World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Physiological alteration of mitochondrial genome in relation to aging on male albino rats

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Received: 16-01-2015 / Revised: 18-02-2015 / Accepted: 20-02-2015

Abstract

There is no doubt that there is a great interest on aging and its causes from human especially women, this increase scientific researches on aging including this increase research, which detect mitochondrial DNA in relation to aging, where mitochondria is the house of energy in the living cell. Sixty male albino rats were divided into three equal groups 1st group, young rats (6 months age) as control, 2nd group, adult rats (12 months age) and 3rd group, aged rats (21, months age). Rats were sacrificed and the brain and liver tissues were preserved in liquid nitrogen immediately for molecular biological analysis to see the different gene expression in the respiratory chain at different ages. Other samples were taken from the liver, brain and serum for the biochemical and physiological analysis to find out the effect of aging on glutathione peroxidase activity and concentration of reduced glutathione, total antioxidant capacity, nitric oxide and finally malondialdhyde. NADH dehydrogenase and cytochrome oxidase gene expression, showed a decreased activity in both liver and brain with aging. Glutathione peroxidase showed non-significant decrease in its activity in liver, but a significant decrease in brain in adult rats, and a significant decrease in both liver & brain in aged rats. Reduced glutathione showed non-significant decrease in its concentration in both liver & brain in adult rats but showed a significant decrease in aged rats. Serum total antioxidant capacity showed non-significant decrease in its concentration in adult rats but significantly decreased in aged rats. Liver and brain nitric oxide showed a significant increase in its concentration in both adult and aged rats. Liver malondialdhyde showed a significant increase in its concentration in both adult and aged rats, but in brain, showed non-significant increase in adult rats while, significantly increased in aged rats. All previous results in compared with young rats (control group).

Keywords: Mitochondrial genome aging, male albino rats liver & brain, NADH dehydrogenase gene expression, cytochrome oxidase gene expression glutathione peroxidase, reduced glutathione, serum total antioxidant, nitric oxide, malondialdhyde.

INTRODUCTION

In cell biology a mitochondrion (plural mitochondrion) is a membrane-enclosed organelle found in most eukaryotic cells. These organelles range from 0.5 to 10 micrometers (μm) in diameter [(1)]. Several characteristics make mitochondria unique. The number of mitochondria in a cell varies widely by organism and tissue type. Many cells have only a single mitochondrion, whereas others can contain several thousand mitochondria [(2)]. Mitochondrial proteins vary depending on the tissue and the species. In humans, 615 distinct types of proteins have been identified from cardiac mitochondria [(3)]. Whereas, in Murinae (rats), 940 proteins encoded by distinct genes have been reported. The mitochondrial proteome is thought to be dynamically regulated [(4)]. Mitochondria are sometimes described as "cellular power plants" because they generate most of the cell's supply of ATP, used as a source of chemical energy [(5)]. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of cell cycle and cell growth [(6)]. Mitochondria have been implicated in several human diseases, including mitochondrial disorders [(7)]. Extant mammalian mtDNAs have retained only 13 polypeptide genes, all of which encode essential components of oxidative phosphorylation. MTDNA also encodes the 12S and 16S rRNA genes and 22 tRNA genes required for mitochondrial protein synthesis. The remaining mitochondrial oxidative phosphorylation proteins,

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the metabolic enzymes, the DNA and RNA polymerases, the ribosomal proteins and the mtDNA regulatory factors are all encoded by nuclear genes, synthesized in the cytosol and then imported into the organelle [8]. The human mitochondrial genome is 16.569 (bp) in length closed, circular molecule. Mitochondrial DNA has two strands, a guanine- rich heavy (H) strand and a cytosine-rich light (L) strand. The heavy strand contains 12 of 13 polypeptide –encoding genes, 14 of the 22 tRNA- encoding genes and all the coding sequences are contiguous [9]. The only non-conding segment of mtDNA is the displacement loop (D-loop), a region of 1121 bp that contains the origin of replication of the H-strand (OH) and the parameters for L and H –strand transcription. The mtDNA is replicated from two origins. DNA replication is initiated at OH using an RNA primer generated from the L-strand transcript.

H-strand synthesis proceeds two –thirds of the way around the mtDNA, displacing the parental H-strand until it reaches the L-strand origin (OL), situated in a cluster of five tRNA genes. Once exposed on the displaced H-strand, OL folds a stem –loop structure and L-strand synthesis is initiated and proceeds beak along the H-strand template. Conseq2uently, mtDNA replication is bidirectional but asynchronous [10]. The mammalian ova contain about 100.000 molecules of mtDNA, while the sperm contains of the order of 100-1500 mtDNA. Sperm mitochondria enter the ova during fertilization but they appear to be lost early in embryogenesis, soon after fertilization, between the two-cell and four cell stages [11]. Mitochondria lack an efficient DNA repair system. Moreover, protective proteins such as histones are missing and mtDNA is physically associated with the inner mitochondrial membrane, where highly somatic mutagenic oxidative phosphorylation[12]. Ageing (British English) or aging (American English) is the accumulation of changes in an organism or object over time [13]. The cumulative accumulation of these somatic mutations during life may cause a bioenergic deficit leading to cell death, or apoptosis and normal aging. In addition to the aging or senescence process somatic mtDNA mutations may be important for determining the onset and progression of mtDNA disease [14]. Aging is associated with common diseases including, Alzheimer's, arthritis, heart attack, cancer, Parkinson's, heart disease, pneumonia, stroke, osteoporosis and diabetes [15]. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. Once formed, these highly reactive radicals can start a chain of reaction, like dominos [16]. Free radicals divided into Reactive Nitrogen Species (RNS) including Nitric Oxide (NO) and peroxynitrite ($ONOO^-$) and reactive oxygen species (ROS), which include radical superoxide (O_2^-) and hydroxyl radical (HO) or non radical species such as hydrogen peroxide (H_2O_2) [17].

