

Research Article

Age Associated Mitochondrial Stress in Rat Liver, Kidney and Heart: Protective Potential of Docosahexaenoic Acid

Dharmjeet Kumar Pandey, Pintu Kumar, Devesh Kumar Joshi, Manisha Choudhary, Abhishek Kumar Singh, Sandeep Tripathi*

Department of Biotechnology, Nims Institute Advanced Science & Technology, Nims University Rajasthan, Jaipur 303121 India

***Corresponding author**

Dr. Sandeep Tripathi

Email: sandeeptripathiphd@gmail.com

Abstract: Aging is a biological phenomenon concerning all living multicellular organisms. It is degenerative process caused by accumulated damaged lipid and protein that leads to cellular dysfunction, tissue failure, and death. In the present study, we attempt to assess the aging induced biochemical and pathological changes in old rats and protective efficacy were tested. Twelve male Wistar rats of two age groups (12 months and 24 months) were selected for normal saline treated control group (n=6) and DHA treated (100 mg / kg bw) experimental groups (n=6). For biochemical assays, lipid peroxidation (LPO), protein carbonyl (PC) and lipofuscin (LIF) and antioxidant levels i.e., superoxide dismutase, (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were investigated in liver, kidney and heart followed by lipid profile and liver and kidney function test. Oxidative stress markers (LPO, LIF and PC) in liver, kidney and heart different organs were significantly increased while the activities of antioxidant enzymes (SOD, CAT, GPx and GSH) in the same organs were significantly depleted. The DHA treated old rats significantly reduces the oxidative stress markers when compared with their respective control. Cellular changes in old control rats correlated with the deteriorated liver and kidney function test and lipid profiles. Our results suggest that increment of the rate of lipid peroxidation, protein oxidation and lipofuscinogenesis are well correlated with the decline in the antioxidant status of the liver, kidney and heart. The increased lipofuscin, formed consequent to mitochondrial residues remaining after lysosomal degradation, also exhibit regional heterogeneity and linear increment with age, while DHA exhibited as an antioxidant and anti-aging properties.

Keywords: Aging, Liver, kidney, heart, DHA.

INTRODUCTION

Aging is the natural phenomenon of growing old and is usually implicated as the gradual decline of the biological and physiological functions which has direct impact on the functional ability of body. Aging leads to decline cognitive ability, neuromuscular, digestion, excretion and cardiovascular functioning. Elderly people are known to have the highest incidence of illness and deficits in cognitive functioning [1-2]. The exact mechanisms that cause this functional decline are unclear. In recent years relatively little effort has been made to investigate the alterations in physiological profiles of this wide group of population [3].

The interplay of biochemical changes, cellular responses and reactions of an organism ultimately lead to the generation of aging phenotypes in which the fitness of the organism is grossly compromised. The phenomena of aging affect cells, tissues and the organism as a whole [4]. The molecular and cellular events involved in aging, especially in a complex multicellular organism [5], are not clearly understood

and this has given rise to a multiplicity of theories which overlap each other to a considerable extent. One of the several explanations put forth to explain the nature of mechanism underlying senescence point towards loss of functional capacity attributable to cellular and molecular damage [6].

The theory of aging is “free radical theory of aging” has gained constant support because it’s able to describe some of the processes that occur with aging. [7]. This theory demonstrated that an increase in the mitochondrial production of free radicals with age due to increased cellular damage [8]. Toxic effects of ROS on cellular components lead to accumulation of oxidative damage which causes cellular dysfunction with age. Mitochondria play central role as an integrator of a many signals within the cells. Mitochondria have different function like modulate energy supply, reactive oxygen species (ROS) signalling, and intrinsic pathways of apoptosis. Therefore, mitochondria have been frequently studied as a target for combating cellular aging [9].

Deposition of oxidatively damaged biomolecules with advancing age and in association with some pathological conditions such as kidney failure, liver cirrhosis and atherosclerosis, among others, has been well documented in many reports [4,10,11]. One major unanswered question have been arrived that whether the level of oxidative stress, i.e., the imbalance between antioxidant capacities vs. oxidant load, remains steady or whether it increases with age. The resolution of this issue is important because an increase in the severity of oxidative stress with age would imply a relatively more rapid rate of accrual of oxidative damage and loss of functional capacity [12].

A frequently employed strategy is the supplementation of food with antioxidants. Although some studies have shown positive effects on life span or severity of certain disease conditions, in other cases, beneficial effects were absent [13,14]. Oxidative stress may be a consequence rather than a cause of aging or the pathological impairments. Docosahexaenoic acid (DHA) is a major component of fish oil. It is a long chain polyunsaturated fatty acid. DHA is vital component of the phospholipids of human cellular membranes, especially those in the brain and retina, necessary for optimal neural development and visual acuity. Earlier some of the studies reported that DHA may have a protective role against oxidative stress as demonstrated by decreased lipid peroxidation, both *ex vivo* and *in vivo* [15,16].

Clinical evidence demonstrates that dietary supplementation with the DHA has beneficial effects in acute and chronic inflammatory conditions [17] (Choudhary *et al.*, 2012) and lowers the risk of death or end-stage renal disease in patients with glomerulonephritis, Which is present at significant concentrations in most human tissues [18]. In the present study protective efficacy of Docosahexaenoic acid (DHA) against oxidative stress in aging were tested by lipid peroxidation and antioxidant profiles in liver, kidney and heart of male albino rats following DHA supplementation.

