International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Volume 3, Issue 7 - 2016

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-7-22

Comparative studies on lens structure and function of newborn rats maternally treated with aspartame, monosodium glutamate and galactose

Hassan IH El-Sayyad*[1], Soad A Khalifa*, Amoura Mohamed Abou-El-Naga*, Eman HM Bakr*, Hamed A N Jala**

*.Zoology Dept., Faculty of Science, Mansoura Univ., Egypt.
**. Zoology Dept., Faculty of Science, Omar El-Moktar Univ., El-Byda, Libya.
*[1].Corresponding Author: **Prof. Dr. Hassan IH El-Sayyad, PhD**. Faculty of Science. Mansoura University, E-mail: *elsayyad@mans.edu.eg*

Abstract

Offspring Wistar albino rats maternally ingested orally 1g/kg. body weight aspartame or monosodium glutamate or galactose throughout gestation and till 21 days post-partum. The lens of offspring were investigated at 1,7,14 and 21 days-postnatal, meanwhile mothers were examined only at the end of experiment. Scanning electron microscopy and biochemical analysis of advanced glycation end product (AGE), protein carbonylation, endothelin-1 (ET-1), adhesion molecules (ICAM-1 & VCAM-1), zinc, iron, sodium and potassium. Comparing with the control, Mother rats and their offspring treated with aspartame or glutamate or galactose showed a marked increase of protein carbonylation and advanced glycation end product, endothelin-1 and adhesion molecules (ICAM-1 & VCAM-1). Also, the lens zinc, iron and sodium contents were markedly increased, however potassium content was markedly decreased. On the other hand, offspring maternally-treated with either aspartame or glutamate or galactose exhibited disorganized and loosely attached lens fibers with increased deformation of ball and socket. Increased deformation of lens fibers were detected in offspring maternally-treated with galactose. The authors finally concluded that maternal administration of aspartame, glutamate or galactose should be restricted to avoid complicatied lesions of retina and lens.

Keywords: Aspartame, glutamatae, galactose, lens, other rats, offspring.

Introduction

The eye lens is an ectodermal organ, developed at the 25th day of gestation. The lens fibers developed and matured after birth with subsequent formation of crystalline protein especially in adult state((Beby *et al*., 2003).. The eye lens is composed of fiber cells that are filled with α-, β- and γ-crystallins. The primary function of crystallins is to maintain the clarity of the lens through ordered interactions as well as through the chaperone-like function of α-crystallin. With aging, the chaperone function of α-crystallin decreases, with the concomitant accumulation of water-insoluble, light-scattering oligomers and crystallin-derived peptides (Santhoshkumar et al., 2011). Cataractous lenses defined as a clouding of the lens parts leading to light scattering of precipitated lens proteins which formed mainly of crystallins (Takata et al., 2007) and divided into congenital or infantile and age-related (Hu et al., 2014; El-Sayyad et al., 2015). Although, lens proteins have very long half-lives, they undergo several modifications such as deamidation, oxidation and glycation which altered protein configuration and development of cataractous lenses (Sakaue et al., 2015).

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a low calorie sweetener with approximately 200 times greater than sugar. Following ingestion, it is metabolized in the intestine to phenylalanine (50%), aspartate (40%) and methanol (10%) **(**Stegink et al., 1981). There is no available work concerning ocular toxicities of aspartame. However, several studies reported a contribution of aspartic acid (N-methyl-D-asparate ,NMDA) to damage of retinal ganglion cells (Nakazawa et al., 2005; Narouko et al., 2013; Hayashi et al., 2015; Sakamoto et al., 2015) and amacrine cells (Metoki et al., 2005). Abnormal increase of phenylalanine and tyrosine and aspartic acid levels in the brain, led to reduction of brain dopamine (Fernstorm *et al.*, 1983) and development of a PKU (phenylketonuria) disorder [14]. PKU is characterized by hyperphenylalanaemia associated with eye abnormalities such as photophobia, cataracts, and corneal opacities (Zwaan, 1983).

Galactose is a hexose sugar that differs from glucose by the configuration of the hydroxyl group at the carbon-4. It is present in milk, dairy products, and many other food types such as fruits and vegetables (Acosta and Gross, 1995). Galactosemia is an autosomal recessive inherited deficiency of one of the three mentioned enzymes especially galactose-1-phosphate uridyltransferase. This enzyme is observed in the normal foetal liver at 10^{th} week of gestation and its deficiency cause congenital abnormality (Laumonier et al., 2005). In human, cataract was reported in two brothers and one sister with a history of galactosaemia and deficiency of galactose-1-phosphate-uridyltransferase (Schmidt et al., 2011). Also, cataract may be attributed to galactokinase deficiency, the enzyme involved in the accumulation of galactitol within the lens (Ai et al., 2000) as well reduction of glutathione which enhanced formation of the advanced glycation end product product (Janzen et al., 2011 ; Jyothi et al., 2011; Singh et al., 2012). The development of cataract remains unclear, although different explanation described its causes. The role of aspartame and glutamate is not available, however several studies touches the role of galactose-treatment. The present study illustrates the structural abnormalities of lens fibers and biochemical markers of lens damage of three widely known nutritive components ; aspartame, monosodium glutamate and galactose.

