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RESEARCH ARTICLE

Investigation of Age-Related Alterations in Brain and Serum Samples in a Healthy Aging Rat Model

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Investigation of Age-Related Alterations in Brain and Serum Samples in a Healthy Aging Rat Model

SUMMARY

Male albino rats of Wistar strain (n=24) were assigned to three groups 2-month-old (GRP I), 9-month-old (GRP II) and 15-month-old (GRP III), (n=8 in each) to investigate age-related changes in serum and brain samples. Malondialdehyde (MDA), reduced glutathione (GSH), acetylcholinesterase (AChE) and paraoxonase (PON) enzyme activities, soluble and aggregated amyloid β1-42 (Aβ1-42) levels were analyzed in brain tissues. Tissues were also stained with Congo-red dye to observe fibrillation. Serum total cholesterol, uric acid, and triglyceride levels, and PON activities were investigated. Serum uric acid levels were significantly decreased ($p<0.05$) whereas total cholesterol and triglyceride levels were increased ($p<0.01$) in GRP III rats. Brain tissue MDA and GSH concentrations did not change significantly among the groups. Both Free Aβ1-42 concentrations and fibrillation levels were significantly increased in brain tissues in GRP III ($p<0.05$). Tissue AChE activities were reduced significantly ($p<0.05$) and PON activities did not change among groups. Our serum results indicated age-related systemic oxidative stress. Brain results did not show oxidative stress in terms of lipid peroxidation but the decreased AChE activities and, unaltered PON activities accompanied with increased amyloidogenesis are accepted as an early response of neurodegeneration in older rats.

Key Words: *Aging, oxidative stress, acetylcholinesterase, paraoxonase, amyloid-β-peptide*

Sağlıklı Yaşlanma Sıçan Modelinde Beyin ve Serum Örneklerinde Yaşa Bağlı Değişikliklerin Araştırılması

ÖZ

Çalışmada 24 adet Wistar albino cinsi sıçan, serum ve beyin örneklerinde yaş-ilişkili değişiklikleri değerlendirmek için kullanılmıştır. Sıçanlar her grupta sekiz adet olacak şekilde GRP I (2 aylık), GRP II (9 aylık)ve GRP (III) (15 aylık) olarak ayrılmıştır. Beyin dokularında ise malondialdehit (MDA), indirgenmiş glutatyon (GSH) derişimleri, asetilkolinesteraz (AChE) ve paraoksonaz (PON) aktiviteleri ile serbest ve fibriler amiloid-β-1-42 peptid düzeyleri belirlenmiştir. Dokularda ayrıca kongo kırmızı boyaması ile fibrilasyon görüntülenmiştir. Serum trigliserit, kolesterol ve asit ile PON enzim aktiviteleri analiz edilmiştir. Serum örneklerinde GRP III sonuçlarında azalan ürik asit derişimleri ($p<0.01$) ile değişen lipid içerikleri ($p<0.05$) yaşa bağlı gelişen sistemik bir oksidatif stres varlığını işaret etmiştir.Beyin dokularında MDA ve GSH derişimlerinde gruplar arası anlamlı bir farklılık gözlenmemiştir. Serbest ve fibrile amiloid-β-1-42 seviyeleri ise GRP III beyin dokularında diğer gruplara önemli derecede artış göstermiştir ($p<0.05$). Doku AChE aktiviteleri yaş ile azalırken PON aktiveleri değişmemiştir. Sonuçlarımız yaşlanma ile birlikte sistemik oksidatif stres gelişimini kanıtlamıştır. Beyin dokularında lipid peroksidasyonu bağlamında gruplar arası bir bir değişiklik gözlenmemiş ancak azalan AChE aktiviteleri,değişmeyen PON düzeyleri ile birlikte artan amiloid-β-1-42 derişimleri beyin dokusunda yaşlanmaya bağlı nörodejenerasyonunun erken bir yanıtı olarak değerlendirilmiştir.

Anahtar Kelimeler: *Yaşlanma, oksidatif stres, asetilkolinesteraz, paraoksonaz, amiloid-β-peptit*

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INTRODUCTION

Aging is an irreversible multifactorial incident that has molecular and cellular phases leading to decrements in various organ functions (Aliabbas, 2021). Especially central nervous system (CNS) and brain may be considered the main targets of aging. Several factors such as oxidative stress, inflammation and mitochondrial dysfunction are known to contribute to the molecular mechanism of brain aging. Progression of neurodegenerative diseases mainly are related to brain aging and so investigating the alterations of cellular protein levels and enzyme activities has a pivotal role in understanding and clarifying the exact mechanisms (Aliabbas, 2021).