MATERIALS AND METHODS

Animals

In the present study young (6 month, weight $270 \pm 2.5g$, $n = 12$) and old (24 month, weight $450 \pm 4.8g$, $n = 12$), male albino rats were taken from the University animal house. Furthermore, all the animals were divided into following subgroups ($N=6$) i.e., Young control (YC), young experimental (YEx), Old control (OC) and Old experimental (OEx). All the animals were separately housed in polypropylene cages in a room, which was maintained at a room temperature of 22 ± 2 °C, humidity of 50 ± 10 % and 12h light dark cycles. They were fed with commercial pellet diet and allowed access to water *ad libitum*. The Institutional Animal Ethics Committee approved the study

(NU/BT/13/94) prior to the initiation of the experiment and also approved all experimental protocols. Docosahexaenoic acid 100 mg/ kg body weight mixed with 1% gum acacia formed an aqueous suspension which was directly introduced into the rat pharynx via a feeding cannula to experimental groups of young and old rats and an equivalent volume of physiological saline was given to control groups for 30 days.

Blood collection and serum separation

Blood samples were collected by ocular punctures into plain centrifuged tubes were kept in inclined position to allow complete clotting of blood and then centrifuged at 2500 rpm for 30 minutes. The resultant clear supernatant was pipette out and preserved in small vials in the freezer until use.

Serum Lipid profiles

Serum total cholesterol, high-density lipoprotein, triacylglycerol were determined using Randox Laboratory kit reagents and VLDL was calculated using the formula $TG/2.2$ mmol/l. Low density lipoprotein (LDL) cholesterol was determined by differential subtraction of the sum of the cholesterol fractions from the total cholesterol.

Liver and Kidney function test

Serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatine and glucose were assayed using commercially available Qualigens kits (Mumbai, India).

Tissue Homogenate Preparation

After 30 of experimental period rats were sacrificed by anesthetic overdose (40mg kg b.w.) and liver, kidney and heart were removed from the body. Ten percent (w/v) homogenate was prepared by of York's homogenizer fitted with Teflon plunger in 0.1 M phosphate buffer (pH 7.1).

Oxidative stress markers

The lipid peroxide levels (thiobarbituric acid reacting substances) were estimated spectrophotometrically at 532 nm and expressed as nmole of MDA /g tissue [19]. Protein carbonyl content (PC) was investigated in the samples by measuring the DNPH adducts at 375 nm. Carbonyl contents were calculated by using a molar extinction coefficient (e) of $22,000 M^{-1} cm^{-1}$. Data were expressed as nmoles carbonyl /mg [20]. Lipofuscin (LIF) was measured using 2:1 chloroform-methanol extraction mixture using fluoro-spectrophotometer at an excitation maximum of 360 nm and emission maximum of 420 nm. The lipofuscin content of the fluorescence was investigated using quinine fluorescence [21]. Data were expressed as U/g tissue.

Measurement of endogenous enzymes

The superoxide dismutase (SOD EC 1: 15.1.1) activity was determined from its ability to inhibit the reduction of NBT in presence of PMS [22]. The reaction was monitored spectrophotometrically at 560nm. The SOD activity was expressed as U/mg protein (1 unit is the amount of enzyme that inhibit the reduction of NBT by one half in above reaction mixture). Catalase (CAT, EC 1.11.1.6) activity was assayed using hydrogen peroxide as substrate; the decomposition of H₂O₂ was followed at 240nm on spectrophotometer. The CAT activity was expressed as U/mg protein [23]. The glutathione peroxidase (GSHPx, EC 1.11.1.0) was assayed using GSH, NADPH and H₂O₂ as reactants. The oxidation of GSH into GSSG was measured in terms of oxidation of NADPH to NADP⁺ and assayed as decrease in the absorbance of reaction mixture at 340 nm on spectrophotometer [24]. The activity of GSHPx was expressed as n moles of NADPH oxidized / min / mg protein. Tissue homogenate was deproteinated with tetrachloroacetic acid, centrifuged and supernatant was used for the estimation of reduced glutathione (GSH) with the help of Ellman reagent (5, 5' dithiobis (2-nitro benzoic acid). The optical density of the pale colour was measured on the spectrophotometer on 412 nm. An appropriate standard (pure GSH) was run simultaneously. The level of GSH was expressed as µg / g tissue [25].

Histopathological studies

After 30 days of experimental treated and control rats were sacrificed by anesthetic overdose (40 mg / kg sodium pentobarbital). Liver, kidney and heart were removed and rinsed with physiological saline. A portion of the tissue samples were fixed with neutral formalin dehydrated with different concentrations of ethyl alcohol and embedded in paraffin. Tissue sections were cut (4 µm) and stained with hematoxylin and eosin for histological grading.

Statistical analysis

The data were summarized as Mean ± SEM. The significance of mean difference between two groups was evaluated by Student's 't' test. The significance were evaluated using one way ANOVA.

