Aging Associated Oxidative Burden in Rat Kidney; Protective Potential of Flax Seeds

[1]Mahfouz Naseer, [2]Anjali Singh, [3]Tarun Gupta

[1,2]Nims institute of advance science and technology, NIMS University, Jaipur (INDIA)-300121
[2]Department of Biotechnology, Mohanlal Sukhadia University, Udaipur, INDIA-313001

***Abstract*: Aging is an extremely complex and multifactorial process that proceeds to the gradual deterioration in functions.. Kidneys are known to be affected by the aging progress. Oxidative stress develops from an imbalance between free radical production often increased through dysfunctional mitochondria formed with increasing age, and reduced anti-oxidant defences. Flax seed is decreasing oxidative stress and so it may prevent kidney diseases. The twenty male albino rats were randomly divided into four groups (n=5) as control old; control experimental; young control; young experimental. Control young and old rats were treated with normal diet. Experimental young and old were treated with flaxseed. In our result significant increase were observed in experimental young & old rats treated with flax seed. Protein and antioxidant enzyme in namely Superoxide dismutase, Catalase shows significant increase in experimental young & old rats.The lipid per oxidation significant decrease in experimental young & old rats. We conclude that anti-aging property of flaxseed helps the albino rat to protect it from free radical damage and it involved the cellular advantage by lipid, protein, SOD and catalase.**

***Keywords*: Aging, Flaxseed, Kidney, Oxidative stress.**

INTRODUCTION

Ageing is a biological phenomenon concerning all living multicellular organisms and it is thought to be degenerative processes that undergo modifications to a different extent as a function of age; noteworthy modifications are observed in renal, respiratory and cardiovascular. The renal function declines with ageing. Degenerative processes caused by accumulated damage that leads to cellular dysfunction, tissue failure, and death. It is now generally accepted that aging and eventual death of multicellular organisms is to a large extent related to macromolecular damage by mitochondrial produced reactive oxygen species, mostly affecting long-lived post mitotic cells. (Piotrowska and Bartnik, 2014).

Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. However, oxidative stress also has a useful role in physiologic adaptation and in the regulation of intracellular signal transduction. Therefore, a more useful definition of oxidative stress may be "a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them .Oxidative damage of biomolecules increases with age and is postulated to be a major causal factor of cellular biochemical senescence.

The cellular-degradation mechanisms to remove damaged structures completely results in the progressive accumulation of garbage, including cytosolic protein aggregates, defective mitochondria, and intralysosomali digestible material (Terman et al., 2010). The mitochondrial free radical theory of aging has taken centre stage for several decades. According to this theory, ROS are considered to be unwanted toxic by-products of aerobic metabolism that induce oxidative damage to various cellular macromolecules due to their high chemical reactivity (Harman Et al.,1956).These free radicals are mainly produced by the mitochondrial respiratory chain as a result of electron transport and the reduction of the oxygen molecule. Toxic effects of ROS on cellular components lead to accumulation of oxidative damage which causes cellular dysfunction with age. The free radical theory has been one of the most popular theories of aging for many years. Aging is associated with increased Oxidative stress. Most of age-dependent changes in the kidney such as excessive fibrosis, a general lack of regenerative ability, and an increase in apoptosis in cells that determine healthy renal functions are often related to excess oxidative Stress (Hall and Unwin, 2007).

Oxidative stress is prevalent in kidney disease patients and is considered to be an important pathogenic mechanism. Oxidative stress develops from an imbalance between free radical production often increased through dysfunctional mitochondria formed with increasing age, and reduced anti-oxidant defences. Perturbations in cellular oxidant handling influence downstream cellular signalling and, in the kidney, promote renal cell apoptosis and senescence, decreased regenerative ability of cells, and fibrosis. These factors have a stochastic deleterious effect on kidney function. Antioxidants are important to protect against many chronic diseases related to oxidative damage (Rubiolo et al., 2008).

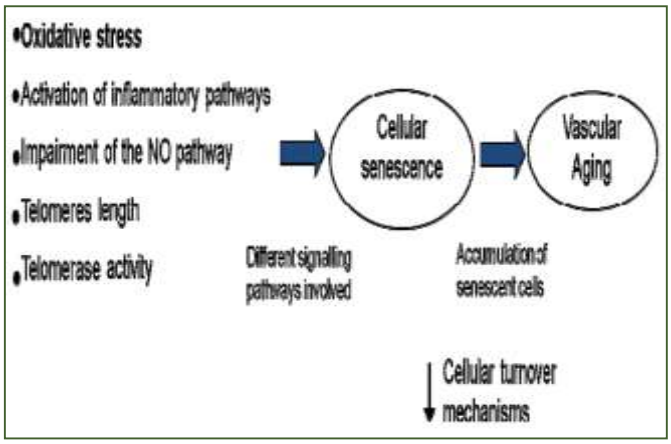


Fig 1. Oxidative stress and aging

Flaxseed is a wonderful, anti-aging superstar food. Flaxseeds are tiny seeds of Linum sitatissimum, which has been widely used for thousands of years as a source of food and clothing. Flaxseeds have become very popular recently, because they are a rich source of the Omega 3 essential fatty acid Alpha Linolenic Acid (ALA) and lignans. The essential fatty acids in flaxseed are largely responsible for its skin-healing powers. Dry skin, acne, rosaceous, eczema, psoriasis, skin cancers etc (Verma, 2011**).** Flaxseed oil (Omega nutrition) suppresses oxygen radical production by white blood cells and improves kidney health. Flax seed is decreasing oxidative stress and so it may prevent kidney diseases (Prasad, 2009 and Xu et al., 2012).