Considering healthy individuals, aging is accepted as the single most important risk factor for Alzheimer's Disease (AD) genesis. Amyloid beta (Ab) peptides and their oligomerization products are the basic hallmarks of AD, dementia, and other related disorders whereas their monomeric forms may behave as antioxidants depending on the concentration (Salazar,2019). Studies have indicated that the accumulation of amyloid peptides is attributed not only to increased synthesis but also decreased brain efflux by age resulting in the elevated accumulation of peptides under normal physiological conditions. Several previous studies report the contribution of amyloid deposition to cognitive impairment in aged rat models (Chiu, 2012; Peng, 2021). The increment of Ab is also known to activate inflammation exacerbating cognitive dysfunction (Xu, 2021).

Cholinergic neurons play a key role in AD, dementia, or normal aging. It is well-established that AD is caused by synaptic dysfunction and amyloidogenesis may result in the death of cholinergic neurons (Schliebs, 2011). Aging-related cholinergic dysfunctions are reported as the important factor in neurological disorders in aging.

Oxidative stress may simply be described as the generation of an imbalance between prooxidants and antioxidants in cells. Reactive oxygen molecules (ROM) are the main contributors to this condition which are highly reactive and has ability to damage many biological macromolecules especially proteins and lipids (Tian, 1998). It is mainly reported that oxidative stress is increased in aging at any stage due to the increased reactive oxygen species (ROS) production (Starke-Reed, 1989; Smith, 1991), and the age-related enhancement of prooxidant production may be the cause of membrane damage in senescent cells. The brain may be considered as a primary target to oxidative damage due to its structural and functional composition with high rate of oxygen utilization (Drivera, 2000; Youdim, 2000). Besides the given, also, neuroinflammation is an important factor in aging and investigated in several reports (Salazar, 2019).

The paraoxonases are ubiquitous Ca-dependent hydrolyses having antioxidant, antiatherogenic, antiinflamatory and, also scavenging properties with broad substrate specificities. Three isozymes, paraoxonase 1 (PON1) and 3 are mainly expressed in the liver and secreted to circulation (Reichert, 2021). Enzymes derive their nomenclature from their ability to hydrolyze paraoxon, whereas PON1 has two activities; paroxonase and arylesterase. Enzyme (PON1) also has known to hydrolyze organophosphates such as chlorpyrifos, diazinon, and also nerve toxins such as sarin, soman having detoxificant activity (Mota, 2019). PON1 is found mainly in high density lipoproteins (HDL) and its main physiological role is to hydrolyse oxidized lipids. It is known that PON1 is transferred via HDL to extrahepatic tissues and suggested that the main antioxidant capacity of HDL comes from its PON1 content (Morris, 2021).

The following study is aimed to investigate the age-related alterations in acetylcholinesterase (AChE), paraoxonase enzyme activities, and Ab peptide levels in a healthy aging rat model considering the possible contribution of oxidative stress.

MATERIALS and METHODS

Chemicals

All chemicals used were obtained from Sigma-Aldrich (St Louis, MO, USA), and Merck (Darmstadt, Germany). Aβ1- 42 ELISA kit was purchased from SunRed-Bio (Shangai, China).

Animals

Wistar albino male rats (*n*=24) were assigned to three groups eight in each group as 2-month-old (GRP I), 9-month-old (GRP II) and 15-month-old (GRP III). All experimental procedures involving animals approved by the Başkent University Institutional Review Board and Ethics Committee, Ankara, Turkey with project number DA11/12. Sacrification was performed by an intracardiac puncture after rats were anesthetized i.p. with ketamine (50 mg/kg) /xylazine (10 mg/kg). After sacrification, brain tissues and serum samples were stored at -86°C for biochemical analysis. All biochemical studies were performed in duplicate by using the left hemisphere of the brain.

Analysis of serum biochemical parameters

Serum uric acid, total cholesterol, and triglyceride concentrations were analyzed autoanalyzer system (Roche Hitachi modular system, Mannheim, Germany) using Roche Diagnostic reagents.