RESULTS AND DISCUSSION

The aging process results in an accelerated decline of functional capacity, but the mechanisms behind this decline are unclear. The free radical theory of aging proposes that mitochondrial production of reactive oxygen species causes an increase in cellular damage with age [26]. This theory has gained strong support because it is consistent with many of the processes and degenerative diseases observed with aging [27].

The objective of this study was to determine whether prolonged treatment with DHA would improve the ability of old animals to tolerate physiological

deterioration of aging. We hypothesized that DHA treatment could enhance physiological process in aged animals by reducing cellular and mitochondrial oxidative damages of the organs. Aging affects organs, tissues and cell functions to different extents, resulting in a decline in the capacity of the whole organism to cope with its environment [4].

In the present study we measure the effect of DHA on lipid profile. Several mechanisms, in addition to various components have been suggested to explain the lipid-lowering effect of DHA. Khaire *et al.*, [28] showed that DHA able to modify cholesterol status by its capacity to bind both cholesterol and bile acids. These include a direct effect on cholesterol metabolism by inhibiting the key enzymes involved in cholesterol and fatty acid synthesis [29]. Our finding is concomitant with the results of Jones *et al.*, [30] they suggested that DHA responsible for improvement in serum lipid profile in leading to decreased TG and cholesterol synthesis in addition to enhanced LDL receptor mediated LDL uptake [31].

Lipid profiles: the lipid profiles were investigated and presented in the table-1. The concentration of cholesterol was found to be significantly ($p < 0.01$) increased in old control rats when compared with the young control rats. The treatment with DHA found to be reduced in old experimental rats as compared to old control rats. The concentration of LDL and VLDL were found to significantly ($p < 0.001$) increased in old control rats when compared with the young control rats while, the treatment with DHA were found to be reduced in old rats as compared to old control rats. The concentration of HDL was found to be reduced significantly ($p < 0.001$) in old control rats when compared with the young control rats. The treatment with DHA found to be increased in old rats experimental rats as compared to old control rats.

In the present study, the serum enzyme levels were found to be elevated in 24 months aged control rats while the co-damination of DHA revealed normal when compared with their respective control. With advancing age numerous physiological changes take place and they are reflected by the physical and biochemical perturbations in the animal, resulting in a difference in proportion of endogenous metabolites excreted in serum and urine. There have been reports of NMR studies providing evidence that ageing reduces the production of energy *via* oxidative phosphorylation due to interference with many key enzymatic processes of TCA cycle (Tripathi *et al.*, 2008) [32]. Moreover, some previous studies in mice have reported age related perturbations in TCA cycle enzymes and impairment in energy pathways.

The data of the liver and kidney function test were investigated and presented in the table-2. ALP, SGOT and SGPT were found to be increased

significantly ($p < 0.001$) in the old rats when compared with the young rats. DHA treatment to aged rats exhibited significant ($p < 0.05$) reduction in APL, SGOT and SGPT. The concentration of urea and creatinin were found to be increased significantly ($p < 0.001$) in old control rats when compared with the young control rats while, the old DHA treated rats exhibited marked reduction in old experimental rats. The concentration of HDL was found to be significantly ($p < 0.001$) decreased in old rats. The DHA administration significantly ($p < 0.01$) increased the concentration of HDL in old experimental rats.

The role of oxidative imbalance in aging is a widely discussed concept (Phillips *et al.*, 2013), and the arguments on the role of oxidative stress and mitochondria may help in understanding the aging as evident by Tripathi *et al.*, [12]. Excessive oxidative stress leads to cell damage and apoptosis [33]. The mitochondria contains array of respiratory chain, where water is produced from the reduction of two molecules of oxygen and produced energy in the form of ATP [34]. Defects in the respiratory chain yield partially reduced free-radical products of oxygen, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and OH^* radicals, which are highly toxic and damage normal cellular constituents by reacting with them [35] and hence they are called reactive oxygen species (ROS) [36].

The lipid peroxide levels were found to be significantly ($p < 0.001$) increased in the liver, kidney and heart of old rats as compared with the young rats. The maximum increment was observed in kidney. The treatment with DHA to old rats found to be significantly ($p < 0.001$) reduced when compared with the old control rats. The protein carbonyl content were found to be significantly ($p < 0.001$) increased in the liver, kidney and heart of old rats as compared with the young rats. The maximum increment was observed in heart followed by kidney and liver. The treatment with DHA to old rats found to be significantly ($p < 0.001$) reduced when compared with the old control rats. The lipofuscin content were found to be significantly ($p < 0.001$) increased in the liver, kidney and heart of old rats as compared with the young rats. The maximum increment was observed in liver followed by kidney and heart. The treatment with DHA to old rats found to be significantly ($p < 0.001$) reduced when compared with the old control rats.

Increased lipid peroxidation (LPO) and protein oxidation are the major consequence associated with oxidative stress. Tissue is highly enriched in long chain polyunsaturated fatty acids (PUFAs) particularly arachidonic acid and docosahexaenoic acid (DHA) which play important role in structural and biological functions. Transport of long chain PUFAs from plasma may important roles because of the limited ability to synthesize long chain PUFAs, in the face of high

demand for them [37,38]. In the present study, peroxidative reactions were evaluated by estimating lipid peroxide levels, protein oxidation and lipofuscin. Rapid increase of lipid peroxides, and conjugated dienes are closely related to peroxidation of lipids. Our result demonstrating increased LPO and their peroxidative products in 12 months and 24 months old rats supported to the findings of the previous studies [39-40].

