

Parameters	Duration	Control group	Aspartame group
Interleukin-1B (pg/mg protein)	2 weeks	10.82±0.2 a,1	11.74±0.45 a,1
	4 weeks	10.59 ±7.00 a,1	15.92±1.05 b,2
	6 weeks	11.63±0.2 a,1	20.02±1.39 c,2
TNF-α (pg/mg protein)	2 weeks	600.23±5.01 a,1	605.23±11.31 a,1
	4 weeks	611.4±11.02 a,1	652.83±12.14 b,2
	6 weeks	589.93±0.7 a,1	669±20.77 b,2
BNDF (pg/mg protein)	2 weeks	107.14±3.0 a,1	124.18±4.6 a,1
	4 weeks	110.106±5.03 a,1	116.69±3.44 a,1
	6 weeks	106.23±3.02 a,1	110.39 ±1.43 b,2

As legend in table(1)
The results summarized in table (3) reported that aspartame administration in the tested dose for 4 and 6 weeks resulted in a significant increase in brain cytokines as interleukin-1 β (IL-1β)and TNF-α production compared to 2 weeks interval and their corresponding controls ($p <0.05$). IL-1β levels increased by 47% and 85% after oral administration of aspartame for 4 and 6 weeks as compared to normal rats and by 35.67% and 70 % as compared to

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aspartame treated group for 2 weeks interval. TNF-α recorded (652.83±11.31 and 669±20 pg/mg protein) in aspartame treated group for 4 weeks and 6 weeks in comparison with (605.23 ±11.31 and 600.23±10.5pg/mg protein) in aspartame treated group for 2 weeks and normal control group. With respect to BNDF and serotonin levels, there were a significant decrease after daily aspartame administration for 6 weeks in comparison with 4,2 weeks intervals and their corresponding controls ($p < 0.05$).

Discussion:

Findings in the present study suggested increased oxidative stress in brain tissue after repeated oral aspartame administration. Moreover, aspartame-induced oxidative stress may depend on the duration of aspartame administration even within the acceptable daily intake dose. This finding derives its importance from the fact that increased oxidative stress in brain has been linked to neurodegenerative diseases as well as to age-related cognitive deficits ***(Besler and Como, 2006).***

Oxidative stress can be the result of increased free radicals production or alternatively decreased endogenous antioxidants. The free radical increase in the present study may be due to the methanol that has been released during aspartame metabolism, as the pathway leading to formate (***Ashok and sheeladevi, 2014).***

In face of free radicals production, the thiol glutathione is the most important cellular free radical scavenging system in the brain ***(Berk et al.,2008***). In this context, brain reduced gulathione is significantly decreased following aspartame administration. These results suggesting that the depletion of this important antioxidant defense mechanism by increased free radicals production can further increase the vulnerability of the brain tissue to other oxidative insult (***Abdelsalam et al., 2012).*** In the same line ***Fries and Kaczynski (2011);Ruszukiew and Albercht (2015)*** reported that the reduction in GSH content may be due to mitochondrial dysfunction and oxidative stress that increase the accumulation of toxic forms of aspartame. The results of ***Patsoukis et al. (2004) and Nikolaos et al. (2004***) confirmed that the decreased protein thiol in brain is due to the oxidative damage which supporting the findings of present study. Also, ***Abhilash et al.,(2011***) reported a similar significant decrease in GSH concentration in rats brain following aspartame consumption . It is essential to point out that even with the FDA approved dosage of aspartame (40 mg / kg) similar alteration in the scavenging system was observed.

Protein carbonyl content is actually the most general indicator and far the most commonly used marker of protein oxidation (***Beal,2003***). In this study, oral aspartame exposure causes a significant increase in protein carbonyl content and a marked reduction in glutathione concentrations in brain. The previous study of ***(Ashok and sheeldevi (2014)*** supported the results of the current study reporting that aspartame exposure resulted in a significant increase in protein carbonyl.