Determination of Tissue Malondialdehyde (MDA) and Reduced Glutathione (GSH) Concentrations

Brain homogenates were prepared in ice-cold 0.15 M KCl (10%, w/v) using an all-glass homogenizer.

Buege and Aust's method was used to determining MDA levels (Buege, 1978). Homogenate samples were incubated with thiobarbituric acid reagent at 100 °C in a water bath for 15 min. After cooling, samples were centrifugated at 1000x g for 10 min and the absorbance of the supernatant was measured spectrophotometrically (Shimadzu UV-1601, Japan) at λ: 535 nm. Concentrations were quantified by using the molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$. The results were expressed as nmol MDA/g tissue. GSH levels were assayed in tissue homogenates according to the method of Ellman (Ellman,1959). Samples were deproteinized and supernatants were used for analysis. Ellman color reagent was added into the supernatants and then the absorbance of the generated color complex was measured immediately at 412 nm against a reagent blank with a spectrophotometer (Shimadzu UV-1601, Japan). Concentrations were calculated by using GSH standard curve and were expressed as μmol GSH/g tissue.

Determination of serum and tissue PON Arylesterase Activity

Tissue homogenates were prepared in Tris-HCl buffer (50 mM), pH 8.0+ 2 mM $CaCl_2$ using an all-glass homogenizer. Homogenates were centrifuged at 15000xg for 10 min and supernatants were used as enzyme sources. PON arylesterase activities of serum and tissue homogenates were analyzed according to the method of Jerzy Beltwoski (Beltwoski,2004). The method is based on the determination of the hydrolysis rate of phenylacetate spectrophotometrically at 270 nm (Shimadzu UV-1601, Japan). Quantitation was performed by using the molar extinction coefficient ($\mathfrak{E}$=1310 $M^{-1}cm^{-1}$). Results were expressed as U/g and U/ml and one unit is defined as the one ml of the enzyme that hydrolyzes 1 mmol of phenylacetate per minute.

Determination of Tissue Acetylcholinesterase Activity

Brain homogenates were prepared in 0.1 M potassium phosphate buffer pH 7.4 with glass homogenizer and supernatants that are obtained after centrifugation at 10000xg were used as enzyme sources. AChE activity was determined by the method of Ellman method (Ellman,1961) with a spectrophotometer (Shimadzu UV-1601, Japan). Assays were carried out at 25^0 C in a mixture containing 84 mM potassium phosphate buffer pH 7.4, 0.1 mM *5,5'-dithiobis-2-nitrobenzoic acid*, and 0.84 ml of enzyme source. The reaction was initiated by the addition of substrate acetylthiocholine. Activity was defined as μmol acetylthiocholine utilized /min/gram tissue (U/g).

Determination of Tissue Aβ1-42 Levels

Tissue Ab1-42 levels were determined by using an ELISA kit (SunRed-Bio, Cat No: 201-11-0094, Shangai, China). Brain homogenates were prepared according to the instructions in the kit. Standards and samples were pipetted onto monoclonal antibody-coated wells of microtiter strips and the assay was carried out as indicated in the instructions of the

manufacturer. The optical densities were measured at 450 nm by a microplate reader (Bio-Tek Instruments, INC.ELX 800, USA). Quantitation was carried out by a standard curve and expressed as ng/g tissue.

Thioflavin T Analysis

Thioflavin T (ThT) is a dye that binds to various peptides, polypeptides, and proteins, and especially the cross-sheet structure, found in most amyloid proteins is a specific binding target of ThT (Khurna, 2005; Biancalana, 2010). ThT fluorescence measurement is generally accepted as an indication of amyloid fibril formation (fibrillation) (Nilsson, 2004; Griffin, 2020). Tissues were homogenized gently in 100 mM sodium phosphate buffer, pH 7.4, using a glass-glass homogenizer (10%, w/v) and the ThT fluorescences of supernatants were determined using 8 μM ThT in 100 mM sodium phosphate buffer, pH 7.4, using a spectrofluorimeter (Shimadzu RF-5301, Japan) at excitation and emission wavelengths 442 and 482 nm respectively. Fluorescence intensities were recorded and expressed as arbitrary units (A.U.).