The decrease in the antioxidative defenses (SOD, CAT, GSHPx, GR and GSH) accompanied with the increased levels of lipid peroxides. On the other hand, co administration with DHA found to be reduced LPO levels in different regions of the brain. Among various antioxidative mechanisms in the body, SOD is thought to be one of the major enzymes which protects against tissue damage caused by the potentially cytotoxic free radicals [41]. It is therefore possible that the decrease in SOD activity with age may be closely related to the mitochondrial stress. It is point out that in general the activity of catalase declines during the maturation of the animal to adulthood. Thus glutathione peroxidase and glutathione reductase plays an important role in maintaining the reducing potential of the cells.

Activity of superoxide dismutase (SOD) were found to be significantly ($p < 0.001$) reduced in the liver, kidney and heart of old rats as compared with the young rats. The maximum decrement was observed in heart followed by kidney and liver. The treatment with DHA to old rats found to be significantly ($p < 0.01$) reduced when compared with the old control rats. Activity of catalase were found to be significantly ($p < 0.01$) reduced in the kidney of old rats as compared with the young rats. The maximum decrement was observed in heart followed by kidney and liver. The treatment with DHA to old rats found to be significantly ($p < 0.05$) reduced when compared with the old control rats. Activity of GPx were found to be significantly ($p < 0.001$) reduced in the liver, kidney and heart of old rats as compared with the young rats. The maximum decrement was observed in heart followed by kidney and liver. The treatment with DHA to old rats found to be significantly ($p < 0.01$) increased when compared with the old control rats. The reduced glutathione content (GSH) were found to be significantly ($p < 0.001$) reduced in the heart, kidney and liver of old rats as compared with the young rats. The maximum decrement was observed in heart followed by kidney and liver. The treatment with DHA to old rats found to be significantly ($p < 0.01$) reduced when compared with the old control rats.

The histopathological (H & E stained) photomicrograph of liver section of young control (A), Young experimental (B), old control (C) and old experimental (D) presented in figure-1. Young control and young experimental rats showed well architecture of the hepatocytes while old control rats exhibited specific changes were seen as mild congestion, diffused

nucleus and necrotic cells in the section of liver micrograph as compared to young control rats. DHA treated old rats showed marked reduction in abnormality in the cellular changes of liver when compared with the old control rats.

The histopathological (H & E stained) photomicrograph of kidney cortex of young control (A), Young experimental (B), old control (C) and old experimental (D) presented in figure-2. Young control and young experimental rats showed well architecture of the cells while old control rats exhibited specific changes were seen as mild congestion in the glomeruli, focal congestion and vacuolar (hydropic) degeneration of tubular cells as compared to young control rats. DHA treated old rats showed marked reduction in abnormality in the cellular changes of kidney when compared with the old control rats.

The supplementation of DHA found to be reversible changes were observed in the old rats. Our results show protection against cellular aging by DHA involving the damage of lipid and protein layer of the cell by the increased production of ROS. It has been shown that DHA exhibited protective effect against aging [42]. A number of studies in rats and mice have examined the changes in the morphology of the kidney, liver and heart with advancing age [43]. Studies in aged rodent models have shown that left ventricular mass

increases and individual ventricular myocytes are hypertrophied across various species. The total number of ventricular myocytes decreases with age in the rat heart, likely as a result of an increase in necrotic and apoptotic cell death. Contractile function also appears to change with age in animal models [44-46].

Age-associated loss of kidney and liver function has been recognized for decades. With aging, many subjects exhibit progressive decreases in glomerular filtration rate (GFR) and renal blood flow (RBF), with wide variability among individuals. In addition, age-related changes in liver function were observed [47] it may be due to increased dilatation and cellular changes observed by histopathology in our study. Namely, in the liver, heart and kidney aging causes oxidative damage, probably as a consequence of significant lowering of antioxidant profile. Our results demonstrated that prolonged DHA supplementation can significantly improve hepatic, renal and cardiac function in old rats. Therefore, these results indicate that DHA is endowed with antiaging and antioxidative property and DHA exert their hepatoprotective, nephroprotective and cardioprotective effect via controlling lipid peroxidation and protection of cellular changes by reducing oxidative stress. Namely, in the liver and kidney aging causes oxidative damage, probably as a consequence of significant lowering of antioxidant profile.

Table-1: Lipid profiles in control and experimental of young and old rats

	YC	YEx	OC	OEx
Total Lipid (mg/ml)	360.5± 38.4	371.5 ± 43.4	347.4 ± 33.2	394.6 ± 36.4
Cholesterol (mg/ml)	87.55 ± 9.7	91.5 ± 12.7	137.2 ± 32.7 [#]	107.6 ± 23.4*
Triglycerides (mg/ml)	75.6 ± 2.5	69.6 ± 2.5	79.4 ± 12.7	74.7 ± 19.7
LDL (mg/dl)	46.6 ± 6.9	49.3 ± 6.8	102.9 ± 5.6 [#]	77.5 ± 6.6*
VLDL (mg/dl)	27.4 ± 1.2	29.2± 1.3	42.4 ± 1.4 [#]	29.4 ± 1.7*
HDL (mg/dl)	59.8 ± 4.3	57.3 ± 5.3	29.9 ± 2.7 [#]	41.5 ± 4.4*

Values are expressed as mean ± SEM six animals (N=6) in each group. he values of total lipid (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and high density lipoprotein (HDL) in young control (YC), Young Experimental (YEx), Old control (OC) and Old experimental (OEx) groups. Sstatistical significance was determined by one way ANOVA followed by Neuman Keules post hoc test between groups. Superscripts relate significant (p< 0.05) comparison to YC with OC (#) and OC with OEx (*).