These compounds are toxic to both the invader and the cell itself, so they are kept in compartments inside the cell. This method is referred to as oxygen – dependent into cellular killing of which there are two types [18]. The first type is the oxygen – dependent production of a superoxide which is an oxygen –rich bacteria – killing substance. The superoxide is converted to hydrogen peroxide and singlet oxygen by an enzyme called superoxide dismutase. Superoxides also react with the hydrogen peroxide to produce hydroxyl radicals which assist in killing the invading microbe [19]. The second type involves the use of the enzyme myeloperoxidase from neutrophil granules. When granules fuse with a phagosome, myeloperoxidase in released into the phagodysosome and this enzyme uses hydrogen peroxide and chlorine to create hypochlorite, a substance used in extremely toxic to bacteria [19]. The membrane – associated NAD (P) H oxidase, cytochrome oxidase and xanthine oxidase[20]. No generation occurs through specific nitric oxide synthase isozymes, including mitochondrial nitric oxide synthase (mtNOS), neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS)[21]. Malondialdhyde mainly exists in the enol form. MDA is generated from reactive oxygen species (ROS) and as such is assayed *in vivo* as a bio. merker of oxidative stress[22]. Malondialdhyde reacts with deoxyadenosine and deoxyguanine in DNA, forming DNA adducts, the primary one being M_1G_1, which is mutagenic. The guandine group of arginine residues condenses with NDA to give aminopyrimidines [23]. An ideal antioxidant should be easily absorbed and counteract free radicals and chelate redox metals at physiologically relevant levels. It should also work in both aqueous and /or membrane domains and have a positive effect on gene expression.

Endogenous antioxidants ploy a crucial role in maintaining optimal cellular Rumetions[24]. Enzymatic antioxidants include superoxide dismutase, catalase and glutathione peroxidase[25]. Non-enzymatic antioxidants include vitamine E and C, thiol antioxidants (glutathione thioredoxin and lipoic acid), carotenoids, natural flavonoids, mdatomin and other compounds [26]. Superoxide dismutase, one the most efficient intracellular enzymatic antioxidants is superoxide dismutase (SOD). SOD is the antioxidant enzyme that catalyses the dismutation of O_2^- to O_2 and to less the reactive species H_2O_2 [27]. Catalase is present in

the peroxisome of aerobic cells and plays a major role in the conversion of hydrogen peroxide to water and molecular oxygen. Catalase has one of the highest turnover rates for all enzymes, one molecule of catalase can convert approximately 6 million molecules of hydrogen peroxide to water and oxygen each minute[24]. Glutathione peroxidase (GPx) had five isoforms of selenium (Se) dependent are found in humans. GPx1, is ubiquitously expressed and a major scavenger for H_2O_2 and lipid hydroperoxides. GPx2, is epithelium –specific and highly expressed in the gastrointestinal tract. GPx3 can uses thioredoxin and glutaredoxin in addition to GSH as electron donors to reduced a board range of hydroperoxide. GPx4 is present in cytosolic, mitochondrial and nuclear forms by alternative splicing, and is a major enzyme preventing oxidation of membrane phospholipids. GPx6 (newly discovered) is localized preferentially in olfactory mucosa and embryonic tissues [28]. This study designed to investigate the gene expression of some mitochondrial respiratory chain complexes at different ages in rats as NADH dehydrogenase (complex I) cytochrome oxidase (complex IV), moreover, determination the activity of glutathione peroxidase enzyme. Also the concentration of reduced glutathione total antioxidant capacity, metric oxide and malondialdhyde were determined at different ages.

MATERIAL AND METHODS

Experimental animals: Sixty male albino rats were obtained from laboratory animal house unit, College of pharmacy, King Soud University, Saudi Arabia, divided into three equal groups, twenty of each. They were kept for two weeks before starting this study in laboratory environment for accommodation. The animals were kept in suitable environmental and nutritional conditions with free access of fresh and clean tap water and a standard commercial chow cake for 90 days. They were divided into three equal each of 20 rats as follows, group 1 as control or young animals (6 months old), group 2 adult (12 months old) and group 3, (24 months, old).

Sampling: After sacrificing one gram of brain and liver were taken immediately and stored in liquid nitrogen container till use for molecular analysis.

Biochemical measurement of glutathione peroxidase (GPx), reduced glutathione (GSH), Nitric oxide (NO) and malondialdhyde (MDA), the tissues from liver and brain were taken and homogenized to obtain their homogenate. Serum samples were prepared to be used for total antioxidant capacity measurement.

Sample preparation:-

Tissue sample preparation: Liver and brain were homogenized in 48 volumes (per weight tissue) of cold buffer (5 mM phosphate buffer, PH 7.0 containing 5 M EDTA and 1 mM 2. mercop to ethanol) and centrifuged at 4000 rpm for 10-20 minutes at 2-8 ^{o}C, then the supernatant were collected and frozen at -80 ^{o}C till use [29].

Serum sample preparation: Blood was collected without using an anticoagulant and was allowed to clot for 30 min. at 25 ^{o}C, the centrifuged at 3000 rpm for 15 min. at 4 ^{o}C. The top yellow serum layer was pipetted without disrupting the white Buffy layer serum was frozen at -80 ^{o}C till use [30].

Molecular determination:-

Gene expression: NADH dehydrogenase (complex-1) quanitative Rt- PCR [31].

RNA extraction from tissues: Total TNA was extracted with E.Z.N.A. spin column RNa extraction kit (Oemga Biotech, Cairo Egypt).

Synthesis of first strand (cDNA): The synthesis of first strand was performed by using (Qiagen long range) RT-PCR kit (20). Mat. No. 1474.

PCR amplification: The PCR amplification was occur using 2XPCR master Mix from (Qiagen long range). PCR was carried out in a volume of 50 µ containing of 1 µg of cDNA, 25 µl of 2X master Mix (Qiagen), forward and reverse primer and completed to 50 µl with water, nuclease –free. The next table shows specific concentration of dNTP and oligonucleatide primer, along with PCR conditions for each gene tested. Oligonucteatide primers were designed with Eugene version 2.2 software (Ambion). A primer pair for β-actin (Ambion, Austin, TX) was used as an internal control for a PCR analysis. All reactions were preformed in 2720 thermocyeler (Applied Biosystem) in which samples underwent a 5 min. initial denaturing step to release DNA polymerase activity (hot start PCR), followed by the number of cycles indicated in table (1) for 30 sec. at 94 ^{o}C, at the annealing temperature indicated in the same table and 45 sec. at 72 ^{o}C. The final extension step was 5- min at 72 ^{o}C [31]. PCR products were separated on a 1.5% agarose gel in Tris acetate EDTA buffer with 0.5 µg/ml. ethdium bromides. The electrophoretic picture was taken by digital camera 3.2 mega pixel and quantitative with gel analyzer 2 software [31].

Biochemical analysis:

Determination of glutathione peroxidase (GPx) activity: Glutathione peroxidase activity was

calorimetrically determined according to the method adopted by paglia and valentine [29].

Determination of Reduced glutathione (GSH) concentration: GSH was measured according to the method of Butler et al. [32].