Congo red staining

Congo-red staining is the traditional qualitative method used for the identification of amyloids. Formalin-fixed and paraffin-embedded brain tissues were used for the histopathological evaluation of cerebral amyloidosis. Hematoxylin and eosin (H&E) followed by Congo-red staining was performed to evaluate the neuronal architecture and amyloid fibrils in brain semi-thin sections. The images were captured using a Leica microscope (Leica DM 400; Wetzlar, Germany) (Puchtler, 1962; Bancroft, 2008).

Statistical Analysis

Data were evaluated with SPSS, Version 17.0 Software. Univariate analysis of variance (ANOVA) coupled with Duncan's posthoc test was performed. Data were expressed as means ± Standard error of the mean (SEM) and *p*-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Serum parameters

In the following study, serum biochemical parameters were evaluated to investigate the response of the organism in a healthy aging process considering the progression of a probable oxidative stress, and results are given in Table 1. As shown in the table, serum uric acid levels were significantly decreased in GRP III when compared to GRP I ($p<0.05$). Triglyceride and total cholesterol concentrations are significantly elevated in GRP III compared to GRP I and GRP II ($p<0.001$). Uric acid, the end product of purine metabolism, is known to behave as a neuroprotective molecule having roles in neuronal activity, brain development, and cognitive functions due to its antioxidant properties (Tang, 2002; Kuzuya, 2002; Liu,2002; Qiao,2021). A previously reported study of Khan et al. indicated a positive relationship between serum uric acid levels with the prevention of onset of a probable dementia and cognitive impairment (Khan,2016). In our experimental model, serum uric acid levels were evaluated as the marker of the total antioxidant status of rats. Our results indicated a significant decrease in aged rats compared to the young group indicating a decrement of the antioxidant capacity of the organisms with aging (Table 1). It is well established that aging is commonly associated with metabolic disorders characterized by serious complications such as the co-existence of dyslipidemia and especially triglyceride levels may be accepted as a biomarker of a healthy aging (Liu, 2015; Deleen, 2016; Abo-Elsoud, 2022). In our experimental model triglyceride and cholesterol levels were significantly increased in aged group (Table 1) similar to the previous reports, and accepted as the response of lipid metabolism to age (Bey, 2001; Ghezzi, 2012; Johnson, 2019).

Table 1. Serum parameters. GRP I (2 months), GRP II (9 months), and GRP III (15 months). Values are mean ± SEM. The sample size of each group is 8. * p<0.05 GRP III vs GRP I; ** p<0.01 GRP III vs GRP I and GRP I

	GRP I	GRP II	GRP III
Uric acid (mg/dl)	1.8 ± 0.27	1.14 ± 0.24	0.9 ± 0.08*
Cholesterol (mg/dl)	59.9 ± 3.61	58.6 ± 4.13	81.1 ± 3.63**
Triglyceride (mg/dl)	36.5 ± 2.17	47.6 ± 4.26	58.8 ± 8.63**

Tissue MDA and GSH concentrations

Tissue MDA and GSH concentrations were analyzed to determine age-related alterations of oxidative stress in brain tissues. Brain MDA concentrations were investigated as the lipid peroxidation index in each group. As shown in Table 2 there was not significant change in MDA concentrations concerning to age in each group. Also, GSH concentrations, determined as the index of the redox status of the cell, did not change significantly among different aged goups.

Aging is a progressive deterioration and is known to enhance susceptibility to free radical reactions (Doğru-Abbasoğlu, 1977). Normal processes of cells, such as mitochondrial respiration, may lead to the formation of free radicals, but the increased rate of these cellular activities in maturing /aging brain may show an enhancement (Drivera, 2000). Considering healthy aging for cells, it is established that oxidative stress is an inevitable cellular modification due to altered cellular metabolism and damaged membrane blood-brain permeability, especially during senescent brain cells (Enciu,2013; Freitas,2022). Several studies have shown the existence of oxidative stress in aging rat models in terms of increased lipid peroxidation (MDA levels) and also decreased GSH concentrations (Cand,1989). Whereas lipid peroxidation index, in terms of MDA levels, is a commonly used marker to investigate oxidative damage, several reports suggest that this may not be the most sensitive method, and direct measurement of basal ROS production and oxidative protein damage must be investigated (Drivera, 2000) to determine the intrinsic age-related differences in the potential to produce free radicals. In our model, brain tissue MDA levels did not change significantly (Table 2) between groups compatible with some previous reports (Doğru-Abbasoğlu, 1977; Cand,1989; Matsuo, 1992). It is reported that, normally, oxidatively modified proteins are major targets of proteolytic system instead of native proteins that are proposed as a secondary free radical defense system (Tian, 1998). Presumably, the mechanism is also valid for the damaged lipids making them as a candidate for rapid degradation and explaining the stable MDA levels among groups, and unaltered oxidative stress in terms of lipid peroxidation (Holmes, 1992).