Materials and Methods

Chemicals:

All of the chemicals used were of highest purity. Monosodium glutamate and galactose were supplied from Sigma-Aldrich Company (USA). Meanwhile aspartame was obtained from pharmacists.

Aspartame, monosodium glutamate and galactose-treatment:

Monosodium glutamate and galactose were dissolved in tap water and orally administered daily at doses of 1g/kg body weight from 6th day of gestation till parturition 21-days post-partum. However, aspartame administered at a dose of 0.1g via the same route of administration.

Experimental work:

Virgin female and male Wistar albino rats were obtained from Ministry of Health, Cairo. They were acclimatizing for 15 days before experimentation. They were made pregnant by mating with fertile male by placing them in plastic cages (1 male: 2 females). Zero date of gestation was determined and pregnant were separated. They were kept in good aerated room with 12 hours light and dark cycle. Standard diet and water were allowed *ad-libitum*. The pregnant rats were divided into four groups (n=20); control**.,** aspartame-, glutamate and galactose--treated groups.

Offspring of studied animals were obtained at 1,7,14 and 21-day old. Both mothers at the end of experiment and their breast-feeding-young were sacrificed by diethyl ether. Ocular regions of both mothers and young were removed and their lenses and retinas were separated and subjected for the following:

1. Scanning electron microscopic investigation:

Lenses of offspring were incised, fixed in 2.5 % buffered glutaraldhyde, dehydrated in ascending ethyl alcohol , dried in a critically carbon dioxide apparatus and coated with a thin layer of gold by DC sputtering and viewed under scanning electron microscope JOEL5300 JSM.

2. Biochemical investigation:

The lens specimens were homogenized in 10% ice-cold 2.5 mM-tris buffer (pH 7.5) and centrifuged at 14000 x *g* for 5 min at 4°C *g*. The supernatant was kept in refrigerator.

2.1. Protein carbonylation & advanced glycation end products:

Lens protein carbonylation and advanced glycation end product (AGE) was determined depending of its

reactive carbonyl groups using Cells Biolabs ELISA Kit (San Diego, CA 92126 USA, Cat. no. STA-317). The colour index of hydrazone protein is determined at wavelengths 360-385 nm.

2.2. Determination of intracellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1:

Lens adhesion molecules (ICAM-1 and VCAM-1) were assayed according to ELISA kit (R&D Systems; Minneapolis, MN). It is determined by conjugating 100 µL antibodies against recombinant human rat ICAM-1 and VCAM-1 and then with horseradish peroxidase followed by adding 100 µL of tetra-methylbenzidine for colour development and measure optical density at a wavelength of 450 nm & 620 nm.
2.4. Endothelin-1: It is determined using ELISA Kit (USCN Life Science Inc, catalogue No. CCA482Hu) using specific antibody followed by conjugation with horseradish peroxidase (HRP). The binding HRP is proportional to the amount of ET-1 in the lens and the absorbance was measured at 450 nm.

2.5. Determination of iron, zinc, sodium and potassuim contents:

Iron, zinc, sodium and potassium were determined in dried tissues after lipid extraction was carried out by a mixture of chloroform and methyl alcohol at ratio of 2:1. A known weight of the dried sample was digested by 1mL of nitric acid at highest purity and diluted with 4mL bi-distilled water and measured by atomic absorption spectrometry (Scancar *et al*., 2000).

3. Statistical analysis:

Data was recorded as mean ± SE and analyzed using SPSS software (version 13) by one way post-hoc analysis of variance between studied groups and the decrease of $p<0.05$ was considered significant.

Results

1. Biochemical assays:

Tables (1-3) illustrates lens protein carbonylation, advanced glycation end product, endothelin, adhesion molecules (ICAM-1& VCAM-1) and zinc,iron,sodium and potassium contents of aspartame, monosodium glutamate and galactose -treated mother rats and their offspring. Comparing with the control, there were marked increase of protein carbonylation (PC) and advanced glycation end product (AGE), endothelin-1 and adhesion molecules (ICAM-1 & VCAM-1). AGE & PC were markedly increased in galactose-treatment compared to other treatments. However, aspartame-treatment possessed marked alterations of the adhesion molecules. Also, the lens zinc, iron and sodium contents were markedly increased, however potassium content was markedly decreased compared to the control..

2. Scanning electron microscopy of lens:

The lens of mother rats and their offspring 2 & 3 weak-old possessed regular concentric layers of densely packed lens fibers with less intercellular spaces in between. Each fiber take poly-or hexagonal shape. The adjacent fibers are interconnected by numerous ball and socket junctions on each planar surface. Normal regular orientation of depressions and the existence of complementary projections are detected (Fig.1 A & A1).Offspring maternally-treated with either aspartame or glutamate or galactose exhibited disorganized and loosely attached lens fibers with increased deformation of ball and socket. Increased deformation of lens fibers were detected in offspring maternally-treated with galactose (Fig1 B-D1).