Total antioxidant capacity: Total antioxidant capacity was determined according to the method of Koraceiv et al., [30].

Determination of nitric oxide (NO) concentration: Nitric oxide (NO) concentration was determined according to the method described by Moshage et al. [33].

Determination L-Malanaldhyde (MDA) concentration: MDA were calorimetrically according to the method adopted by Esterbauer et al., [34].

Statistical analysis: The data were analyzed using one way ANOVA to determine the statistical significance of difference among groups [35].

RESULTS

Molecular determination:

β-actin, mRNA expression of rats liver and brain:- The obtained results were statistically and analysed in table (2) and illustrated in Figs. (1 & 2).

Table (1): Oligoncleotide primer sequences and concentration (NDI from Jove et al., 2003, [36], β-actin and COX 3 from Dai et al., 2008) [37].

Gene name	Forward primer	Reverse primer	Size (bp)	Primer conc. (nM)	Annealing Temp.	Cycles No.	dNTP conc. (µm)
β-actin	`5CCTcTATGTCCAACACAGTGC`3	`5GTACTCCTGCTTCGTGATCC`3	211	200	58 ^{o}C	40	80
NADH dehydrogenase (complex I)	`5CGGCCCCATTCGCGTTATTAC`3	`5TTGATAACGTAACGGAAGCGA`3	201	200	60 ^{o}C	28	80
cytochrome oxidase (compled IV)	`5CAGCCTAGTTCCTACCCACGAC`3	`5CCCGTTGCTATGAAGAATGTTG`3	294	200	61 ^{o}C	40	80

Table (2): β-actin (211 bp) mRNA expression of rats liver and brain.

Group / Parameters	Expression level of liver β-actin	Expression level of brain β-actin
Control (young rats)	185	159
Adult rats	181	156
Aged rats	172	151

Table (3): NADH dehydrogenase (201 bp) mRNa expression of rat liver and brain.

Group / Parameters	Expression level of liver NADH dehydrogenase	Expression level of brain NADH dehydrogenase
Control (young rats)	91	135
Adult rats	87	130
Aged rats	81	125

Table (4): Cytochrom oxidase (294 bp) mRNA expression of rats liver and brain.

Group / Parameters	Expression level of liver Cytochrom oxidase	Expression level of brain Cytochrom oxidase
Control (young rats)	165	195
Adult rats	152	185
Aged rats	120	181

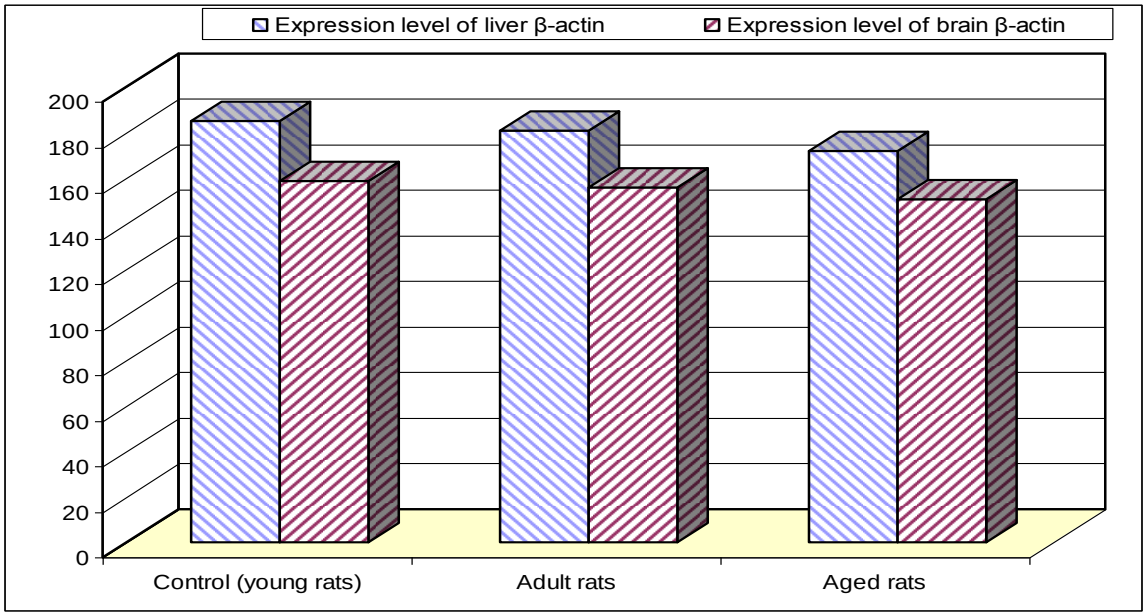

Fig. (1): β-actin (211 bp) mRNA expression of rats liver and brain.

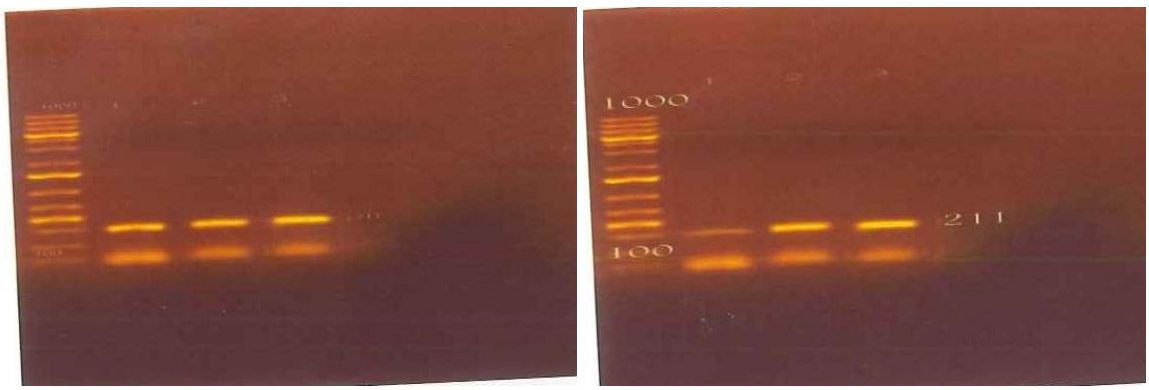

Fig. (2): The electropherograph of β-actin (211 bp) mRNA expression of rat liver (A) and brain (B).
1- Young rats group (control); 2- adult rats. 3- Aged rats group; M: 100 bp DNA ladder; N.B. Molecular weight where 211 refers to specific amplified product for β-actin according to synthesized primer.