Thiol redox status (TRS) is accepted as a marker of the antioxidant defence capacity of cells. TRS includes the reduced and oxidized forms of several thiols (protein/non-protein). The most abundant non-protein thiol, GSH has two roles in the antioxidant system. It either behaves as an antioxidant itself or has a role as a substrate in enzymatic antioxidant defense mechanisms. Recent studies highlight the redox status as a marker of aging and neurodegeneration and the reduced form of GSH is generally determined to investigate the antioxidant status of the cell (Grintazalis, 2022; Chen,2022). However many several studies report the decline in GSH levels in aging brain tissues (Zhua,2006), our results did not show a significant difference among groups compatible with the findings of Schultz et al. in the human brain and rat brain (Schulz,2000, Chen,2022).

Table 2. Tissue MDA and GSH concentrations. GRP I (2 months), GRP II (9 months), and GRP III (15 months). Values are mean ± SEM. The sample size of each group is 8.

	MDA (nmol/g)	GSH (mmol/g)
GRP I	22.25 ± 1.83	2.19 ± 0.31
GRP II	24.01 ± 4.28	1.83 ± 0.11
GRP III	20.51 ± 3.14	2.03 ± 0.28

Tissue amyloidogenesis

In monomeric forms, Ab peptides are known to have antioxidant roles depending on their local concentration (Kontush, 2001) but at high concentrations (mM) they exhibit toxic properties linked to methionine-35 mediated radical generation and also their tendency to aggregate (Sehar, 2022). By having these two-faced functions, it is mainly suggested that Ab behaves as a stress-related protein. In the present study, Ab1-42 monomeric levels and also accompanied aggregate formation, due to the predisposition of Ab1-42's to aggregate, are significantly increased indicating cellular stress in the aged group as an acute phase response of organism (Table 3) (Kontush, 2001; Butterfield, 2001; Cheignona, 2018; Özturan-Özer, 2020; Salazar, 2022;). The accumulation of beta peptides is caused by not only increased synthesis but also depends on the decreased removal. The efflux of Ab peptides is mediated by blood-brain barrier associated p-glycoprotein and it is mainly reported that the concentration and expression of this protein is decreased with age. Also, it is reported that the expression of beta-secretase enzymes, which are responsible for the production of pathological amyloid peptides, is increased during aging. So, we may conclude that the inevitable increment of Ab formation and also aggregation during the aging process is caused by the imbalance between synthesis and removal mechanisms in the brain (Chiu, 2012). The unaltered GSH levels were considered as a compensatory mechanism due to the generated cellular stress resulting from elevated amyloid plague formation in the aging process (Hussain, 1995; Mandal, 2022).

Table 3. Tissue Ab1-42 concentrations and fibrillation levels. Values are mean ± SEM. The sample size of each group is 8. * p< 0.05; GRP III vs GRP I and GRP II

	Ab1-42 (pg/g)	Fluorescence intensity (A.U.)
GRP I	365 ± 29.5	3 ± 1.6
GRP II	384 ± 48,1	14 ± 4.2
GRP III	453 ± 33.4*	39 ± 6.4*

Histopathological analysis, Congo-red staining

Our biochemical findings about amyloidogenesis were confirmed by the histological ultrastructural studies (Figure 1). The Congo red stained semi-thin brain sections showed the aggregates of amyloid plaques as brick-red cloudy materials in brain tissues (Figure 1C) (Setti,2021; Addi,2022) also accumulation in arterial vessels. This accumulation forms cerebral amyloid angiopathy due to impaired clearence of Ab peptides mostly through perivascular drainage pathways with age (Figure 1D) in older rats (Tanner, 2022).

AChE activities

The alterations of AChE activities with age are given in Figure 2. As shown in the figure, brain AChE activities were significantly decreased in aged rats. GRP I activities were significantly higher than GRP II and GRP III enzyme activities (665 ± 62.3; 404 ± 65.4; 511 ± 59.1 respectively).