Aging is a biological phenomenon concerning all living multicellular organisms and it is thought to be a degenerative process caused by accumulated damage that leads to cellular dysfunction, tissue failure, and death. Free radical-derived reactive oxygen species (ROS) are constantly generated in most living tissue and can potentially damage DNA, proteins and lipids. "Oxidative stress" occurs if ROS reach abnormally high concentrations. Harman was the first to propose that the damaging effects of ROS may play a key role in the mechanism of aging. However, ROS are not only a cause of structural damage, but also physiologically important mediators in biological signalling processes (Droge, 2003).

Oxidative stress is defined as the imbalance between biochemical processes leading to production of reactive oxygen species (ROS) (Song et al., 2014). The oxidative stress theory of aging states that declines in organism function, that characterize the aging process, result from a progressive accrual of oxidative damage to cellular constituents. Tissues that become subject to oxidative stress witness steady state levels of ROS mediated damage to all bio macromolecules (polynucleotides, proteins, lipids, and sugars) that can lead to a critical failure of biological functions and ultimately cell death (Garofalo et al.,2013).

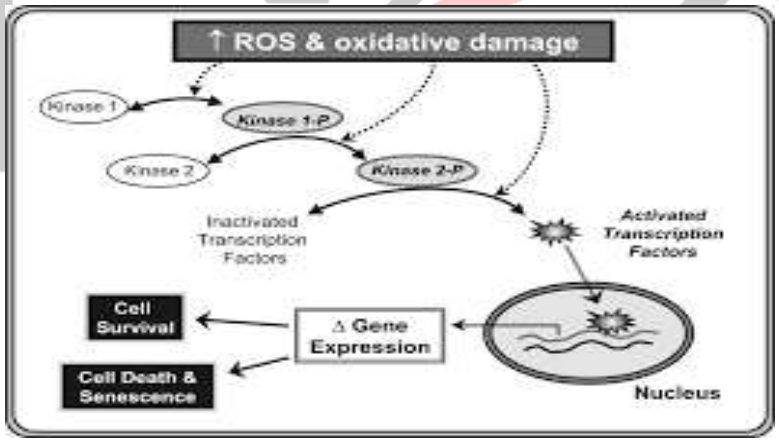


Figure 2. Oxidative stress and ROS

AGING AND OXIDATIVE STRESS MECHANISM

Aging is considered as a biological process characterized by a progressive deterioration in physiological functions and metabolic processes that drive to morbidity and mortality. In agreement with the free radical theory of aging, reactive oxygen species (ROS), generated as byproducts of biological oxidations, induce casual and cumulative oxidative damage to macromolecules inducing to cellular dysfunction with age and eventually cell death [30]. Mitochondria seem to be closely involved in the aging process because these organelles are considered the main intracellular source of superoxide anion (O2 −), as well as the major target of free radical attack. ROS produced by the mitochondrial respiratory chain damage mitochondrial constituents, including proteins, lipids, and mitochondrial DNA (mtDNA). Progressive accumulation of oxidant-induced somatic mutations in mtDNA during an individual's lifetime leads to a deterioration in the bioenergetics function of mitochondria and contributes to the aging process. Low levels of

ROS are generated during mitochondrial respiration under physiological conditions. Progressive oxidative damage to mtDNA with age may induce to DNA strand breaks and to the phenomenon of somatic mtDNA mutations.

Accumulation of these mtDNA alterations may lead to impairment of the respiratory chain complexes, leading to a vicious cycle with an increase in mitochondrial ROS production and a subsequent accumulation of more mitochondrial DNA mutations. This chain reaction has been proposed to be involved in the increased oxidative damage during aging that induces to the progressive decline in cellular and tissue function as a result of insufficient supply of energy and/or increased susceptibility to apoptosis. Age related increase in oxidative damage to DNA, lipids, and proteins has been well documented , as well as evidence supporting increased mtDNA deletions and mitochondrial dysfunction with aging (Francesca et al.,).