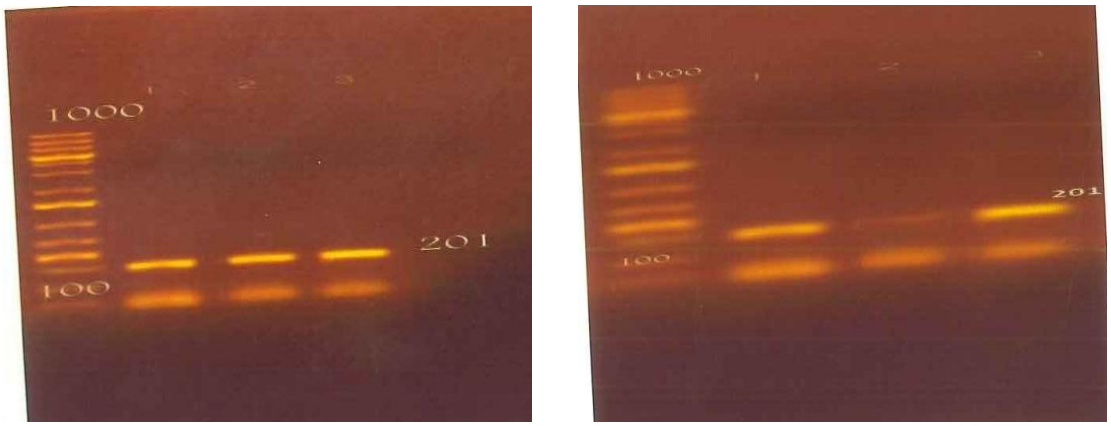

Fig. (3): The electropherograph of NADH dehydrogenase (201 bp) mRNA expression of rats.
A. liver. B- brain.
1- Control or young rats group. 2- Adult rats group. 3- Aged rats group.
N.B. Molecular weight where 201 refers to specific amplified product for NADH dehydrogenase according to synthesized primer.

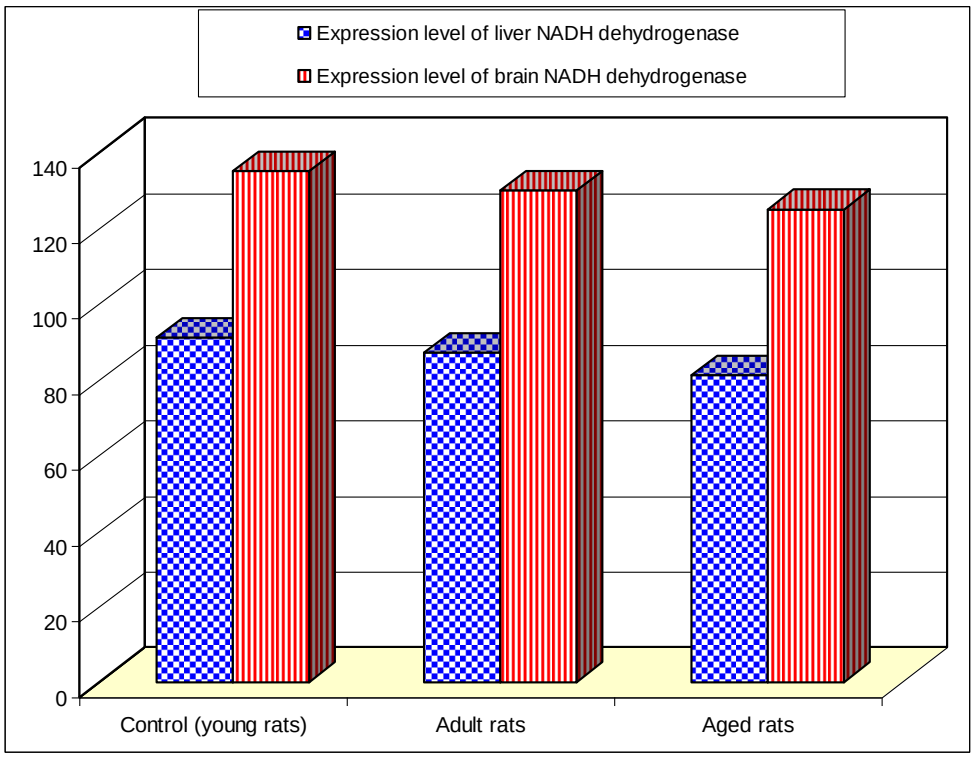

Fig. (4): The gene expression of NADH dehydrogenase level in both liver and brain showed marked decrease with age.

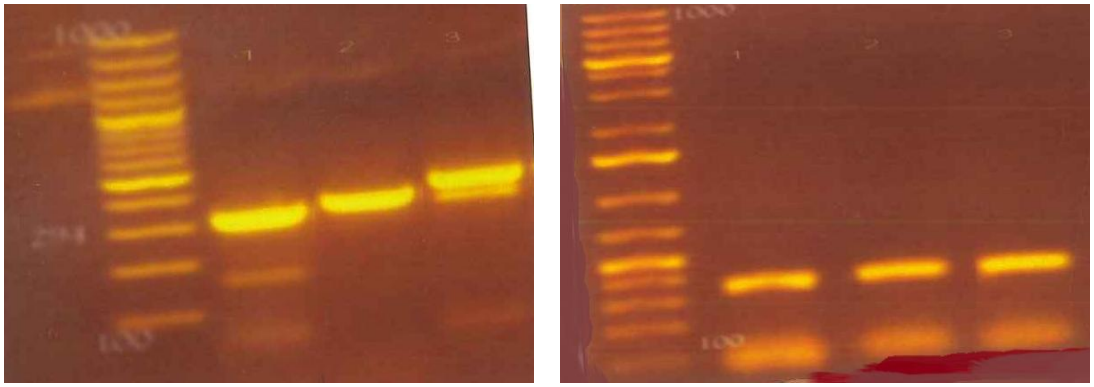

Fig. (5): The electropherograph of cytochrome oxidase (294 bp) mRNA expression of rats.
A- Liver. B- Brain.
1- Control (young rats) group. 2- Adult rats group. 3- Aged rats group.
M : 100 – 1000 bp DNA ladder.
N.B. Molecular weight where 294 refers to specific amplified product for NADH cytochrome oxidase synthesized primer.