Whereas oxidative stress and related pathologies are accepted as the main hallmarks of aging, cholinergic dysfunction is also seriously important. These alterations generally are associated with cognitive decline, neurobehavioral deficits, and susceptibility to immune disorders (Benfante, 2021). In progressive aging, the decrease of acetylcholine levels whether caused by decreased enzyme activity or damage of cholinergic neurons is a well-known phenomenon. This decrease is reported to be associated with the construction and maintenance of learning memory in the brain with aging (Schliebs, 2011). Several reports emphasize the importance of AChE activities for nervous systems and mostly it is reported that an decrease in AChE activity can be accepted as an indicator of neurodegeneration (Haider, 2014). In our study, the decreased AChE levels in GRP II and GRP III with age were consistent with the previous studies (Liu,2022). It is abundantly evident that Abs trigger cholinergic dysfunction in several ways: affecting a-7 nicotinic acetylcholine receptors, affecting nerve growth factor signaling, and also interacting with the peripheral an-

ionic site of acetylcholinesterase (Sultzer, 2022). In the following study, it is clear that the decreased AChE levels in the GRP III group are also accompanied by the enhanced levels of Ab1-42, which is much more prone to aggregate and displays higher neurotoxicity *in vivo* (Haider,2014).

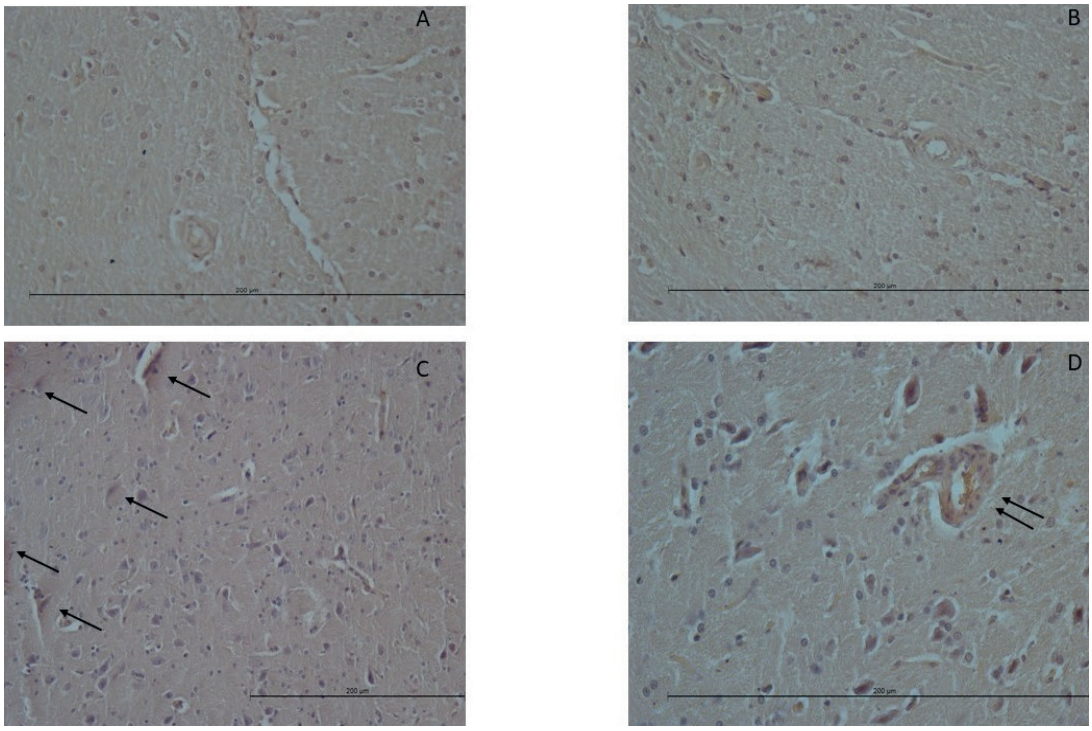

Figure 1. Histopathology of rat cerebral sections. Microscopic image of Congo red staining of **of 2 month(A),** 9 month (B) and 15 months (C and D) age of rate brain sections. **B**. 9 month **C and D**. 15 months age of rat brain sections. Sections were counterstained with H&E. All the pictures were taken at magnification x 40. Scale bars, 200 mM. Black single arrows indicate aggregated Ab plaques and double arrows indicate the Ab deposition in vessel walls (Addi, 2002; Tanner, 2022).