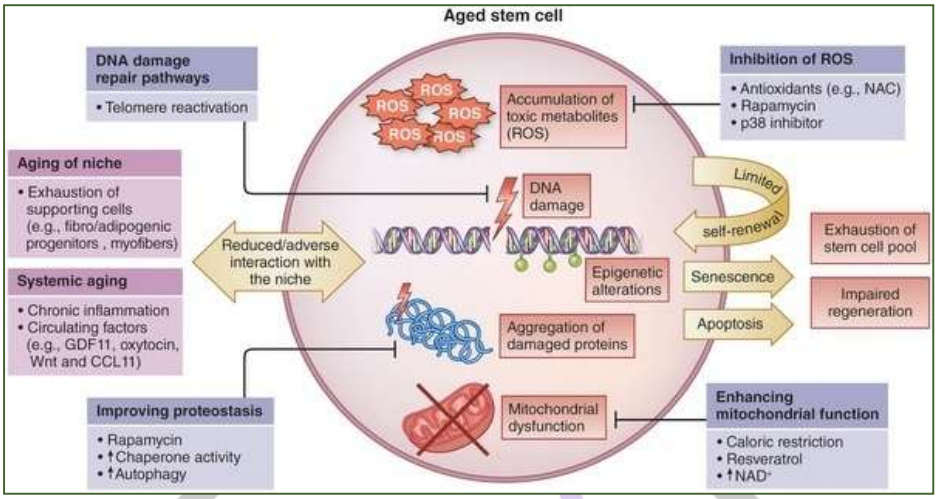


Fig 3.Aged stem cell

THE ROLE OF MITOCHONDRIA IN AGING

Mitochondria regulate a multitude of different metabolic and signaling path ways and also play an important role in programmed cell death. The primary function of mitochondria is to produce ATP through the process of oxidative phosphorylation, which is conducted by the four Respiration Chain complexes (complexes I–IV) and the ATP synthase (complex V), all located in the inner mitochondrial membrane . Mitochondria are unique among the cellular organelles, as they contain their own genetic information, mtDNA, a double-stranded circular molecule of 16.5 kb encoding 13 proteins, 22 transfer RNAs (tRNAs), and 2 ribosomal RNAs in mammals. The 13 mtDNA-encoded proteins are all components of the RC or the ATP synthase, and oxidative phosphorylation collapses in the absence of mtDNA expression(Larsson et al,1998).

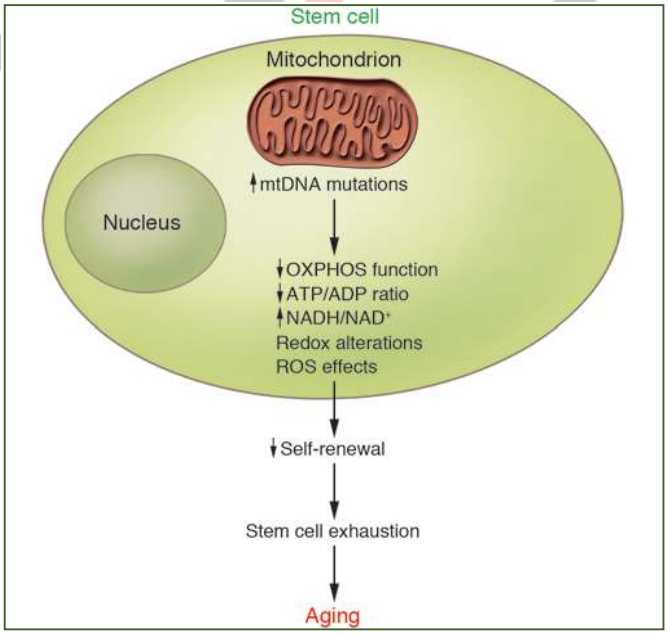


Figure 4. mtDNA mutations in the stem cell hypothesis of aging

Mitochondrial oxidant stress can arise from either extrinsic or intrinsic sources. Extrinsic oxidant stress can arise when non–mitochondrial processes, such as lysosomal degradation of proteins, generate reactive oxygen species (ROS) that enter mitochondria (Surmeier et al., 2011; Kubota, 2009). Intrinsic oxidant stress can arise when ROS are generated by electron leakage from the electron transport chain (Abou–Sleiman et al., 2006). This oxidant stress can be amplified by genetic or pharmacological perturbations that alter the balance between ROS generation and clearance. Mitochondrial Ca^{2+} entry de–represses enzymes of the tricarboxylic acid cycle, increasing the production of reducing equivalents for the electron transport chain and respiration (McCormack et al., 1990). However, the precise mechanism by which mitochondrial Ca^{2+} augments oxidant generation is not fully established.

Mitochondrial dysfunction and free radical-induced oxidative damage have been implicated in the pathogenesis of PD and AD,(Zhu et al.,2004) as well as other neurodegenerative disorders. Usually considered as the chief instigator of oxidative stress damage, the hydroxyl radical reacts non-discriminately with all bio macromolecules at diffusion-controlled rates, i.e., within nm distances from its site of generation. Hydroxyl radical can be produced by gamma radiation, but is most commonly generated physiologically by the Fenton reaction between reduced transition metals (usually iron (II) or copper (I)) and H_2O_2. Re-reduction of the resulting oxidized transition metal ions (iron (III) or copper (II)) can be effected by cellular reluctant such as vitamin C. In contrast to hydroxyl radical, (Hata et al., 2015) superoxide radical is chemically unreactive, except at lower pH, where it exists as the hydroperoxy radical. However, superoxide can serve as the reductant of oxidized metal ions for the production of hydroxyl radical from H_2O_2, the so-called Haber-Weiss reaction.