Discussion

Maternal-treatment with aspartame or glutamate or galactose increase the formation of advanced glycation end products and protein carbonylation. Similar finding of altered protein structure was reported by Sippel (1966). In vitro and in vivo studies of high galactose-treatment led to disruption of redox balance mediating Maillard reaction during cataractogenesis (Saxena *et al*., 1996). C57Bl6 mice injected daily with D-galactose 8 weeks developed advanced glycation end product-specific fluorescence in lens (Ida *et al*., 2004). Galactose showed high tendencies of forming adducts with the amino acids lysine, glycine, alanine, glutamate and aspartate and altered protein configuration and glycation products (Ramakrishnan *et al*.,1997). Aged human lenses showed increased average of d-beta-aspartic acids suspecting a role of cataract formation(Fujii *et al*., 2000). Mild posterior subcapsular cataract was detected in rabbit received intravitreal Injection of 100µL of 1% methanol or formaldhyde or formate (Hayasaka *et al*., 2001). In human, advanced glycation endproducts play a significant role in cataract formationthrough modification of lens proteins such as N(6)-Carboxymethyl lysine, N(6)-carboxyethyl lysine, N(7)-carboxyethyl arginine,

Table1. Biochemical markers of lens function of aspartame-treated mother rat and their offspring.

							Minerals			
		AGE nmol/mg	Enothelin Pg/100mg	ICAM-1 (ng/100mg)	VCAM-1 (ng/100mg)	PC nmol/mg	Fe (ng/mg)	Zn (ng/mg)	Na Mmol/gm	K Mmol/gm
M	C	304.59±3.69	6.39±0.039	1.02±0.17	1.32±0.07	2.23±0.18	92.25±3.16	8.25±1.63	121.27±2.26	8.32±0.35
	Asp	*356.54±3.40	*9.14±0.18	*1.51±0.12	*1.46±0.15	*3.05±0.27	*103.66±2.73	*10.35±0.72	*145.58±2.18	*4.54±0.33
1D	C	227.32±2.72	5.16±0.18	1.14±0.07	1.29±0.08	1.24±0.24	64.36±2.45	6.56±0.72	98.42±3.06	6.39±0.25
	Asp	*248.60±3.13	*7.518±0.18	*1.34±0.14	*1.40±0.11	*1.81±18.0	*78.09±3.15	*8.05±0.65	*123.758±2.47	*3.18±0.29
1W	C	227.41±3.16	6.01±0.25	1.10±0.04	1.27±0.07	1.46±0.15	66.98±4.019	7.14±0.571	107.73±2.09	6.86±0.03
	Asp	*261.66±3.25	*7.6±0.22	*1.35±0.08	*1.44±0.11	*1.95±0.27	*79.64±2.95	*9.61±0.58	*129.34±2.12	*3.51±0.22
2W	C	227.89±4.049	6.11±0.20	1.2±0.09	1.35±0.07	1.47±0.20	65.05±4.27	7.43±0.99	108.87±3.495	6.56±0.18
	Asp	*265.34±2.72	*7.52±0.22	*1.38±0.07	*1.46±0.07	*2.03±0.18	*87.06±3.31	*9.91±0.65	*131.50±2.85	*3.79±0.30
3W	C	228.99±6.72	6.35±0.20	1. 25±0.10	1.31±0.07	1.46±0.22	69.61±3.46	7.42±0.61	110.81±2.52	6.72±0.27
	Asp	*267.65±1.84	*7.66±0.22	*1.41±0.07	*1.48±0.07	*2.28±0.22	*93.96±2.90	*9.43±0.59	*135.38±2.55	*3.57±0.31
F-test		137.015	3.128	4.797	1.462	4.118	7.468	1.245	4.823	1.269
P<0.05		0.000	0.006	0.000	0.195	0.000	0.000	0.296	0.000	0.284

Each result represent M±SE (n=5), AGE, advanced glycation end product; Asp, aspartame; C, control; M, mother; 1D, 1day; 1W, 1week; 2W, 2week; 3W,3week ; ICAM-1,intrercellular adhesion molecule; VCAM-1,vascular cell adhesion molecule; Fe, iron; Zn, zinc; Na, sodium; K, potassium, * Significant at $P < 0.05$.

Table 2. Biochemical markers of lens function of glutamate-treated mother rat and their offspring.