Table-2: Clinical chemistry test in control and experimental of young and old rats

	YC	YEx	OC	OEx
ALP	28.3 ± 3.9	26.9 ± 4.9	57.1 ± 4.5 [#]	38.5 ± 4.1 *
SGOT	17.5 ± 2.7	16.7 ± 3.5	36.4 ± 3.8 [#]	27.6 ± 2.3*
SGPT	13.8 ± 2.8	14.2 ± 2.8	31.1 ± 11.6 [#]	18.0 ± 2.2*
Urea	34.2 ± 4.8	33.8 ± 5.1	65.2 ± 15.5 [#]	55.2 ± 5.8*
Creatinin	0.68 ± 0.6	0.72 ± 0.6	1.72 ± 0.6 [#]	0.93 ± 0.6*
Glucose	146.5 ± 23.3	147 ± 27.5	98.5 ± 29.5 [#]	124.2 ± 23.3*

Values are expressed as mean ± SEM for eight animals (N=8) in each group. The concentration of Alkaline phosphate (ALP; KA), serum oxaloacetic acid transaminase (SGOT; U/ml), serum glutamine pyruvate transaminase (SGPT; U/ml), Urea, Creatinin and glucose in serum of young control (YC), Young Experimental (YEx), Old control (OC) and Old experimental (OEx) groups. Sstatistical significance was determined by one way ANOVA followed by Neuman Keules post hoc test between groups. Superscripts relate significant (p< 0.05) comparison to YC with OC (#) and OC with OEx (*).

Table-3: Oxidative stress parameters in control and experimental of young and old rats

		YC	YEx	OC	OEx
LPO	Liver	258.0 ± 12.4	262.1 ± 13.2	378.5 ± 22.2 [#]	288.4 ± 13.2 [*]
	Kidney	222.0 ± 11.6	220.0 ± 9.4	342.0 ± 14.4 [#]	299.0 ± 11.2 [*]
	Heart	234.0 ± 9.8	243.0 ± 10.5	345.0 ± 9.4 [#]	319.0 ± 10.4 [*]
PC	Liver	12.85 ± 1.2	13.45 ± 1.3	18.85 ± 1.8 [#]	13.45 ± 1.1 [*]
	Kidney	14.85 ± 1.1	13.45 ± 1.2	23.85 ± 2.1 [#]	17.45 ± 1.6 [*]
	Heart	15.6 ± 1.2	14.9 ± 1.3	25.3 ± 2.2 [#]	18.2 ± 1.9 [*]
LIF	Liver	3.7 ± 0.12	3.6 ± 0.13	16.4 ± 0.22 [#]	12.1 ± 0.15 [*]
	Kidney	4.2 ± 0.14	4.0 ± 0.15	14.2 ± 0.21 [#]	8.5 ± 0.14 [*]
	Heart	5.4 ± 0.18	5.1 ± 0.16	18.3 ± 0.25 [#]	13.2 ± 0.12 [*]

Values are expressed as mean ± SEM for eight animals (N=8) in each group. The concentration of lipid peroxide levels (LPO; nmole MDA / g tissue), protein carbonyl (PC; nmole carbonyl content/mg protein) and lipofuscin (LIF; unit / g tissue) in young control (YC), Young Experimental (YEx), Old control (OC) and Old experimental (OEx) groups. Sstatistical significance was determined by one way ANOVA followed by Neuman Keules post hoc test between groups. Superscripts relate significant (p< 0.05) comparison to YC with OC (#) and OC with OEx (*).

Table-4: Antioxidant profiles in control and experimental of young and old rats

		YC	YEx	OC	OEx
SOD	Liver	5.1 ± 0.12	5.4 ± 0.13	4.1 ± 0.15 [#]	5.0 ± 0.19 [*]
	Kidney	4.9 ± 0.15	5.1 ± 0.17	3.8 ± 0.12 [#]	4.6 ± 0.14 [*]
	Heart	4.2 ± 0.14	4.3 ± 0.13	3.2 ± 0.11 [#]	4.6 ± 0.12 [*]
CAT	Liver	2.7 ± 0.08	2.9 ± 0.04	2.2 ± 0.06 [#]	3.2 ± 0.03 [*]
	Kidney	2.3 ± 0.07	2.5 ± 0.05	1.9 ± 0.05 [#]	3.1 ± 0.07 [*]
	Heart	2.7 ± 0.08	2.9 ± 0.08	1.6 ± 0.04 [#]	1.2 ± 0.04 [*]
GPx	Liver	99.2 ± 8.8	102.3 ± 9.8	84.6 ± 5.8 [#]	105.6 ± 9.8 [*]
	Kidney	82.4 ± 7.8	85.2 ± 9.8	67.3 ± 5.8 [#]	94.3 ± 7.8 [*]
	Heart	66.2 ± 6.8	71.3 ± 7.8	52.5 ± 6.6 [#]	67.3 ± 6.4 [*]
GSH	Liver	6.2 ± 0.8	6.4 ± 0.9	5.2 ± 0.3 [#]	6.8 ± 0.4 [*]
	Kidney	6.2 ± 0.5	6.4 ± 0.8	4.9 ± 0.4	5.8 ± 0.5 [*]
	Heart	4.5 ± 0.3	5.0 ± 0.4	3.8 ± 0.3 [#]	4.9 ± 0.4 [*]