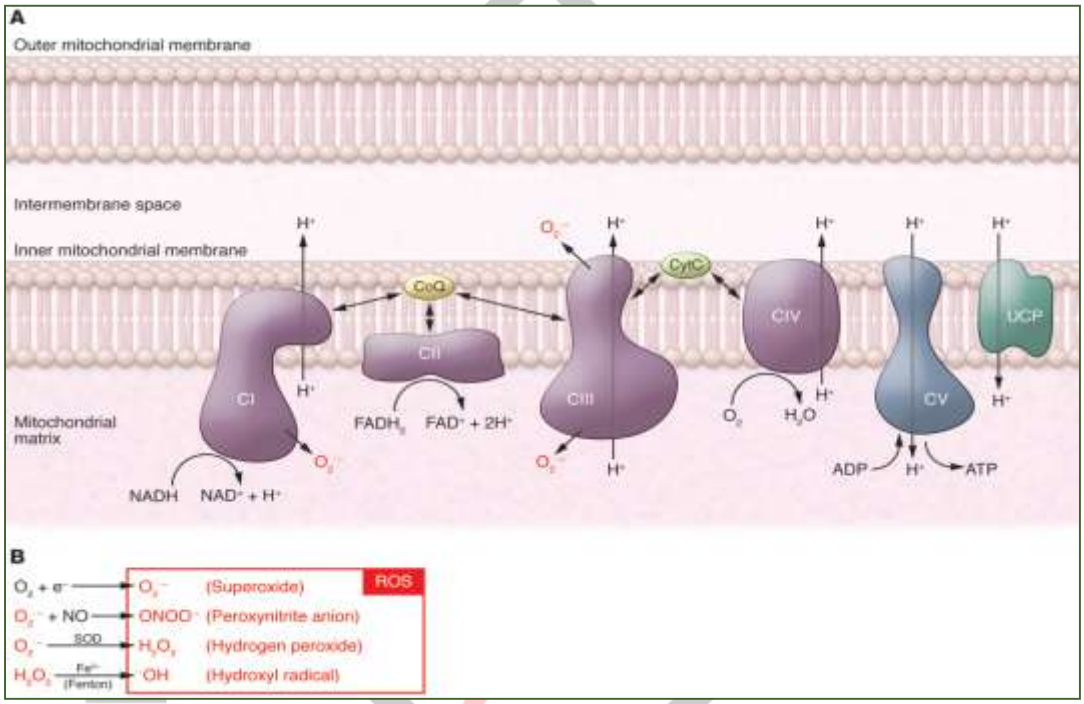


Figure 5. Schematic model of the oxidative phosphorylation system and the production of ROS. Schematic model of the oxidative phosphorylation system and the production of ROS.

AGING ASSOCIATED OXIDATIVE BURDEN IN RAT KIDNEY

OS causes tissue damage by different mechanisms including promoting lipid per oxidation, DNA damage, and protein modification. These processes have been implicated in the pathogenesis of several systemic diseases including kidney.

The kidney is one of the most important organs in the regulation of systemic hemodynamic with higher susceptibility to the development of age dependent tissue damage. It is a highly vulnerable organ to damage caused by ROS, due to the abundance of long-chain-polyunsaturated Fatty acids. Klotho is a recently identified anti-aging gene (Wang and Sun 2009). It encodes a single-pass transmembrane protein with a long extracellular domain and a short cytoplasmic tail. Klotho protein is predominantly expressed in distal convoluted tubules in the kidneys (Kurosu, et al). However, a secreted form of the protein has been described and is found in the blood. Genetic mutation of klotho results in extensive aging phenotypes resembling human aging, including shortened life span, growth retardation, infertility, arteriosclerosis, skin and muscle atrophy, osteoporosis, pulmonary emphysema (Kuroo, et al. 1997), over expression of the klotho gene extends the life span in mice (Kurosu, et al. 2005). Klotho expression in the kidneys has been shown to be reduced in patients with chronic renal failure (Koh et al. 2001) and with acute renal failure in ischemia reperfusion injury murine models (Sugiura et al. 2005). These findings would imply that the reduction of klotho protein may be relevant to the pathophysiology of renal failure. It is not known, however, if klotho expression is altered in kidneys of aged animals, An increase in superoxide production or oxidative stress may contribute to aging (Krause and Nunez et al, .2007).

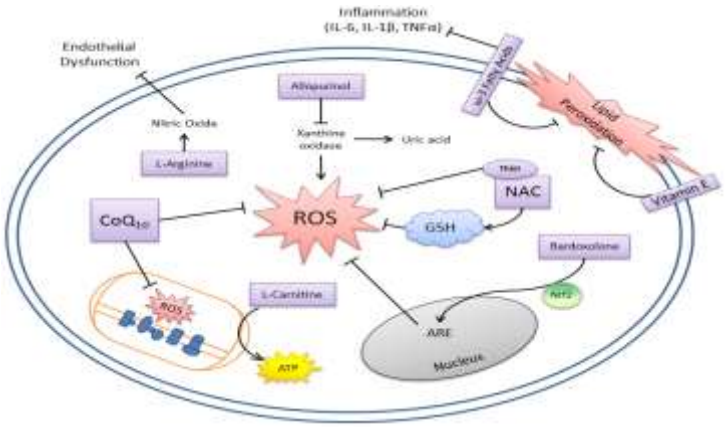


Figure 6.Oxidative Stress and Antioxidant Therapy in Chronic Kidney

PROTECTIVE POTENTIAL OF FLAXSEEDS IN KIDNEY

Flaxseed is a wonderful, anti-aging super star food. Flaxseed is tiny seeds of Linum usitatissimum, which has been widely used for thousands of years as a source of food and clothing. Flaxseeds have become very popular recently, because they are a rich source of the Omega 3 essential fatty acid Alpha Linolenic Acid (ALA) and lignans.

Flaxseed contains 30-40% oil (including 36-50% alpha Linolenic acid, 23-24% Linolenic acid- Omega-6 fatty acids), mucilage (6%), protein (25%), B group vitamins, lecithin, selenium, calcium, foliate, magnesium, zinc, carotene, sulphur, potassium, phosphorous, vitamins A and E and all essential amino acids. three power components of flaxseed—omega-3 fatty acids, lignans, and antioxidants—also have the ability to benefit the health of our skin. Here are some examples:

- Fatty acids keep skin hydrated, moist, and smooth, preventing drying and reducing the look of fine lines, wrinkles, and dullness.

- Antioxidants help protect the skin from free radicals, slowing oxidative damage and protecting from UV rays.