							Minerals			
		AGE nmol/mg	Endothelin Pg/100mg	ICAM-1 ng/100mg	VCAM-1 ng/100mg	PC nmol/mg	Fe (ng/mg)	Zn (ng/mg)	Na (Mmol/gm)	K (Mmol/gm)
M	C	304.59±3.69	6.39±0.03	1.02±0.17	1.32±0.07	2.23±0.18	92.25±3.16	8.25±1.63	121.27±3.69	8.32±0.35
	Glu.	*376.01±3.40	*9.44±0.22	*1.58±0.03	*1.51±0.07	*3.231±0.316	*108.03±2.84	9.33±0.65	*143.33±2.37	*5.31±0.20
1D	C	227.324±2.72	5.16±0.184	1.14±0.07	1.29±0.087	1.24±0.24	64.36±2.45	6.56±0.72	98.42±2.72	6.39±0.25
	Glu.	*287.15±3.30	*7.44±0.15	1.36±0.03	1.41±0.079	2.17±0.17	*81.36±2.91	*8.23±0.53	*125.58±1.84	*3.29±0.27
1W	C	227.41±3.16	6.01±0.25	1.10±0.04	1.27±0.079	1.46±0.158	66.98±4.01	7.14±0.57	107.07±3.16	6.86±0.037
	Glu.	*291.1±3.22	7.61±0.20	1.47±0.04	1.45±0.066	*2.27±0.184	*83.15±2.94	8.37±0.47	*126.07±2.28	*3.08±0.24
2W	C	228.89±4.04	6.11±0.20	1.21±0.09	135±0.079	1.47±0.202	65.05±4.27	7.83±0.99	108.87±4.049	6.56±0.18
	Glu.	*295.26±3.2	*7.71±0.20	1.48.0413	1.49±0.07	*2.31±0.246	*87.45±3.13	*9.28±0.51	*137.86±3.25	*3.69±0.25
3W	C	228.99±6.72	5.35±0.20	1.25±0.1	1.11±0.071	1.48±0.228	69.61±3.46	7.12±0.61	110.81±6.72	6.72±0.272
	Glu.	*311.43±2.72	*7.81±0.22	1.53±0.08	1.47±0.079	*2.62±0.228	*89.43±2.39	*9.35±0.59	*136.18±2.72	*3.66±0.228
F-test		129.222	4.246	1.216	0.965	2.816	7.986	1.151	1.158	1.178
P<0.05		0.006	0.001	0.313	0.483	0.012	0.000	0.352	0.347	0.335

Each result represent M±SE (n=5),AGE, advanced glycation end product; C, control; Glu, glutamate; M, mother; 1D, 1day; 1W, 1week; 2W, 2week; 3W, 3week ; ICAM-1,intrercellular adhesion molecule; VCAM-1,vascular cell adhesion molecule; Fe, iron; Zn, zinc; Na, sodium; K, potassium. * Significant at $P < 0.05$.

Table 3. Biochemical markers of lens function of galactose-treated mother rat and their offspring.

							Minerals			
		AGE (nmol/mg)	Endothelin (Pg/100mg)	ICAM-1 (ng/100mg)	VCAM-1 (ng/100mg)	PC (nmol/mg)	Fe (ng/mg)	Zn (ng/mg)	Na (mmol/gm)	K (mmol/gm)
M	C	304.59±3.69	6.39±0.039	1.02±0.17	1.32±0.07	2.23±0.18	92.25±3.16	8.259±1.63	121.59±3.69	8.32±0.35
	GA	*376.93±2.98	*9.607±0.275	*1.49±0.09	1.48±0.11	*2.79±0.21	*115.31±2.26	*10.15±0.73	*143.33±2.43	*5.09±0.21
1D	C	227.324±2.72	5.16±0.18	1.14±0.07	1.29±0.08	1.24±0.24	64.36±2.45	6.56±0.72	98.42±2.72	6.39±0.25
	GA	*271.58±3.11	*7.28±0.228	1.39±0.07	*1.39±0.07	*1.97±0.27	*76.04±3.25	*7.63±0.57	*125.58±2.65	*3.80±0.24
1W	C	227.41±3.16	6.01±0.25	1.10±0.04	1.27±0.07	1.46±0.15	66.98±4.019	7.14±0.571	107.73±3.16	6.86±0.03
	GA	*286.46±2.28	*7.36±0.18	*1.36±0.07	1.33±0.07	1.88±0.25	*81.36±3.02	*8.875±0.63	*126.07±3.35	*3.6±0.22
2W	C	228.89±4.04	5.11±0.20	1.2±0.09	1.25±0.07	1.47±0.20	65.05±4.27	7.83±0.89	108.87±4.04	6.56±0.18
	GA	*279.61±3.16	*7.306±0.25	*1.42±0.06	1.34±0.07	1.98±0.25	*87.41±3.63	*9.43±0.85	*137.86±2.54	*3.59±0.24
3W	C	228.99±6.72	5.35±0.20	1.25±0.10	1.31±0.07	1.48±0.22	69.61±3.46	7.42±0.614	110.81±6.72	6.72±0.27
	GA	*271.64±2.98	*7.45±0.20	*1.54±0.03	*1.48±0.07	*2.25±0.22	*95.69±2.732	*10.25±0.68	*136.81±2.91	*3.69±0.22
F-test		170.885	3.918	2.745	2.170	4.712	7.554	1.404	28.512	1.761
P<0.05		0.000	0.001	0.013	0.045	0.000	0.000	0.219	0.000	0.107

Each result represent M±SE (n=5), AGE, glycation and product C, control; GA, galactose; M, mother; 1D, 1day; 1W, 1week; 2W, 2week; 3W,3week ; ICAM, intrercellular adhesion molecule; VCAM, vascular cell adhesion molecule; Fe, iron; Zn, zinc; Na, sodium; K, potassium, * Significant at $P < 0.05$.