Values are expressed as mean ± SEM for eight animals (N=8) in each group. The activity of concentration of the superoxide dismutase (SOD; unit / mg protein), catalase (CAT; unit / mg protein), and glutathione peroxidase (GPx; NADPH oxidized / min mg protein) and reduced glutathione (GSH; µg / g tissue) in young control (YC), Young Experimental (YEx), Old control (OC) and Old experimental (OEx) groups. Sstatistical significance was determined by one way ANOVA followed by Neuman Keules post hoc test between groups. Superscripts relate significant (p< 0.05) comparison to YC with OC (#) and OC with OEx (*).

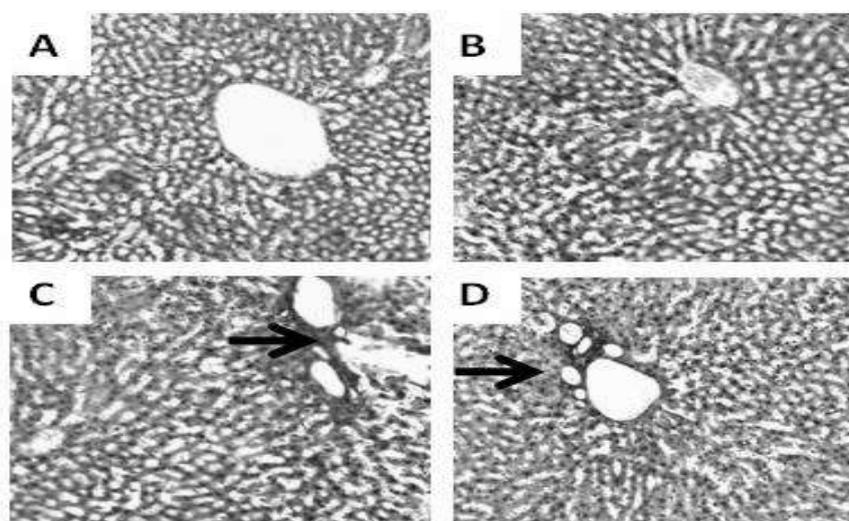


Fig-1: Histopathology (H&E stained, 100X) photograph of liver of young control (A), Young Experimental (B), Old control (OC) and Old control (C) Old experimental (D)

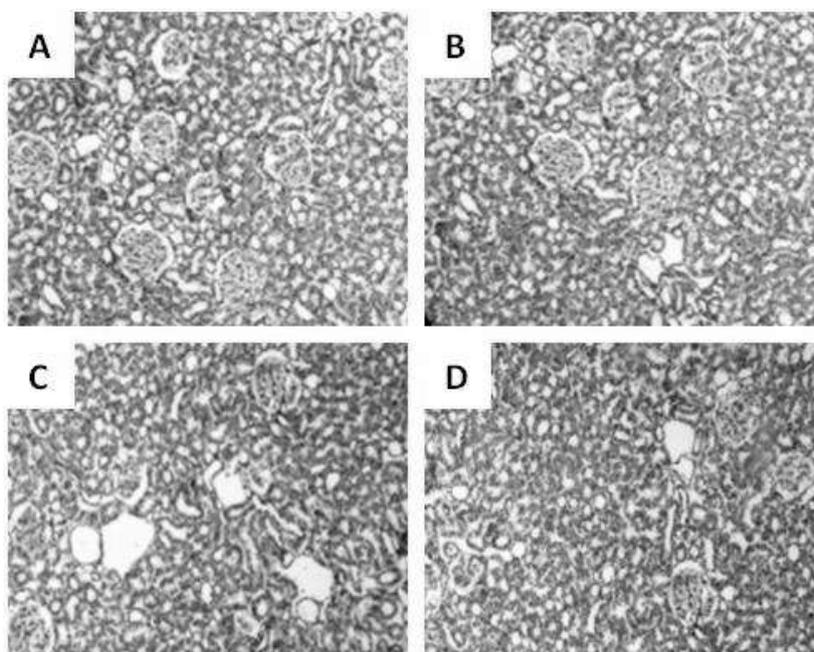


Fig-2:Histopathology (H&E stained, 40X) photograph of kidney of young control (A), Young Experimental (B), Old control (OC) and Old control (C) Old experimental (D).

CONCLUSION

In the present study, the marked biochemical and pathological changes were observed in the kidney, heart and heart of old rats than those of young animals and they are more susceptible for oxidative stress induced cell death, it may be advancing age. The remarkable finding was pointed out that DHA supplementation recovers the aging induced changes in liver, kidney and heart. Therefore, DHA play crucial role for the protection of oxidative stress with advance aging of animals. However, further studies are required in other models to unravel the role of DHA in the pathophysiology of aging. However we recommend the anti-aging and antioxidant property of DHA.