- The anti-inflammatory properties help reduce skin irritation and inflammation, cutting down on redness and rashes. In fact, flaxseed oil is often recommended for the treatment of rosacea, psoriasis, and eczema.

Flax seeds may have a protective role against oxidative stress as demonstrated by decreased lipid per oxidation, both ex-vivo and in-vivo (Nowak, 2013).

Flaxseed oil (Omega nutrition) suppresses oxygen radical production by white blood cells and improves kidney health. Flaxseed is decreasing oxidative stress and so it may prevent kidney diseases (Prasad, 2009 and Xu et al., 2012). Flaxseed oil also produces a protective effect against sodium nitroprusside- induced nephrotoxicity and oxidative damage in male rats (Khan et al. 2012). Flaxseed has demonstrated useful anti-inflammatory and anti oxidative properties. Flaxseed may also inhibit sclerosis and formation of scar tissue. It can play a beneficially therapeutic role in kidney disease, which often involves destructive inflammatory, oxidative and sclerotic processes. Flaxseed rich in lignans retards the development and progression of chronic renal disease.

METHODS:

Animals: Ten old rats (24 months) and ten young (6 months) male albino rats will be taken from NIMS University animal house. The animals will be separately housed in polypropylene cages in a room, which will maintained at a temperature of 22±20C, relative humidity of 50±10 % and 12hrs light dark cycles. They will fed a commercial pellet diet and allow access to water ad labium. The Institutional Animal Ethics Committee have already been approved this study. Each group of animals will be divided in two subgroups namely control and Experimental.

Experimental procedure:

We will purchase the milled flax or we will grind it by our self. After that will prepare an aqueous suspension which will directly introduced into the rat experimental groups and an equivalent volume of control groups for 30 days.

- Total no. of Animals – 20
- Group 1 - CONTROL -Young Rats (6 months)- treated with normal saline(YC)
- Group 11 - EXPERIMENTAL - Young Rats (6 months) – treated with Flaxseeds(YEx)
- Group 111- CONTROL – Aged Rats (24 Months) – treated with normal saline (OC)

- Group 1V - EXPERIMENTAL– Old Rats (24 Months) – treated with Flaxseeds(OEx)

The doses will be given through diet 100mg/ kg/b.w. Thereafter rats will be sacrificed by Aesthetic over dose (40mg/kg b.w. sodium pentobarbital by intraperitonialn) and remove kidney. Kidney will be dissected for the chemical and morphological study.

KIDNEY FUNCTION TEST

Kidney function testing done if we have other conditions that can harm the kidneys, such as diabetes or high blood pressure.

- **Types of Kidney Function Tests**

Urinalysis

A urinalysis screens for the presence of protein and blood in the urine. There are many possible reasons for protein in your urine, not all of which are related to disease. Infection increases urine protein, but so does a heavy physical workout.

When higher-than-normal amounts of protein are in the urine, it is called **proteinuria**. Proteinuria is often a sign of kidney damage and disease. The test does not show what kinds of protein are in the urine. To determine this, your doctor may also order tests such as a serum and urine protein electrophoresis. The test also does not show the cause of the protein.

Protein levels may be higher during the day than the night. Other factors, such as extreme stress, may also influence the test results

Serum Creatinine Test

This blood test examines whether creatinine is building up in your blood. The kidneys usually completely filter creatinine from the blood. A high level of creatinine suggests a kidney problem.

Estimation of creatinine

Creatinine is eliminated by glomerular filtration through the kidneys and excreted in urine without tubular reabsorption. In renal dysfunction, the ability of the kidneys to filter creatinine is diminished leading to a rise in serum creatinine. Therefore, serum creatinine level is used as an indicator of renal function.
Normal value of serum creatinine:

• For adult male: 0.3 – 0.9 mg/dl.

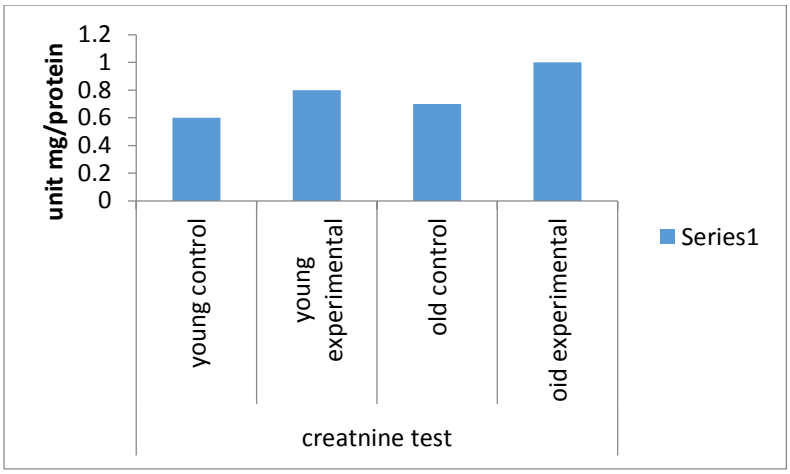
Principle: NaOH Creatinine + picric acid Creatinine picrate (Yellow) (alkaline medium) (Orange) The orange color can be measured colorimetrically, where the intensity of the obtained color is directly proportional to the concentration of creatinine in the sample.