AChE is an important regulatory enzyme, which rapidly hydrolyses neurotransmitter acetylcholine (ACh) found mainly in the brain, muscles, erythrocytes, and cholinergic neurons (***Ahmed et al., 2006***). AChE activity is sensitive to exogenous factors, including diets (***Kaizer et al.,2004***). Normal activity of (AChE) in brain is essential for brain healthy function, and changes in AChE activity are reported to be accompanied by clear signs of neurobehavioral toxicity (***Babadi et al.,2014).*** The results of this study demonstrated that aspartame inhibit AChE activity and therefore prevent AChE from working normally leading to a high level of ACh to persist in the brain . Similar results obtained by ***Simintizi et al.(2007)*** showing that incubation of hippocampal homogenates with each or sum of ASP metabolites resulted in significant reduction in AChE activity . The observed AChE inhibition in rat brain of aspartame treated group may be due an increase in reactive oxygen species production and in intracellular ca^{+} concentration caused by the metabolites as reported previously by ***(Simintzi et al.,2007).***

Serotonin , also called 5-hydroxytryptamine (5HT) has multiple physiologic roles, including pain perception, regulation of sleep, appetite, temperature, blood pressure, cognitive functions and mood ***(Harvey,2011).*** The results showed that serotonin concentration was significantly decreased in aspartame treated rats as compared to normal control rats. These results are in accordance with the results of ***(Abdel-Salam et al., 2012)*** showing that aspartame caused dose-dependent inhibition of brain serotonin. ***Sharma et al.(1987***) explained the reduction in the concentrations of serotonin may be due to increased supply of phenylalanine which may be responsible for a decrease in tryptophan uptake by the brain tissue or for a depression in tryptophan conversion to serotonin. Also, ***Tsakiris et al .(2006***) reported that excess of phenyl alanine, will saturate this carrier system excluding other amino acids such as tyrosine and tryptophane from entery into the brain .Thus, high level of phenylalanine interfere with the conversion of tyrosine into adrenaline and serotonin possibly affecting AChE activity .

Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family (consisting of nerve growth factor, BDNF, neurotrophin-3, and neurotrophin-4), has been intensely studied concerning its positive effect on survival promotion and synaptic regulation in the central nervous system ***(Numakwa,2014)*** . BDNF is the most prevalent growth factor in the central nervous system (CNS). It is essential for the development of the CNS and for neuronal plasticity. Because BDNF plays a crucial role in development and plasticity of the brain, it is widely implicated in psychiatric diseases ***(Autry and monteggia,2012) .*** Also***, Kimpoton (2012)*** postulated that the reduction in BNDF is directly involved in the pathophysiology of depression. The results of the current study showed that daily oral administration of ASP (40mg/kg b.wt/day) for 6 weeks significantly reduced BDNF level in brain suggesting that loss or alterations in it. On the other hand, no significant change observed after oral administration of aspartame for 2 or 4 weeks. This decrease in BNDF may contribute to the pathogenesis of neuronal dysfunction which may be related to the high or toxic concentrations of the sweetener metabolites (***Abdel-Salam et al., 2012***). The results of the current study are in harmony with that obtained by ***Saleh (2015)*** who observed the significant reduction in BNDF in rat cerebral cortex after aspartame administration.

Inflammatory cytokines have potent effects in the brain on altering mood, cognitive function, endocrine and behavioral responses (***Dantzer and Kelley, 2007).*** Many studies have showed links between elevated levels of inflammatory cytokines including elevated levels like IL-1β and TNF-α and mood disorder ***(Hashmi et al.,2013).*** In the present study, aspartame administration caused an elevation in the levels of the pro-inflammatory cytokines IL-1β

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and TNF-α in brain homogenate. The role of inflammatory pathways in the pathophysiology of depression explained by ***Catena-Dell'Osso et al.(2013***). The increase in pro-inflammatory cytokines would lead to increased oxidative damage with a production of tryptophane catabolites and consequent reduced availability of tryptophane and serotonin. The results of the current study are in accordance with the results of ***Saleh (2015)*** who reported that aspartame administration resulted in a significant increase in TNF-α level in the rat cerebral cortex. These results indicate that aspartame neurotoxicity may be due to induction of inflammatory process.

Conculsion:

From these results it is concluded that aspartame ingestion could alter the brain antioxidant status and increase oxidative damage in brain proteins.These abnormal changes may induce neurochemical alterations and neuro-inflammation leading to neurotoxicity and depression which become more prominent following long term consumption.