REFERENCES

1. Mahdi AA, Annarao S, Tripathi S, Nawab A, Mahdi F, Hasan M, Roy R, Khetrpal CL; Correlation of Age-Related Metabonomic Changes in ¹H NMR Serum and Urine Profiles of Rats with Cognitive Function. *Open Magnetic Resonance*, 2008; (1); 71-76.
2. Tripathi S, Mahdi AA, Hasan M, Mahdi F, Bajpai VK; Protective potential of Bacopamonniera extract on aluminum induced cerebellum toxicity and neuromuscular decline in aging rats. *Cell Mol Biol (Noisy-le-grand)*. 2011; 57(1): 3-15.
3. da Silva VL, Diniz CG, dos Santos SG, Gomes RM, Nicoli JR, Magalhães PP, Mendes EN, de Carvalho MA, Farias LM; Physiological alterations of a *Fusobacterium nucleatum* strain exposed to oxidative stress. *J Appl. Microbiol.* 2007;103(1):20-6.
4. Joshi DK, Chaoudhary M, Tripathi S, Negi MPS, Mahdi AA; Age dependent relative risk of aluminum toxicity: Levels of metals and enzymic and non enzymic antioxidants status in liver, kidney and brain of aluminum treated young and old rats. *Int. J Biol. Pharm. Res.* 2013; 4(3): 176-185
5. Donato AJ, Morgan RG, Walker AE, Lesniewski LA; Cellular and molecular biology of aging endothelial cells. *J Mol Cell Cardiol.* 2015; S0022-2828(15)00034-6.
6. Chen JH, Hales CN, Ozanne SE; DNA damage, cellular senescence and organismal ageing: causal or correlative? *Nucleic Acids Res.* 2007;35(22):7417-7428.
7. Harman D; Free radical theory of aging. *Mutat Res.* 1992; 275 (3-6): 257-266.
8. Cadenas E, Davies KJ; Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med.* 2000; 29 (3-4): 222-230.
9. Hepple RT; Mitochondrial involvement and impact in aging skeletal muscle. *Front Aging Neurosci.* 2014;6:211.
10. Levine RL, Stadtman ER; Oxidative modification of proteins during aging. *Exp Gerontol*, 2001;36 (9):1495-1502.
11. Beckman KB, Ames BN; The free radical theory of aging matures. *Physiol Rev*, 1998;78(2):547-81.
12. Tripathi S, Mahdi AA, Nawab A, Chander R, Hasan M, Siddique MS, Mahdi, F, Mitra K, Bajpai VK; Influence of age on aluminium induced lipid peroxidation and neurolipofuscin in frontal cortex of rat brain: A behavioral, biochemical and ultrastructural study. *Brain Res.* 2009; 1253: 107-116.
13. Sumien N, Heinrich KR, Sohal RS, Forster MJ; Short-term vitamin E intake fails to improve cognitive or psychomotor performance of aged mice. 2004;36:1424-1433.
14. Meydani M; Nutrition interventions in aging and age-associated disease. 2001; 928:226-235.