.
CREATININE TEST= RESULTS
Young control= 0.6

Young experimental= 0.8

Old control= 0.7

Old experimental= 1.0



Graph 1.Creatinine test results

URINALYSIS

Biochemical Estimations:

Tissue Homogenate Preparation

The kidney will be removed and weighed individually. Ten percent (w/v) homogenate of the kidney will be prepared using York's homogenizer fitted with Teflon plunger in 0.1 M phosphate buffer (pH 7.1). The whole homogenate was first centrifuged at 2500 x g for 10 minutes in a refrigerated centrifuge. The pellet consisting of mitochondria fraction will be taken for biochemical investigation.

PROTEIN ESTIMATION :

It is the most commonly used method for determination of protein in cell free extracts, because of its high sensitivity and quantities as low as 20µg of protein concentration be measured. The peptide bond in poly peptide chain reacts with copper sulphate in an alkaline medium to give a blue coloured complex. In addition, tyrosine and tryptophan residues phosphor tungstate component of folin indicator reagent to give blue product which contribute towards enhancing the sensitivity of this method.

Reagent:

- **Alkaline Na_2CO_3 reagent-A:** Prepare 2% of Na2Co3 in 0.1 NaOH and makeup the volume up to 100 ml with 0.1N NaOH
- **Copper sulphate reagent-B:** Prepare 0.5% CuSO4 in1% Na-K titrate solution
- **Alkaline copper sulphate solution-C:** Add 50 ml reagent A and 2ml reagent B to prepare reagent C. This mixture is unstable and make freshly.
- **Folin's reagent (1:2):** Folin + TDW (freshly prepared).

LIPID PEROXIDEATION: (Ohkawa et al,. 1979).

Principle:

Acetic acid detached lipid and protein in the reaction mixture is dissolved by the addition of SDS, 2- thiobarbituric acid (TBA) reacts with MDA, form colours additives with absorption mixture at 532 nm.

R**eagents:**

- **S**odium deodylsulphate (SDS) = 8.0% (8 gm/100ml)
- Thiobarbituric acid (TBA)= 0.8% (0.8gm/100ml)
- Trichloro acetic acid (TCA)=10%
- Acetic acid glacial

SUPER OXIDE DISMUTASE: (**Cord & Fridorich., 1969**)

Principle:

NADH (Nicitinamide dinucleotide reduce) in the presence of phenozinemethosulphate (PMS) gives Super Oxide radicals (O_2). These Oxygen free radicals reduce Nitro blue tetrazolium (NBT) and form Farm zone having dark blue colour. When SOD source (biochemical sample) is added to above reaction mixture, these participate in another reaction to neutralize O_2 into H_2O_2 and therefore first reaction (reduction of NBT) showed down and indicatly give a measure of SOD in test samples.

REAGENTS:

- Sodium pyrophosphate buffer - 909 mg/dl in TDW
- Nitro blue tetrazolium (NBT) – 12.86 mg/ 10 ml in buffer
- NADH (2.34 m mole) – 16.59 mg/ 10 ml
- PMS (93 µ mole) – 2.8 mg / 100 TDW

CATALASE :(Aebi, 1974)

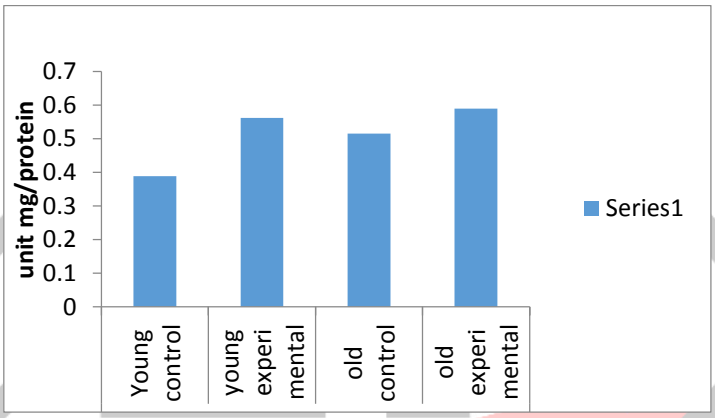
Principle:

In UV range H_2O_2 shows a continual increase in adsorption with decreasing wavelength and maximum at 240 nm. The decomposition of H_2O_2 can be followed directly by the decrease in extinction 240 nm (E = 240 = 40cm^2 µmol). The decrease in extinction 240 nm (E = 240 = 40 cm^2 µ mol). The decrease in extinction (E= 240) per unit time is the measured of the catalase activity.

Reagents:

- H_2O_2 = 0.2M
- Phosphate Buffer = 0.01 M KH_2PO_4 =681 mg /dl + k_2HPO_4 = 1.225 gm /155ml

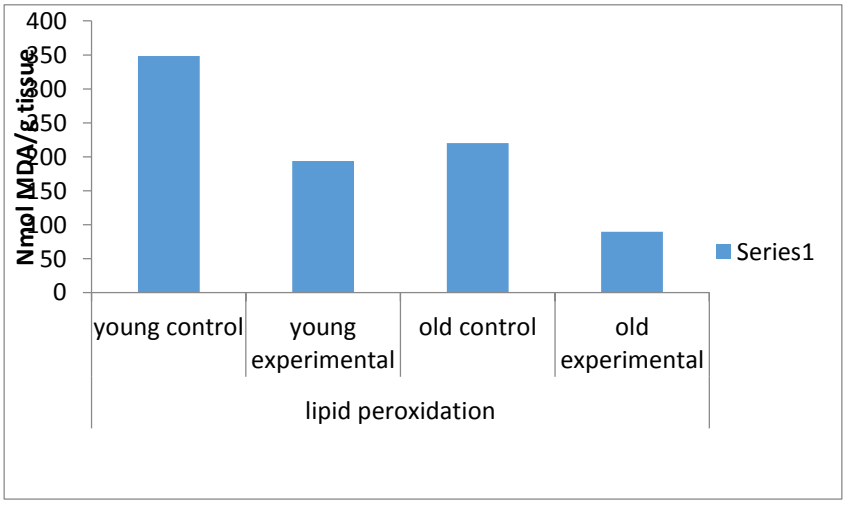
SUPEROXIDE DISMUTASE



Graph 2 .the SOD level was expressed as mean ± SEM of control and experimental groups. Data were analysed using Intat 3.0 software by one way ANOVA followed by Numen Keul posthoc test. The significance of p vale is considered (p>0.05, p<0.01, p<0.001).