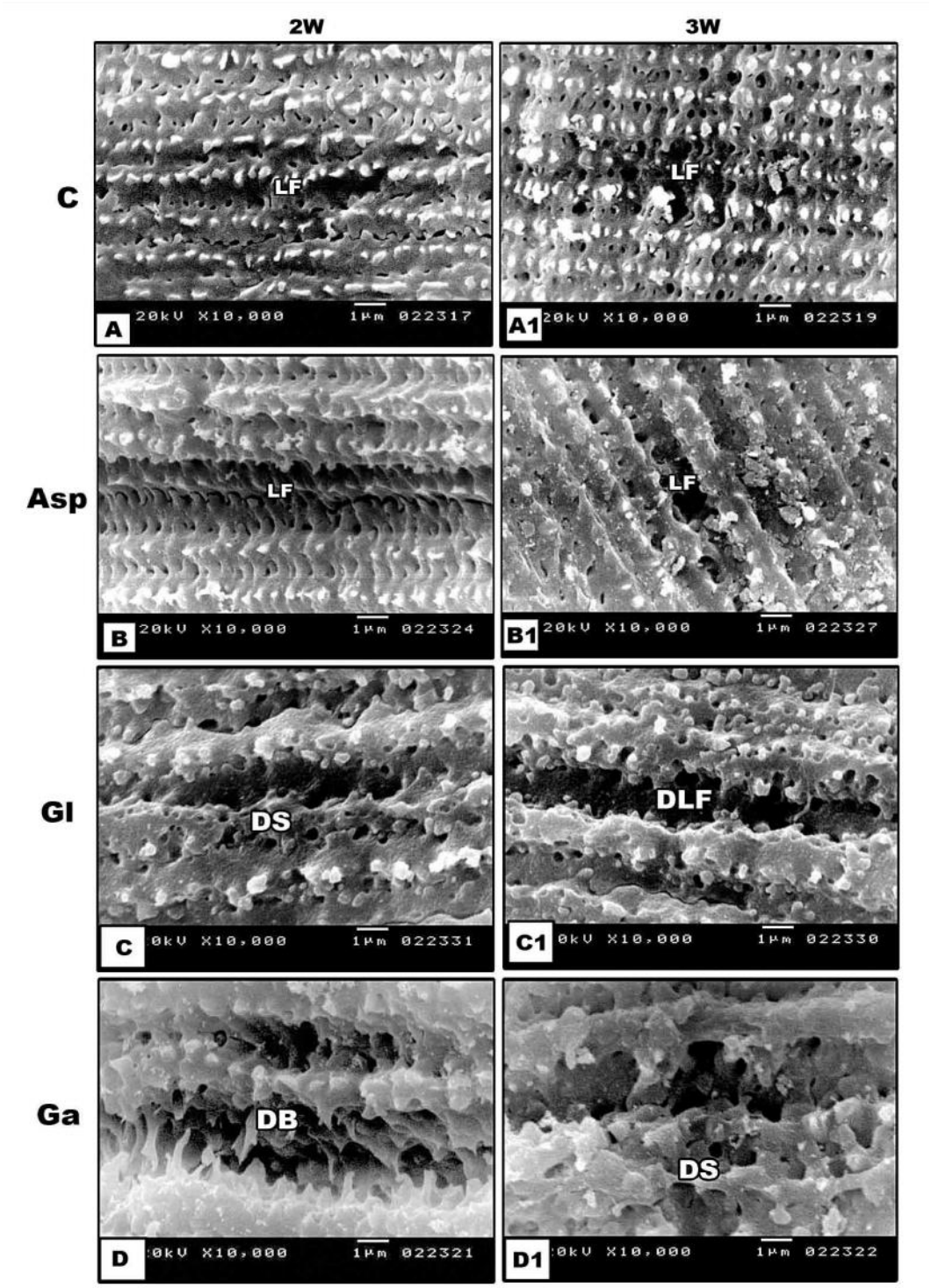

Fig.1. scanning electron micrographs of lens fibers. A &A1. Control 2 &3 week-old offspring showing normal orientation of tightly arranged lens fibers with obvious ball and socket. B &B1. 2&3 week-old offspring maternally-treated with aspartame showing fragile and disoriented lens fibers. C&C1. 2&3 week-old offspring maternally-treated with glutamate showing damaged and loosely attached lens fibers. D &D1. 2&3 week-old offspring maternally-treated with galactose showing marked deformation of lens fibers Abbreviations; DB, degenerated ball; DLF, degenerated lens fibers; DS, degenerated socket; LF, Lens fibers.

methylglyoxal hydroimidazolone 1, and N(6)-lactoyl lysine following Maillard protein modifications (Smuda *et al*., 2015). D-galactose-treatment in mice for a period of six weeks significantly increased lipid peroxidation and impaired mitochondrial complex (I, II and III) enzymes activities Ji et al., 2015). Also, accumulation of AGE and protein carbonylation in lenses was contributed in human lenses for mediate aberrant crosslinking of extracellular matrix proteins and disrupting ATP production and enhancing oxidative stress(Kandarakis et al., 2014).