15. Chaudhary M, Joshi DK, Tripathi S, Kulshrestha S, Mahdi AA; Docosahexaenoic acid ameliorates aluminum induced biochemical and morphological alteration in rat cerebellum. *Annals of Neurosci.* 2014; 21(1), 5-9.
16. Lee LK, Shahar S, Rajab N, Yusoff NA, Jamal RA, Then SM; The role of long chain omega-3 polyunsaturated fatty acids in reducing lipid peroxidation among elderly patients with mild cognitive impairment: a case-control study. *J Nutr Biochem.* 2013;24(5):803-808.
17. Choudhary M, Pal AK, Joshi DK, Tripathi S, Kulshrestha S, Modi DR; Docosahexaenoic Acid Prevents Aluminum Induced Neuronal Cell Death. *Sch. J. App. Med. Sci.*, 2013; 1(3):142-146
18. Arterburn LM, Hall EB, Oken H; Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr.* 2006;83(6):1467S-1476S.
19. Ohkawa H, Ohishi N, Yagi K; Assay for lipid peroxides in animal tissue by thio-barbituric acid reaction. *Anal Biochem.* 1979; 95: 351-358.
20. Liu R, Liu IY, Thompson RF, Doctrow SR, Malfroy B, Baudry M; Reversal of age related learning and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. *Proc Natl Acad Sci USA.* 2003;100:8526–8531.
21. Sohal, R.S, Donato HJ; Effect of experimental prolongation of life span on lipofuscin content and lysosomal enzyme activity in the brain of the housefly, *Musca domestica*. *J. Gerontol.* 1979; 34:489-496.
22. McCord JM, Fridovich I; SOD enzyme function for erythrocyte. *J. Biol. Chem.* 1969; 224: 6049-6055.
23. Aebi H; Catalase. In: Bergmeyer HU (ed). *Methods of Enzymatic Analysis.* NY Acad Press Inc. 1974; 2: 673–684.
24. Paglia DE, Valentine WN; Studies on the qualitative and quantitative characterization of erythrocyte GPx. *J. Lab. Clin. Med.* 1967; 20:150-168.
25. Ellman GL; Tissue sulfhydryl groups. *Arch Biochem Biophysics.* 1959; 82- 70.
26. Harman D; Free radical theory of aging: an update. *Annals of the New York Academy of Sciences.* 2006; 1067(1):10-21.
27. Julian D, Leeuwenburgh C; Linkage between insulin and the free radical theory of aging. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2004; 286: R20–R21.
28. Khaire A, Rathod R, Kale A, Joshi S; Vitamin B12 and omega-3 fatty acids together regulate lipid metabolism in Wistar rats. *Prostaglandins Leukot Essent Fatty Acids.* 2015;99:7-17.
29. Mayol V, Duran MJ, Gerbi A, Dignat-George F, Lévy S, Sampol J, Maixent JM. Cholesterol and omega-3 fatty acids inhibit Na, K-ATPase activity in human endothelial cells. *Atherosclerosis.* 1999;142 (2):327-33.
30. Jones PJ, Senanayake VK, Pu S, Jenkins DJ, Connelly PW, Lamarche B, Couture P, Charest A, Baril-Gravel L, West SG, Liu X, Fleming JA, McCrea CE, Kris-Etherton PM; DHA-enriched high-oleic acid canola oil improves lipid profile and lowers predicted cardiovascular disease risk in the canola oil multicenter randomized controlled trial. *Am J Clin Nutr.* 2014;100(1):88-97.
31. Weintraub HS; Overview of prescription omega-3 fatty acid products for hypertriglyceridemia. *Postgrad Med.* 2014;126 (7):7-18.
32. Tripathi S, Somashekar, Mahdi AA, Mahdi F, Hasan M, Roy R, Khetrapal CL; Aluminum mediated metabolic changes in rat serum and urine: A proton magnetic resonance study. *J Bio Mol Toxicol* 2008; 16; 22 (2):119-127.
33. Steele AD, Hetz C, Yi CH, Jackson WS, Borkowski AW, Yuan J, Wollmann RH, Lindquist S; Prion pathogenesis is independent of caspase-12. *Prion.* 2007;1(4):243-7.
34. Wang SB, Murray CI, Chung HS, Van Eyk JE; Redox regulation of mitochondrial ATP synthase. *Trends Cardiovasc Med.* 2013;23(1):14-8.
35. Srinivasan S, Avadhani NG; Cytochrome c oxidase dysfunction in oxidative stress. *Free Radic Biol Med.* 2012;53(6):1252-63.
36. Reeder BJ; The redox activity of hemoglobins: from physiologic functions to pathologic mechanisms. *Antioxid Redox Signal.* 2010;13(7):1087-123.
37. Pandey A, Bani S. Hydroxychavicol inhibits immune responses to mitigate cognitive dysfunction in rats. *J Neuroimmunol.* 2010;226 (1-2):48-58.
38. Milton VJ, Sweeney ST. Oxidative stress in synapse development and function. *Dev Neurobiol.* 2012;72 (1):100-10.
39. Newairy AS, Salama AF, Hussein HM, Yousef MI; Propolis alleviates aluminum induced lipid peroxidation and biochemical parameters in male rats. *Food Chem Toxicol.* 2009;47:1093-1098.
40. Sharma D, Sethi P, Hussain E, Singh R; Curcumin counteracts the aluminium-induced ageing-related alterations in oxidative stress, Na⁺, K⁺ ATPase and protein kinase C in adult and old rat brain regions. *Biogerontology.* 2009;10:489-502.
41. Wang HH, Chowdhury KK, Lutt WW; A synergistic, balanced antioxidant cocktail, protects aging rats from insulin resistance and absence of meal-induced insulin sensitization (AMIS) syndrome. *Molecules.* 2015;20(1):669-682.
42. Florent S, Malaplate-Armand C, Youssef I, Kriem B, Koziel V, Escanyé MC, Fifre A, Sponne I, Leininger-Muller B, Olivier JL, Pillot T, Oster T; Docosahexaenoic acid prevents neuronal apoptosis induced by soluble amyloid-beta oligomers. *J Neurochem.* 2006;96(2):385-395.
43. Höhn S, Dallérac G, Faure A, Urbach YK, Nguyen HP, Riess O, von Hörsten S, Le Blanc P, Desvignes N, El Massioui N, Brown BL, Doyère V; Behavioral and in vivo electrophysiological evidence for presymptomatic alteration of prefrontostriatal processing in the transgenic rat model for huntington disease. *J Neurosci.* 2011;31(24):8986-8997.

44. Hacker TA, McKiernan SH, Douglas PS, Wanagat J, Aiken JM; ge-related changes in cardiac structure and function in Fischer 344× Brown Norway hybrid rats. *Am. J. Physiol*, 2006;290:H304-11.
45. Dibb KM, Rueckschloss U, Eisner DA, Isenberg G, Trafford AW; Mechanisms underlying enhanced cardiac excitation contraction coupling observed in the senescent sheep myocardium. *J Mol. Cell. Cardiol.* 2004;37:1171-81.
46. Fares E, Howlett SE; Proceedings of the Australian Physiological Society. 2009;40:1-9.
47. Frith J, Jones D, Newton JL; Chronic liver disease in an ageing population. *Age Ageing.* 2009;38(1):11-8.