The protein content of the kidney was found to be significantly increase by 37% in experimental group (flaxseed treated) as compared to the control group. The maximum recovery was found to the flaxseed treated group compared to control group

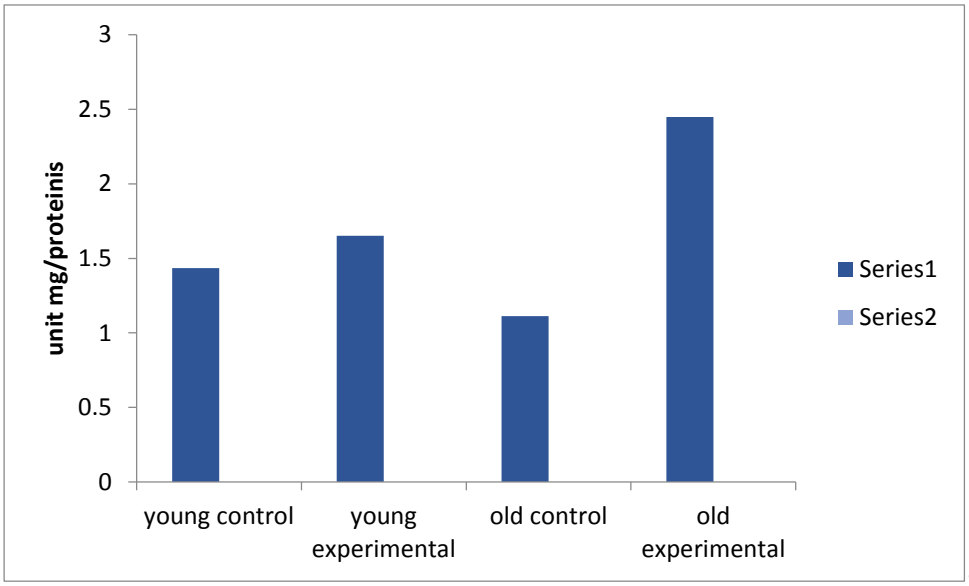
LIPID PEROXIDATION



Graph 3. The SOD level was expressed as mean ± SEM of control and experimental groups. Data were analysed using Intat 3.0 software by one way ANOVA followed by Numen Keul posthoc test. The significance of p vale is considered (p>0.05, p<0.01, p<0.001).

The protein content of the kidney was found to be significantly increase by 37% in experimental group (flaxseed treated) as compared to the control group. The maximum recovery was found to the flaxseed treated group compared to control group.

PROTEIN ESTIMATION



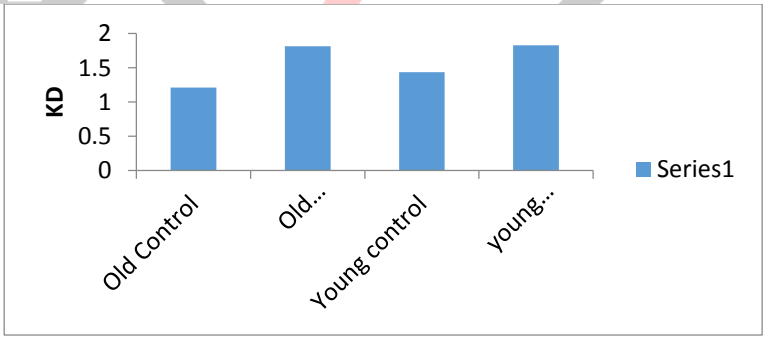
4.

Graph 4. The total protein content was expressed as mean ± SEM of control and experimental groups. Data were analysed using Instat 3.0 software by one way ANOVA followed by Numen Keul's posthoc test. The significance of p value is considered ($p > 0.05$, $p < 0.01$, $p < 0.001$) as in superscript of the data

The Protein content of the kidney was found to be significantly increase by 37% in experimental group as compared to the control group. The maximum recovery was found to the flaxseed treated group compared to control group.

CATALASE

The protein content of the kidney was found to be significantly increase by 27% in experimental group (flaxseed treated) as compared to the control group. The maximum recovery was found to the flaxseed treated group compared to control group



Graph 5. The Catalase level was expressed as mean ± SEM of control and experimental groups. Data were analysed using Intat 3.0 software by one way ANOVA followed by Numen Keul posthoc test. The significance of p vale is considered ($p > 0.05$, $p < 0.01$, $p < 0.001$).

DISCUSSION

In present study, we investigated the potential protective effect of flax seeds against age associated oxidative stress induce cognitive and biochemical disorders in rat liver. Aging induce the over production of the free radicals resulting in oxidative stress. Oxidative stress is either increase in free radical generation or decrease in anti oxidant enzyme or both. At the cellular level, such imbalance can result in structural damage due to oxidative modifications of proteins and lipids. So, we estimated the levels of antioxidant enzyme such as Superoxide dismutase and catalase which regulate the level of free radicals.