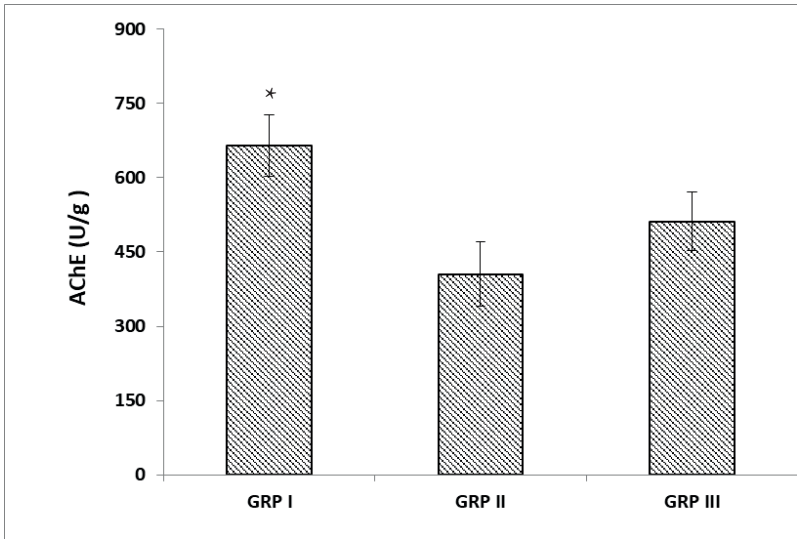

Figure 2. Tissue AChE activities Values are mean ± SEM. The sample size of each group is 8.

* p< 0.05; GRPI vs GRP II and GRP III

PON activities

Serum and brain PON activities are shown in Figure 3. As seen in the Figure 3A, there was no significant variation in brain PON activities among groups whereas Figure 3B shows that serum PON activities were significantly higher in the aged group with respect to other two groups ($p<0.01$) (92.3 ± 6.44; 78.14± 2.29; 118.7 ± 8.86 respectively).

PON enzymes, especially PON arylesterase are defined to be a critical defense system against lipid peroxidation, and a decrease in activity is a marker of increased systemic oxidative stress (Menini, 2014; Bassu, 2022; Ayada, 2022; Wang, 2022). Many clinical and experimental studies report a significant decrease in serum PON arylesterase activity in several pathological conditions such as dementia (Menini, 2014), rheumatoid arthritis (Erre, 2022), coronary artery diseases (Sofflai, 2022), cancer (Elseadya, 2002; Jasinski, 2022; Thompson, 2022), COVID-19 (Gabaldo,2022) and also in a rat model (Dube, 2022). On the contrary, in our healthy aging model, serum PON levels were significantly increased in older rats accompanied by a decrement in uric acid levels. In general, it is known that aging triggers elevated levels of cholesterol, and triacylglycerols that are associated with cardiac and vascular diseases (Sofflai, 2022). In the following study, the increased paraoxonase arylesterase activities were accepted as a response of the organisms against to generated systemic oxidative stress (decrement of uric levels) and also a protection mechanism. Also, this increase was found to be positively correlated with serum cholesterol levels ($r=0.593$). This association was regarded as a prevention mechanism by paraoxonase enzyme against the oxidation of lipoproteins (Mackness, 2021).

PON family members are lactonases with broad substrate specificity and tissue distribution. PON1 and PON 3 are expressed in the liver and their protein product can be found in circulation. Among the three ones, PON-2 is the only one expressed in nervous tissues. Although it is believed there is no PON1 and PON3 gene expression in the brain; studies report the existence of these enzymes in brain's regions indicating a possible role in modulating the brain oxidative stress. Also, Salazar et al reported the abundant expression of PON1 and PON 3 surrounding Ab plaques (Salazar, 2021). Another study showed the existence of PON1 in the brain suggesting and supporting the idea of transfer of PON enzymes from blood circulation to the central nervous system whenever needed (Levy, 2019; Salazar, 2021). It is known that the cellular modifications in neurodegeneration in the brain, such as Ab deposition, lead to the production of ROS by activated astrocytes, oligodendrocytes, and microglia resulting in generating oxidative stress. In our model, there was no increase in PON activity and also MDA and GSH levels with age suggesting no alteration of apperant oxidative stress.