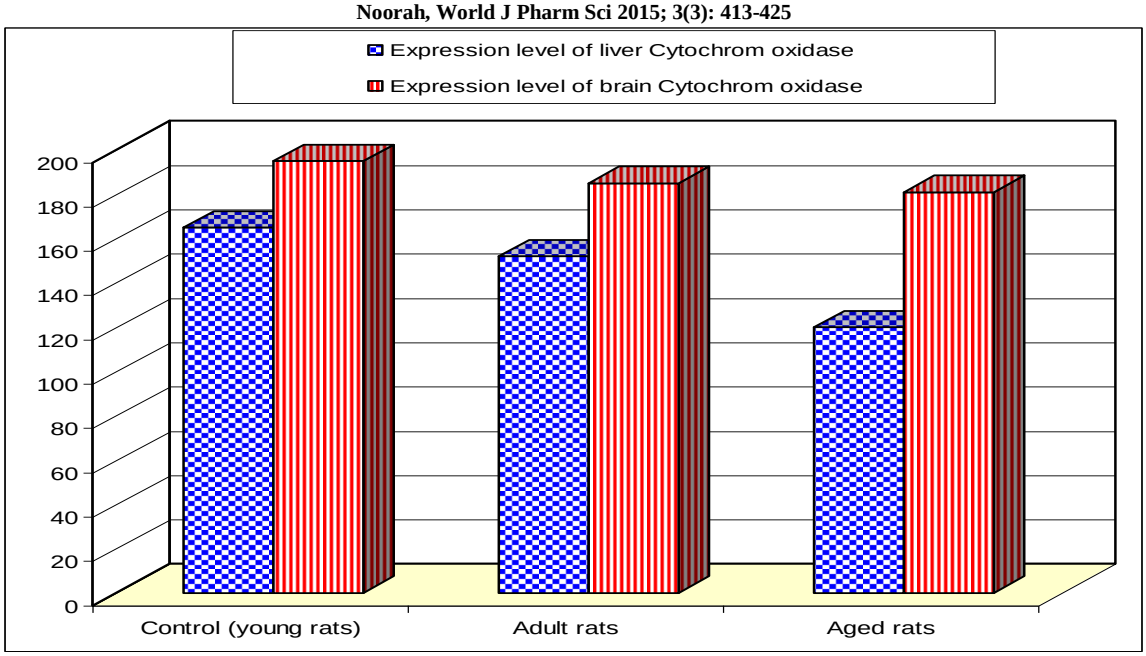

Fig. (6): The gene expression of cytochrome oxidase level in both liver and brain showed decrease with age.

Liver and brain glutathione peroxidase (GPx):
In table (5), liver glutathione oxidase showed no significant decrease in its activity in adult (12 months) rats, which significantly decrease in both liver and brain in aged (21 months) rats in compared with young rats group (Fig. 7).

Table (5): Liver and brain glutathione peroxidase (GPx) activity (u/gm) of rats.

Group / Parameters	Liver glutathione peroxidase (GPx) activity (U/gm)	Brain glutathione peroxidase (GPx) activity (U/gm)
Control (young rats)	22.55 ± 1.25 [a]	20.30 ± 1.55 [a]
Adult rats	17.40 ± 0.95 [b]	12.60 ± 0.85 [b]
Aged rats	13.25 ± 0.75 [c]	5.75 ± 0.15 [c]

Mean with different superscripts letters in the same column are significantly different at ($P \leq 0.05$).

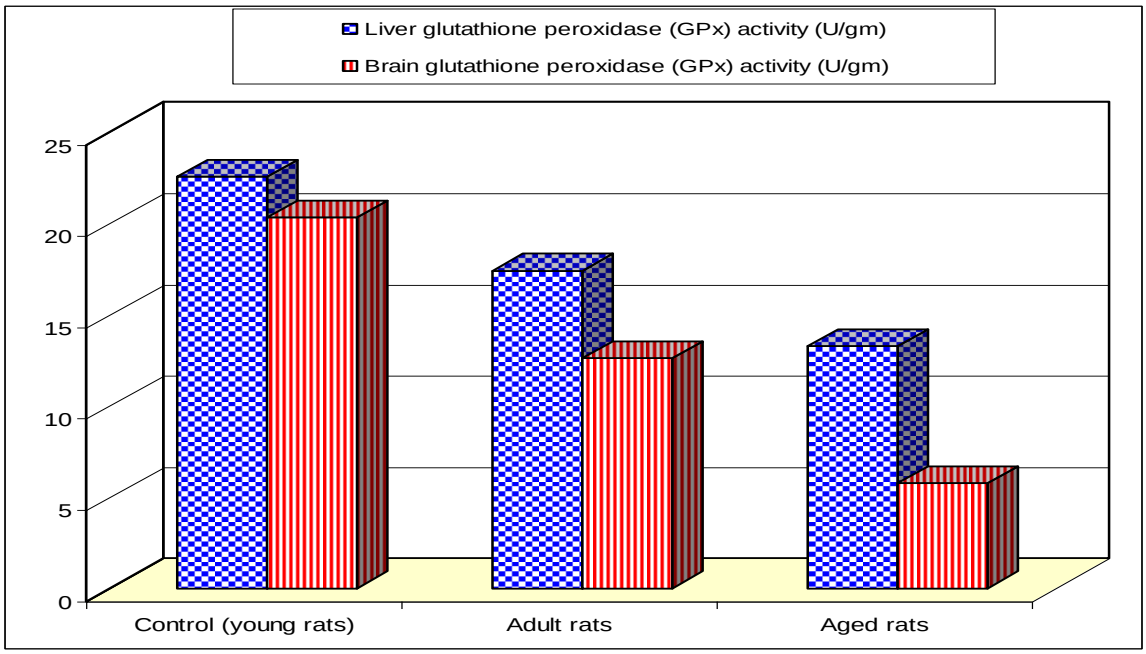

Fig. (7): Liver and brain glutathione oxidase (GPx) activity (U/gm) of rats.

Liver and brain reduced glutathione (GSH)): In table (6), the liver and brain reduced glutathione concentration showed no significant decrease in adult (12 months) rats. This decrease became significant in aged (21 months) in compared with control group. Fig. (8).

Table (6): Liver and brain reduced glutathione (GSH) concentration (mg/gm tissue) of rats group.

Group / Parameters	Liver reduced glutathione(GSX) concentration (mg/gm tissue)	Brain reduced glutathione concentration (mg/gm tissue)
Control (young rats)	89.45± 3.95 [a]	60.20± 4.30 [a]
Adult rats	82.15± 3.35 [b]	56.40 ± 2.60 [b]
Aged rats	73.10± 0.65 [c]	43.35± 3.25 [c]

Mean with different superscripts letters in the same column are significantly different at ($P \leq 0.05$).