The observed findings revealed marked increase of endothelin-1 and adhesion molecules (ICAM-1 & VCAM-1) in either aspartame or glutamate or galactose-treatment lenses. This was coincides with increase lens sodium and decrease of potassium content. Endothelin-1 (ET-1) was found to cause inhibition of lens active Na-K transport, keeping water balance for promoting lens function. Activation of ET receptors led to marked increase of cytoplasmic calcium concentration in cultured lens epithelial cells, the markers of cataracts (Okafor and Delmare, 2001). Sippel (1966) observed hydrated cataractous lenses in female rats fed on diet containing galactose for 12-14 days The osmotic vacuolization facilitated cataractous formation and lens membrane deterioration. Increased lens sodium and decrease of potassium confirmed alteration of sodium-potassium pump as a result of alterations of endothelin as well as facilitated water accumulation. Similar findings were reported in galactosaemic cataracts(Ramana et al.,2007) and lens of aged rats (El-Sayyad et al., 2012).

Moreover, Aspartame or glutamate or galactose-treatment possessed a detected increase of adhesion molecules especially ICAM-1 parallel with increased endothelin level. Adhesion molecule ICAM-1 is a cell surface glycoprotein, overexpressed in lens epithelium by increased fructose level (Glushakova *et al*., 2008) which led to a decrease in lens epithelial proliferation and enhanced the progression of diabetic cataract(Fan *et al*., 2012).

The present data exhibited that the applied aspartame or glutamate or galactose-treatment exhibited marked increase of zinc and iron content. Similar findings of increased lens zinc and iron content were reported by Dawczynski et al.(2002) and Gündüz et al. (2003)] in diabetic and senile human cataractous lenses. As we know that the superoxide dismutase (SOD) is dependent upon zinc and copper ions for promoting its activity and scavenging superoxide anion which is important part in oxidative stress(Lobo et al., 2010). Increased zinc content seemed to be attributed to disrupted lens metabolism. Also, transferrin and Fe concentrations was markedly at higher level in the intraocular fluids in diseased conditions and tend to be accumulated in lens during ocular inflammation. There are two ways of picking iron by tissues, receptor-medicated endocytosis of diferric transferrin and cell membrane mediated by an oxido-reductase (McGahan et al.,1995). C57BL/6 mice received subcutaneous injection of D-galactose (100mg/kg once daily) for 14 weeks altered ferroportin and hepcidin genes expression of iron in the cortex and hippocampus leading to iron accumulation and increased cognitive function (Wei et al., 2014).

The observed biochemical changes in lenses of offspring maternally-treated with aspartame, glutamate and galactose was confirmed by degeneration, disorganized orientation of lens fibers and deformation of ball and socket. Similar findings were reported in cataractous lenses (Tag-Eldin, 2016).Cataractous lenses were reported in galactose-treated experimental animals (Lackner et al.,1997; Ohta et al.,1999), monosodium-L-glutamate (Kawamura and Azuma, 1992). Offsprings maternally fed on galactose developed cataractous lenses associated with increased galactitol accumulation (Tsutsumi et al., 1992), increase osmotic swelling and cataract formation (Berry, 1995) and apoptosis of lens cells (Takamura et al.,2003). Finally the authors concluded that ingestion of diet containing overload of aspartame, glutamate or galactose, facilitated lenticular changes of lens fibers characteristic of cataract assisted by increase of its biomarker levels.

References

Acosta PB, Gross, KC (2000) Hidden sources of galactose in the environment. Eur. J. pediatr 154(7 suppl 2): S 87-S92.

Ai Y, Zheng Z, O'Brien-Jenkins A, Bernard DJ, Wynshaw-Boris T, Ning C, Reynolds R, Segal S, Huang K, Stambolian D (2000) A mouse model of galactose-induced cataracts. Hum Mol Genet 9(12):1821-27.

Beby F, Morle L, Michon L, M B, Edery P, et al. (2003) The genetics of hereditary cataract. J Fr Ophtalmol 26: 400-408.

Berry GT(1995) The role of polyols in the pathophysiology of galactosemia. Eur J Pediatr 154(Supplement 2):S45–9.

Bowen J, Evangelista MA (2002) Brain cell damage from amino acid isolates: a primary concern from aspartame-based products and artificial sweetening agents. http://www.wnho.net/ aspartame_brain_ damage.htm

Dawczynski J, Blum M, Winnefeld K, Strobel J (2002) Increased content of zinc and iron in human cataractous lenses. Biol Trace Elem Res 90: 15-23.