REFERENCE

1-Ahmed M, Rocha JBT, Correˆa M et al (2006): Inhibition of two different cholinesterases by tacrine. Chem Biol Interact 162: 165–171 2- Abhilash, M., Paul, M.V. , Varghese, M.V., Nair,R.H(2011): Effect of long term intake of aspartame on antioxidant defense status in liver. Food Chem Toxicol ;49(6):1203-7. 3-Ablilash,M.,Paul,M.V.,Varghese,M.V.,Nair,R.H(2013):Long term consumption of aspartame and brain antioxidant defense status. Drug Chem Toxicol .,39:135-140 4- Abdel-Salam, O.M.E., Salem, N.A., El-Shamarka, M.E.S., Hussein, J.S., Ahmed, N.A.S., El-Nagar, M.E.S.,(2012): Studies on the effects of aspartame on memory and oxidative stress in brain of mice. Eur. Rev. Med. Pharmacol. Sci. 16 (15), 2092–2101. 5-Autry,A.E and Lisa M. Monteggia,L.M (2012): Brain-Derived Neurotrophic Factor and Neuropsychiatric Disorders Pharmacol Rev., 64(2): 238–258 6- Ashok ,L.,Sheeladevi,R (2014):Biochemical response and mitochondrial mediated activation of apoptosis on long term effect of aspartame in rat brain.Redox boil.,.2:820-831 7-Babadi,V.Y., Sadeghi,L., Shirani,K., Malekirad,A.A., and Mohammad Rezaei,M(2014): The Toxic Effect of Manganese on the Acetylcholinesterase Activity in Rat Brains. Journal of Toxicology.,2014 , 4 pages 8- Beal, M.F(2002):. Oxidatively modified proteins in aging and disease. Free Radic Biol Med;32(9):797– 803 9- Berk M., Dean O and Bush., A.I. (2008): Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol.,11:851–876 10- Besler,H.T. and Como,S (2006):Lipoproteins oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerosis.Nutr.Neurosci;6:189-196 11-Beutler, B. ; Greenwald, D. and Hulmes, J.D. (1985): Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature, 316: 552-554. 12-Catena-Dell'Osso ,M., Rotella ,F., Dell'Osso ,A., Fagiolini, A and Marazziti, D(2013): Inflammation, serotonin and major depression. Curr Drug Targets. May 1;14(5):571-7. 13- Choudhary, A.K., Devi, R.S., (2015): Longer period of oral administration of aspartame on cytokine response in Wistar albino rats. Endocrinología y Nutrición. 62 (3), 114-122 14-Curzon,G and Green,A.R.(1970):Rapid method for the determination of 5-hydroxytryptamine and 5- hydroxyindolacetic acid in small regions of rat brain. Br J Pharmacol.,39:653-658. 15-Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun. 2007;21:153–160 16- Den Blaauwen DH, PoppeWA, Tritschler W (1983): Acetylcholinesterase with acetylthiocholine iodide as substrate: references depending on age and sex with special reference to hormonal effects and pregnancy. J ClinChemClinBiochem., 21: 381–386 17- Engblom, D., Saha, S., Ericsson-Dahlstrand, A., Jakobsson, P.J., Blomqvist, A.,(2002): Prostaglandins as inflammatory messengers across the blood-brain barrier. J. Mol. Med. ., 80 (1), 5–15. 18- Fries, G.R and Kapczinski, F., (2011):N-acetylcysteine as a mitochondrial enhancer: a new class of psychoactive drugs? Rev. Bras. Psiquiatr.,33 (4): 245–255. 19- Grassi, J. ; Roberge, C.J. and Frobert, Y. (1991): Determination of IL-1α, IL-1β and IL-2 in biological media using specific enzyme immunometric assay. Immunol. Res., 119: 125-145. 20-Harvey, R.A. and Ferrier, D.R (2011): Lippincott's Illustrated Review:Biochemistry, Fifith Edition: Lippincott Williams and Wilkins. 21- Humpheries,p., Pretorus,E. and Naude,H (2008): Direct and indirect cellular effects of aspartame on the brain .Eur.J.Clin .Nut .,62,451 22-Kaizer RR, Silva AC, Morsch VM et al (2004) Diet-induced changes in AChE activity after long-term exposure. Neurochem Res., 29:2251–2255 23-Kimpton ,J (2012): The brain derived neurotrophic factor and influences of stress in depression. Psychiatr Danub., 24 Suppl 1:S169-71 24-Levine,R.L.,Garland,D.,Oliver, C.N.,Amici, A.,Climent,I.,Lenz,A.G.,Ahn,B.,Shaltiel,S and Stadtman,E.R(1990):Determination of carbonyl content in oxidatively modified proteins methods in Enzymology.,186:464-478 25- Lu, B., Nagappan, G., Guan, X., Nathan, P.J., Wren, P.