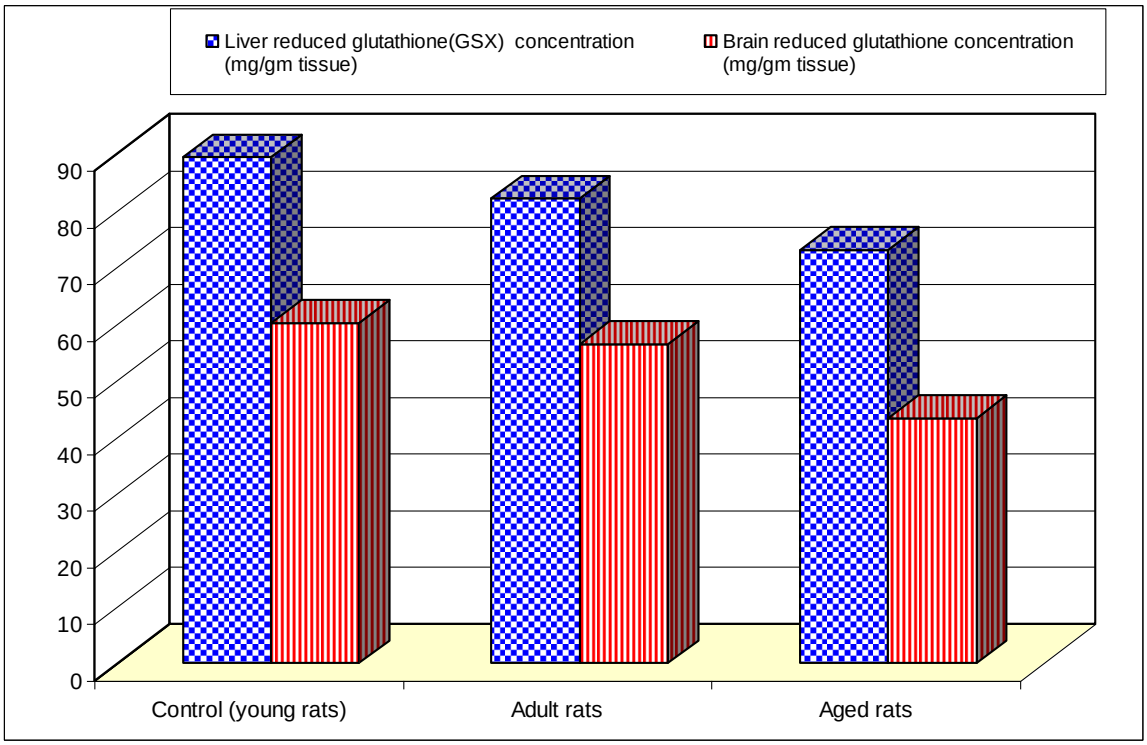

Fig. (8): Liver and brain reduced glutathione letters in the same column are significantly different at ($P \leq 0.05$).

Serum total antioxidant capacity:-

In table (7), the serum total antioxidants concentration none significantly in adults (12 months), rats, while, the decrease became significant in aged (2 months), rats in compared with young (6 months) rats. Fig. (9).

Table (7): Serum total antioxidant concentration (mM/L) of rats groups.

Group / Parameters	Serum total antioxidants concentration (mM.L)
Control (young rats)	6.10± 0.25 [a]
Adult rats	5.00± 0.45 [a]
Aged rats	3.65 ± 0.20 [b]

Mean with different superscripts letters in the same column are significantly different at ($P \leq 0.05$).

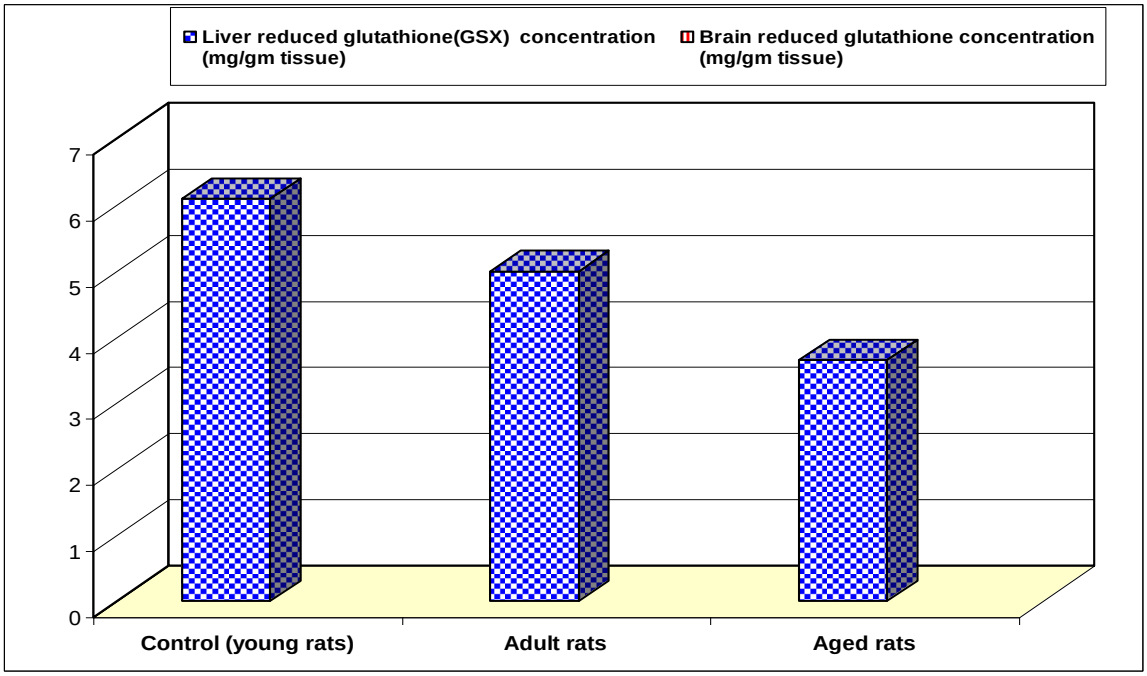

Fig. (9): Serum total antioxidant concentration (mM/L) of rats group

Liver and brain Nitric oxide (NO): In table (8), the liver and brain nitric oxide concentration significantly increased in adult and aged rats in compared with young rats. (Fig. 10).

Table (8): Liver and brain Nitric oxide (NO) concentration (µMol/gm tissue) of rats groups.