El-Sayyad HI, Khalifa SA, El-Sayyad FI, Maylod EE (2012) Biochemical and ultra structural changes in lens during aging albino rats**.** Br J Med Med Res 2: 105-121.

El-Sayyad HIH, Bakr EHM, El-Ghawet HA , El-Desoky T M G E (2015) Overview of congenital, senile and metabolic cataract. J Ocular Biol 3(2):1-12.

Fan H, Suzuki T, Ogata M, Nakagawa Y, Onouchi H, et al. (2012) Expression of PCNA, ICAM-1, and vimentin in lens epithelial cells of cataract patients with and without Type 2 Diabetes. Tokai J Exp Clin Med 37: 51-56.

Fernstrom JD (1994) Dietary amino acids and brain function. *J Am Diet Ass* **94** (1), 71–77.

Fujii N, Takemoto LJ, Matsumoto S, Hiroki K, Boyle D, Akaboshi M.(2000) Comparison of d-aspartic acid contents in alpha A-crystallin from normal and age-matched cataractous human lenses. Biochem Biophys Res Commun 278(2):408-13.

Glushakova O, Kosugi T, Roncal C, Mu W, Heinig M, et al. (2008) Fructose induces the inflammatory molecule ICAM-1 in endothelial cells. J Am Soc Nephrol 19: 1712-1720.

Gündüz G, Gündüz F, Yucel i, Şentürk UK (2003) Levels of zinc and magnesium in senile and diabetic senile cataractous lenses. Biol Trace Elem Res 95: 107-112.

Hayasaka Y, Hayasaka S, Nagaki Y (2001) Ocular changes after intravitreal injection of methanol, formaldehyde, or formate in rabbits. Pharmacol Toxicol 89(2):74-8.

Hayashi I, Aoki Y, Asano D, Ushikubo H, Mori A, Sakamoto K, Nakahara T, Ishii K (2015) Protective Effects of Everolimus against N-Methyl-D-aspartic Acid-Induced Retinal Damage in Rats. Biol Pharm Bull 38(11):1765-71.

Hu Y, Gao L, Feng Y, Yang T, Huang S, Shao Z, Yuan H.(2014) Identification of a novel mutation of the gene for gap junction protein α3 (GJA3) in a Chinese family with congenital cataract. Mol Biol Rep 41(7):4753-8.

Ida H, Ishibashi K, Reiser K, Hjelmeland LM, Handa JT.(2004) Ultrastructural aging of the RPE-Bruch's membrane-choriocapillaris complex in the D-galactose-treated mouse. Invest Ophthalmol Vis Sci 45(7):2348-54.

Janzen N, Illsinger S, Meyer U, Shin YS, Sander J, Lücke T, Das AM (2011) Early cataract formation due to galactokinase deficiency: impact of newborn screening. Arch Med Res 42(7):608-12.

Ji ZH, Liu C, Zhao H, Yu XY(2015) Neuroprotective effect of biatractylenolide against memory impairment in D-galactose-induced aging mice. J Mol Neurosci 55(3):678-83.

Jyothi M, Sanil R, Shashidhar S (20110 Influence of galactose cataract on erythrocytic and lenticular glutathione metabolism in albino rats.Indian J Ophthalmol 59(4):287-90.

Kandarakis SA, Piperi C, Topouzis F, Papavassiliou AG (2014) Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. Prog Retin Eye Res 42:85-102.

Kawamura M, Azuma N (1992) Morphological studies on cataract and small lens formation in neonatal rats treated with monosodium-L-glutamate. Ophthalmic Res 24(5):289-97.

Lackner PA, Rodriguez L, Sato S, Lizak MJ, Wyman M, Kador PF (1997) Age-dependent lens changes in galactose-fed dogs. Exp Eye Res.;64(3):431-6.

Laumonier E, Labalette P, Morisot C, Mouriaux F, Dobbelaere D, Rouland JF (2005) Vitreous hemorrhage in a neonate with galactosemia. A case report. J Fr Ophtalmol 28(5):490-6.

Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4(8):118-126.

McGahan MC, Grimes AM, Nasisse MP, Fleisher LN (1995) Iron uptake by cultured lens epithelial cells. Graefes Arch Clin Exp Ophthalmol. 233: 354- 359.

Metoki T, Ohguro H, Ohguro I, Mamiya K, Ito T, Nakazawa M (2005) Study of effects of antiglaucoma eye drops on N-methyl-D-aspartate-induced retinal damage. Jpn J Ophthalmol 49(6):453-61.

Nakazawa T, Shimura M, Endo S, Takahashi H, Mori N, Tamai M.(2005) N-Methyl-D-Aspartic acid suppresses Akt activity through protein phosphatase in retinal ganglion cells. Mol Vis 11:1173-82.