(2013): BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. Nat. Rev. Neurosci.,14 (6);401–416. 26-MagnusenMagnusen,B.A.,Burdock,G.A.,Doull,J.,Kores,R.M.,Marsh,G .M., Pariza,M.W.,Spencer,P.S.,Waddell,W.J.,Walker,R and Williams,G.M (2007): Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. Crit Rev Toxicol., 37:629–727. 27- Mehl-Madrona, L (2005): Autism an overview and theories on its causes.http://www.healingarts.org/mehl-madrona/autism accessed:17 December 2005 28-Mourad,I.M.,Noor,N.A(2011):Aspartame (a widely used artificial sweeteners) and oxidative stress in the rat cerebral cortex.In.J.Pharm.Biomed.Sci.,2(1):4-10 29-Nikolaos ,P., George ,Z., Nikolaos, T.P., Christos, D.G., Fevronia., A and Nikolaos A.M (2004): Thiol redox state (TRS) and oxidative stress in the mouse hippocampus after pentylenetetrazol-induced epileptic seizure. Neurosci. Lett., 357 83–86 30- Numakawa,T (2014): Possible protective action of neurotrophic factors and natural compounds against common neurodegenerative diseases. Neural Regen Res.,15; 9(16): 1506–1508. 31- Oswald C, Smits SH, Hoing M, Sohn-Bosser L, Dupont L, Le Rudulier D, Schmitt L, Bremer E (2008): Crystal structures of choline/acetylcholine substrate-binding protein chox from sinorhizobiummeliloti in the liganded and unliganded–closed states. J BiolChem 283: 32848–32859. 32- Patsoukis, N., Zervoudakis, G. , Panagopoulos, N.T., Georgiou, C.D., Angelatou, F.,Matsokis,N.A(2004): Thiol redox state (TRS) and oxidative stress in the mouse hippocampus after pentylene tetrazol-induced epileptic seizure, Neuroscience Letters., 357 : 83–86. 33- Pastore, A.; Piemonte, F.; Locatelli, M.; Gaeta, L.; Tozzi, G. and Federici, G. (2001): Determination of Blood Total, Reduced and Oxidized Glutathione in Pediatric Subjects. Clin. Chem. 47(8): 1467-1469. 34- Reeves, G.; Nielsen, F.H. and Fahey J.R.G.C. (1993): AIN-1993 reformation of the AIN-76: purified diet for laboratory rodents: Final Report of The American Institute of Nutrition, purified AdHOC writing committee on the Reformation of the AIN-76 A rodent diet. American Institute, J. Nutr. 26: 1939-1951 35- Rios, M.; Fan, G.; Fekete, C.; Kelly, J.; Bates, B.; Kuehn, R.; Lechan, R. M.; Jaenisch, R.(2001): Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Molec. Endocr. 15: 1748-1757 36-Ruszkiewicz, J.and Albrecht, J.(2015): Changes in the mitochondrial antioxidant systems in neurodegenerative diseases and acute brain disorders. Neurochem. Int. http://dx.doi.org/10.1016/j.neuint. 2014.12.012. 37-Saleh,A.A.S (2015):Anti-neuroinflammatory and antioxidant effects of N-asetyl cysteine in long- term consumption of artificaial sweetner aspartame in the rat cerebral cortex.The journal of basic and applied science.,72:73-80 38- Sharma, R.P., and R.A. Coulombe, Jr. (1987): Effects of repeated doses of aspartame on serotonin and its metabolite in various regions of the mouse brain. Food Chem. Toxicol. 25:565-568. 39- Sharma,R.P., Tun, N.and Grayson, D.R (2008): Depolarization induces downregulation of DNMT1 and DNMT3a in primary cortical cultures Epigenetics, 3 (2) (2008), pp. 74–80 40- Simintzi,I., Schulpis,K.H., Angelogianni,P.,Liapi,C and Tsakiris,S (2007): The effect of aspartame on acetylcholineestaerase activity in hippocampal homogenates of suckling rats .Pharmacological research ,56:155-159 41- Soffritti, M., Belpoggi, F., Esposti, D., Lambertini, L., Tibaldi, E., Rigano, A. (2006):First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to Sprague-Dawley rats. Environ. Health Perspect. 114 (3), 379–385. 42-Tsakiris,s.,Giannoulia-Karantana,A .,Simintzi,I.,Schulpis,K.H (2006): The effect of aspartame metabolites on human erthyrocyte membrane acetyl-cholineesterase activity .Pharmacol Res,53:1-5