Group / Parameters	Liver antioxidant (NO) concentration (µMol/ mg tissue)	Brain antioxidant (NO) concentration (µMol/ mg tissue)
Control (young rats)	0.18± 0.05 [b]	0.22± 0.01 [b]
Adult rats	0.42± 0.45 [a]	0.35± 0.05 [a]
Aged rats	0.44± 0.010 [a]	0.39± 0.03 [a]

Mean with different superscripts letters in the same column are significantly different at ($P \leq 0.05$).

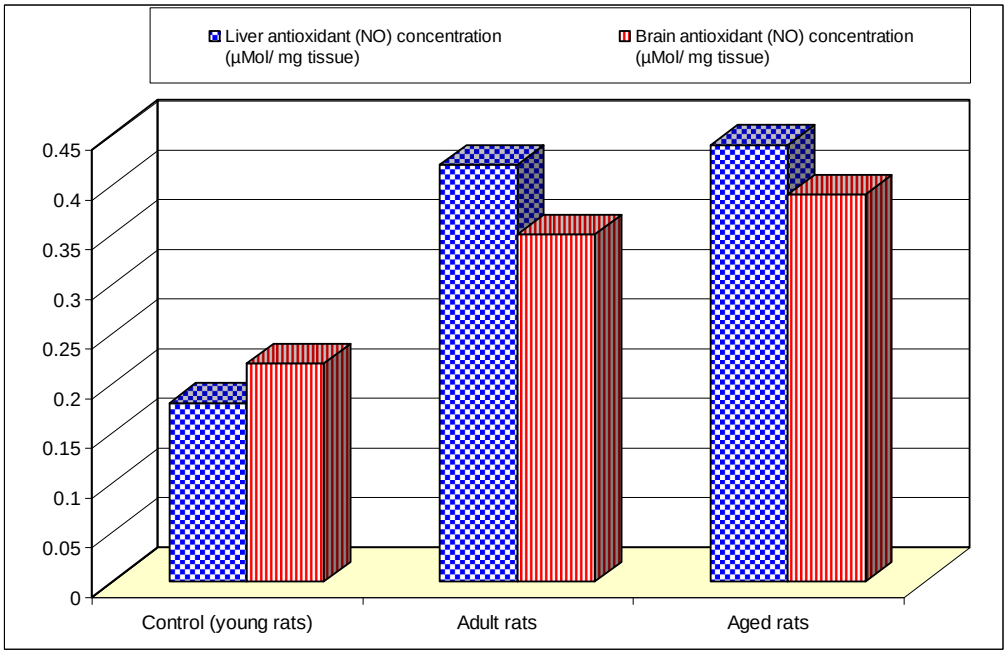

Fig. (10): Liver and brain Nitric oxide (NO) concentration (µMol/gm tissue) of rats.

Liver and brain malondialdehyde (MDA): In table (9), liver malondialdehyde (MDA) concentration showed a significant increase in both adult and aged rats but in brain tissue showed no significant increase in adult while its concentrations significantly increased in aged rats in compared with control group. Fig. (11).

Table (9): Liver and brain malondialdehyde (MDA) concentration (µmol/gm tissue) of rats group.

Group / Parameters	MDA concentration (µMol/ mg tissue)	MDA concentration (nMol/ mg tissue)
Control (young rats)	36.55 ± 0.45 ^{c}	45.35± 1.40 ^{b}
Adult rats	43.40 ± 0.50 ^{b}	47.25± 0.80 ^{b}
Aged rats	45.45± 0.65 ^{a}	54.35± 1.55 ^{a}

Mean with different superscripts letters in the same column are significantly different at ($P \leq 0.05$).

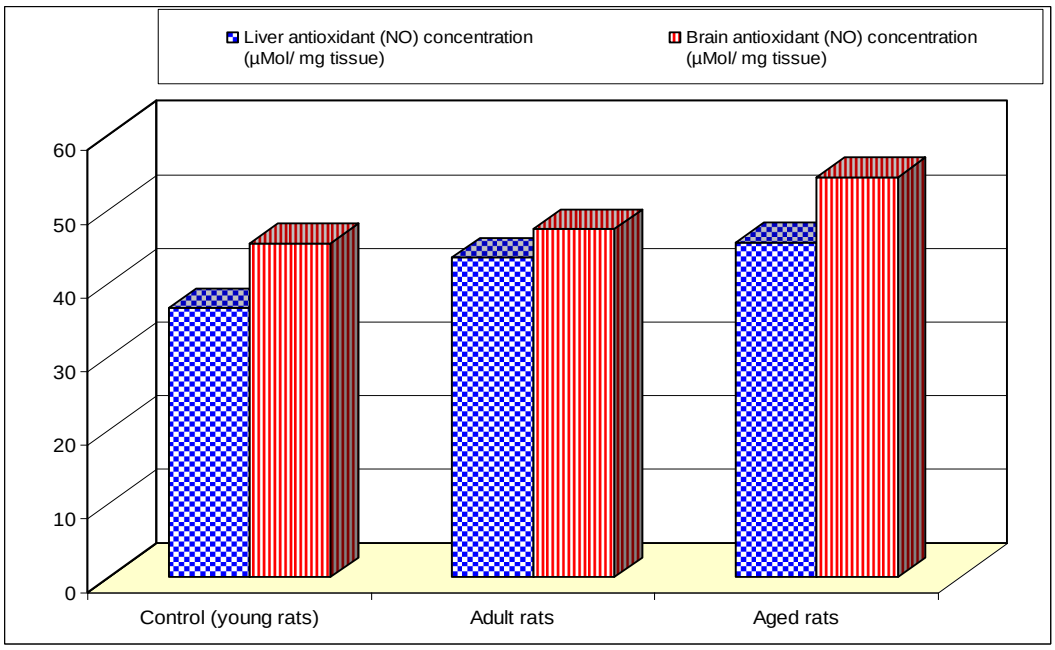

Fig. (11): Liver and brain malondialdehyde (MDA) concentration (µmol/gm tissue) of rats group.

DISCUSSION

Aging refers to a multidimensional process of physical, psychological and social changes. Some dimensions of aging grow and expand over time, while others decline. Reaction time, e.g. may slow with age, while the knowledge of world events and wisdom may expand. Researchers showed that even late in life, potential exists for physical, mental and social growth and development. Aging is an important part of all human societies reflecting the biological changes that occur, but also reflecting cultural and societal conventions.