Naruoka T, Nakahara T, Tsuda Y, Kurauchi Y, Mori A, Sakamoto K, Nishihira J, Ishii K (2013) ISO-1, a macrophage migration inhibitory factor antagonist, prevents N-methyl-D-aspartate-induced retinal damage. Eur J Pharmacol 718(1-3):138-44.

Ohta Y, Yamasaki T, Niwa T, Goto H, Majima Y, Ishiguro I (1999) Cataract development in 12-month-old rats fed a 25% galactose diet and its relation to osmotic stress and oxidative damage.

Ophthalmic Res 31(5):321-31.

Okafor MC, Delamere NA (2001) The inhibitory influence of endothelin on active sodium-potassium transport in porcine lens. Invest Ophthalmol Vis Sci 42: 1018-1023.

Ramakrishnan S, Sulochana KN, Punitham R (1997) Free lysine, glycine, alanine, glutamic acid and aspartic acid reduce the glycation of human lens proteins by galactose. Indian J Biochem Biophys.;34(6):518-23.

Ramana BV, Raju TN, Kumar VV, Reddy PU (2007) Defensive role of quercetin against imbalances of calcium, sodium, and potassium in galactosemic cataract. Biol Trace Elem Res 119(1):35-41.

Sakamoto K, Kuroki T, Okuno Y, Sekiya H, Watanabe A, Sagawa T, Ito H, Mizuta A, Mori A, Nakahara T, Ishii K (2014) Activation of the TRPV1 channel attenuates N-methyl-D-aspartic acid-induced neuronal injury in the rat retina. Eur J Pharmacol 733:13-22.

Sakaue H, Takata T, Fujii N, Sasaki H, Fujii,N (2015) Alpha B- and βA3-crystallins containing d-aspartic acids exist in a monomeric state. Biochim Biophys Acta 1854(1):1-9.

Santhoshkumar P, Raju M, Sharma KK (2011) αA-crystallin peptide 66 SDRDKFVIFLDVKHF80 accumulating in aging lens impairs the function of α-crystallin and induces lens protein aggregation. PLoS One 6(4):e19291.

Scancar J, Milacic R, Benedik M and Bukovec P (2000) Determination of trace elements and calcium in bone of the human iliac crest by atomic absorption spectrometry. Clin Chim Acta 293: 187-197.

Schmidt D, Shin YS, Auw-Haedrich C,; Tacke U (2011) Congenital ocular malformations (lens subluxation, pupillary displacement, cataract, myopia) and classic galactosaemia associated with Q188R and/or G1391A mutations. Acta Ophthalmol 89(5):489-94.

Saxena P, Saxena AK, Monnier VM.(1996) High galactose levels in vitro and in vivo impair ascorbate regeneration and increase ascorbate-mediated glycation in cultured rat lens. Exp Eye Res.;63(5):535-45.

Sippel TO (1966) Changes in the water, protein, and glutathione contents of the lens in the course of galactose cataract development in rats. Invest Ophthalmol Vis Sci 5: 568-575.

Smuda M, Henning C, Raghavan CT, Johar K, Vasavada AR, Nagaraj RH, Glomb MA(2015) Comprehensive analysis of maillard protein modifications in human lenses: effect of age and cataract. Biochemistry 54(15):2500-7.

Stegink LD, Filer LJ, Baker GL (1983) Plasma amino acid concentrations in normal adults fed meals with added monosodium L-glutamate and aspartame. J Nutr 113(9):1851-1860.

Tag-Eldin YM (2016). Comparative molecular and developmental studies in cataractous lenses of pediatric and senile patients.PhD. thesis, Mansoura Univ., Egypt.

Takamura Y, Kubo E, Tsuzuki S, Akagi Y (2003) Apoptotic cell death in the lens epithelium of rat sugar cataract.Exp Eye Res 77(1):51-7.

Takata T, Oxford JT, Brandon TR, Lampi KJ (2007) Deamidation alters the structure and decreases the stability of human lens betaA3-crystallin. Biochemistry 46(30):8861-71.

Tsutsumi M, Matsumoto Y, Mori K, Ikebe H, Terubayashi H, Akagi Y, Tanimoto T (1992) Formation of congenital cataract in the offspring of galactosemic rats. Nippon Ganka Gakkai Zasshi 96(1):22-7

Wei S, Shi W, Li M, Gao Q.(2014) Calorie restriction down-regulates expression of the iron regulatory hormone hepcidin in normal and d-galactose–induced aging mouse brain. Rejuvenation Res 17(1):19-26.

Zwaan J (1983). Eye findings in patients with phenylketonuria. Arch Ophthalmol.;101(8):1236-7.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Animal Sciences

How to cite this article:
Hassan IH El-Sayyad, Soad A Khalifa, Amoura Mohamed Abou-El-Naga, Eman HM Bakr, Hamed A N Jala. (2016). Comparative studies on lens structure and function of newborn rats maternally treated with aspartame, monosodium glutamate and galactose. Int. J. Adv. Res. Biol. Sci. 3(7): 145-154.