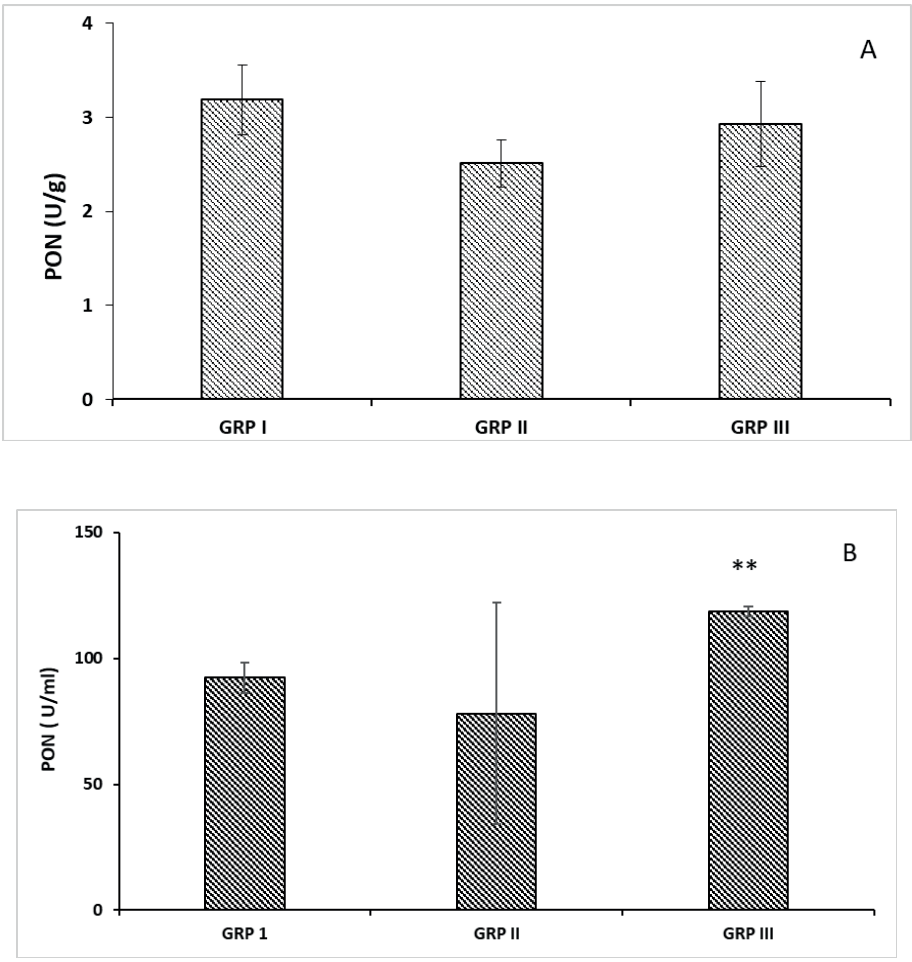

Figure 3. (A) Brain (B) Serum PON activities. GRP I (2 months), GRP II (9 months),and GRP III (15 months). Values are mean ± SEM. The sample size of each group is 8.

**p<0.01 GRP III vs GRP I and GRP II

CONCLUSIONS

Aging causes complex and irreversible cellular modifications. Senescent cells mainly suffer from mitochondrial dysfunction and also oxidative stress (Zhang, 2022). In our study, the systemic antioxidant status of aged group rats; uric acid; was significantly decreased with age as expectedly. Also, the generated dyslipidemia with age was consistent with the situation indicating a systemic oxidative damage. The brain results were confusing as there was no alteration of tissue markers in terms of lipid peroxidation and PON activities. But also, the decrease in AChE levels during aging indicated neurodegeneration in the brain. This decrease accompanied by the increased amyloid levels suggested that the cholinergic system is the first target of brain aging. However, the increased levels of amyloid plaque formation may contribute cellular stress in our model, we may suggest that amyloid monomers have shown a two-faced role as antioxidants preventing probable oxidative stress and, but not properly effective considering the decreased AChE levels. So we may conclude that the brain has its protection mechanisms against systemic oxidative stress and Ab peptide levels must be strictly evaluated to determine probable stress in brain tissue.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

EO: %100.

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