Roughly, 100.000 people word wide die each day of age –related causes [38]. Numerous studies have suggested that age –associated deterioration in body function can be related to oxidative damage by reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced during mitochondrial oxidative phosphorylation. As the major cellular source of ROS mitochondrial are also the main targets of their damaging effects [39]. The delicate balance between both reactive species (ROS) and reactive nitrogen species generation and elimination is maintained by many complex mechanisms and a dysfunction of any of these mechanisms could load to alterations in cellular redox status [40]. Antioxidants are defense system against the harmful effect of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

They include enzymes like superoxide dismutase, catalase and glutathione peroxidase. Antioxidant defenses a bit as a balanced or coordinated system and each relies on the action of the other. In health, the balance lies slightly in favour of the reactive species so that they are able to fulfill their biological roles. [40]. Age –related mutations of mitochondrial DNA, reduced mRNA expression and translation of age coding for subunits of enzymes complex were demonstrated in various

tissues [41]. This results in agree with our results table (2) & Fig. (1 & 2) in liver & brain tissues. The effect of aging in mitochondrial DNA ensured by Sastre who pointed out that the role of old mitochondrial in cell aging has been emphasized by the finding that cells microinjected with mitochondria isolated from fibroblasts of old rats degenerate to a much greater extent than those microinjected with mitochondrial from young rats [41].

NADH dehydrogenase (complex I), the first enzyme of the mitochondrial transport chain which translocates 4 protons across the inner membrane per molecule of oxidized NADH, helping to build the electrochemical potential used to produced ATP [42]. The obtained results showed that NADH dehydrogenase (complex I) decreased in its activity in liver and brain of both adult and aged rats in compared with youngs rats table (3) and Fig. (3 & 4). These results are in agreement with that of **Preston et al.** They reported that 42-46 % reduction in complex I activity was demonstrated in hearts of 24 months old rats. These reductions may be contributed to the reduced energetic reserves and increased susceptibility of the aged heart to withstand the stress and oxidative injury[43]. Cytochrom oxidase (complex IV), the last enzyme in the respiratory electron transport chain on the mitochondria. It receives an electron from each of four cytochrom C molecules, and transfers them to one oxygen molecule, converting molecular oxygen to two molecules of water (Table 4 and fig. 6). [44].

The present findings of cytochrom oxidase (complex IV) showed a decline in its activity in liver and brain of both adult and aged rats when compared with control rats. The findings are in consistence with some studies that showed a marked decline in the activity of complex IV with age [16]. The obtained results of glutathione peroxidase showed no significant decrease in its activity in liver of adult rats. This decrease became significant in aged rats glutathione peroxidase showed a significant decrease in compared with control rats (table 5 & Fig. 7). The obtained results are in consistent with the study of **Day (2009)** who reported decreases in the activity of glutathione peroxidase. This was attributed to the increased generation of ROS as a function of age and could be due to increased level of free radicals in older population as compared to young **(45)**. Reduced glutathione (GSH) is known to play a crucial role in the detoxification reactive intermediates arising from endogenous substances or exogenous xenobiotic during the course of their metabolism. The concentration of reduced glutathione (GSH) in both liver and brain of adult rats. This decrease became significant in aged rate in compared with young rats (control) (Table, 6 and Fig. 8). These results are in agreement with those reported by De La Asunction et al. [46] who stated that mitochondrial reduced glutathione content showed a general decline in the liver, kidney and brain of aged rats and mice. Antioxidants counter the action of free radicals by several mechanisms, which include enzymes that degrade free radicals, protein such as transferring that can bind metals which stimulate the production of free radicals and antioxidants such as vit. C. and E that acts as free radicals scavengers [47]. The results of serum total antioxidants capacity showed no significant decrease in its concentration in adult rats, while in aged rats its concentration significantly decrease in compared with young rats (Table 7, Fig. 9).

These results are in agreement with that results of anther who pointed out that no aging effect was observed in the antioxidant capacity of serum and brain in the adult and old rats, moreover, some studies demonstrated that total antioxidant capacity of plasma remained unchanged in the aged rats [47]**.** Nitric acid (NO) is generated in biological tissues by specific nitric oxide synthesis (NOSs). Cells of the immune system produce nitric oxide during the oxidative burst triggered during inflammatory processes under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion (ONOO-), which is an oxidizing free radicals that can cause DNA fragmentation and lipid peroxidation. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal functions [48]**.** The obtained results of nitric oxide (NO) showed a significant increase in its concentration in both liver and brain of adult and aged rats in compared with young rats (Table 8 and Fig. 10).

These results are in consistence with this anther results that increase in the NO^- and /or superoxide production in mitochondria with age; this may be became deleterious to the mitochondrial respiratory enzymes. In contrast, aging causes a significant decrease of NOS I expression and NOD activity in the old rats, which may be one of the mechanisms leading to erectile dysfunction associated with aging. In the same aspect also reported an impairment of circadian rhythmicity in the elderly, which has been suggested to cause age –associated diseases such as atherosclerosis and hypertension and also reported endothethelium –derived nitric oxide (NO) is a critical regulator of cardiovascular homeostasis, but its production declines with aging, there by including vascular dysfunction [49]. Malondialdehyde (MDA), the direct result of

oxidative stress taken as biomarker of lipid peroxidation of different tissues. The findings of MDA in the present investigation showed a significant increase in its concentration in liver of both adult and aged rats in compared with young rats.

This increase was non-significant in the brain of adult rats and became significant in the brain of aged rats in compared with young rats (Table, 9 and Fig. 11). This findings are in agreement with the anther had reported in Shaal sheep the lowest concentration of MDA, which is widely used as a biomarker of lipid peroxidation, was also seen in the youngest group. It seems that in the early growth phase [(50).] In contrast to the previous findings, the age-related variation in oxidant status and lipid damage and occluded that the mitochondrial lipid peroxidation index was not different in young and old mice [(51)].

CONCLUSION

From this paper results, it can be concluded that aging has a great harmful effect on mitochondrial DNA, that can be detected from the obtained results: decreases in the activity of NADH dehydrogenase (complex I) and cytochrom oxidase (complex IV) , which correlated with age, decease in the activity of glutathione peroxidase and in concentration of both reduced glutathione and total antioxidant capacity and increase in the concentration of nitric oxide and malondialdhyde with age.

Recommendation: The molecular mechanism of the antioxidants that enables the body to face the free radicals harmful effects should be more clarified. Increase the human awareness at the beginning of the aging with the danger of free radicals and how to decrease its side effects and consequently decrease aging disease and change the human life style, which is easier in youth and aims to avoid the harmful effect of free radicals.

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