In our study, the significant increase in the SOD and catalase activity and decrease in lipid per oxidation level was found when compared to their normal rats. The increase in SOD and catalase activity indicate that flax seed induce the over production of free radicals leads to the oxidation of lipid, protein and other bio molecules.

The present study demonstrated that the supplementation of dietary omega-3 fatty acid to the aged rats significantly increase the activity of anti oxidant enzymes (SOD, catalase). Thus the supplementation of dietary omega-3 fatty acid neutralized When the ROS is excessive, the homeostasis will be disturbed, resulting in oxidative stress, which plays a critical role in liver diseases and other chronic and degenerative disorders.The free radicals, generated during aging. These free radicals increase the oxidative stress. Aging is associated with increased Oxidative stress. Most of age-dependent changes in the kidney such as excessive fibrosis, a general lack of regenerative ability, and an increase in apoptosis in cells that determine healthy renal functions are often related to excess oxidative Stress (Hall and Unwin, 2007). Flaxseeds are essential in fatty acid that could improve the kidney dysfunction observed in the hypertension condition. There is significant decrease in lipid per oxidation in experimental young & old rats. The protein will be observed Significant increase in Experimental young & old rats. Antioxidant enzyme in namely Superoxide dismutase and Catalase shows significant increase in experimental young & old rat.

CONCLUSION

The finding of the present study indicate that the treatment with flax seed could increase the antioxidant profile and reduced the oxidative stress in terms of lipid per oxidation and protein carbonyl content, which due to experimental conditions have a great role in the sparing of produced free radicals. These observations led to the postulation that the rapid sparing processes of the free radicals produced during aging, requires increased utility of flax seed. We conclude that anti-aging property of flaxseed helps the albino rat to protect it from free radical damage and it involved the cellular advantage by lipid, protein, SOD and catalase.

REFRENCES

[1] Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105:121-6.

[2] Abou-Sleiman PM, Muqit MM, Wood NW. Expanding insights of mitochondrial dysfunction in Parkinson's disease. Nat Rev Neurosci. 2006; 7: 207–19

[3] Droge W. Oxidative stress and aging .2003; 543: 191-200.

[4] Garofalo C, Capuano G, Sottile R, Tallerico R, Adami R, Reverchon E, Carbone E, Izzo L, Pappalardo D. Different Insight into Amphiphilic PEG-PLA Copolymers: Influence of Macromolecular Architecture on the Micelle Formation and Cellular Uptake. Bio macromolécules. 2014 ; 15(1):403-15.

[5] Hata AN, Engelman JA, Faber AC. The BCL2 Family: Key Mediators of the Apoptotic Response to Targeted Anticancer Therapeutics .Cancer Discover. 2015; 5(5):475-87.

[6] HARMAN D. Aging: a theory based on free radical and radiation chemistry. J. Gerontol.1965 (3):298-300.

[7] Hall AM, Unwin J. The not so 'mighty chondrion': emergence of renal diseases due to mitochondrial dysfunction. Nephron Physiol. 2007; 105(1):p1-10.

[8] Khan R, Apewokin S, Grazziutti M, Yaccoby S et al,. Renal insufficiency retains adverse prognostic implications despite renal function improvement following Total Therapy for newly diagnosed multiple myeloma. Leukemia. 2015 ; 29(5):1195-1201.

[9] Kurosu H, Yamamoto M, Clark JD et al. Suppression of aging in mice by the hormone Klotho. Science. 2005; 309(5742):1829-33.

[10] Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI. Mutation of the mouse *klotho* gene leads to a syndrome resembling ageing. Nature.1997; 390(6655):45-51.

[11] Koh HS, Chun TY, Yoo HS, Zhang YP, Wang J, Zhang M, Wu CH. Mitochondrial Cytochrome b Gene Sequence Diversity in the Korean Hare, Lepus coreanus Thomas (Mammalia, Lagomorpha). Biochem Genet. 2001; 39(11-12):417-29.

[12] Kubota C, Torii S, Hou N, Saito N, Yoshimoto Y, Imai H, Takeuchi T. Constitutive Reactive Oxygen Species Generation from Autophagosome/Lysosome in Neuronal Oxidative Toxicity. J Biol Chem. 2010; 285(1):667-74.

[13] Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS, Clayton DA. Mitochondrial transcription factor A is necessary for mtDNA maintance and embryogenesis in mice. Nat Genet. 1998; 18(3):231-6.

[14] Mendoza-Núñez VM, Ruiz-Ramos M, Sánchez-Rodríguez MA, Retana-Ugalde R, Muñoz-Sánchez JL.Tohoku J Exp Med. 2007 ; 213(3):261-268.

[15] McCormack JG, Osbaldeston NJ. The use of the Ca2(+)-sensitive intramitochondrial dehydrogenases and entrapped fura-2 to study Sr2+ and Ba2+ transport across the inner membrane of mammalian mitochondria. Eur J Biochem. 1990 ;192(1):239-244.

[16] McCord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocu- prein. J Biol Chem. 1969 ; 244(22):6049-55.

[17] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.Anal Biochem. 1979 ; 95(2):351-8.

[18] Piotrowska A, Bartnik E. The role of reactive oxygen species and mitochondria in aging. Postepy Biochem. 2014; 60(2):240-7.

[19] Rubiolo JA, Mithieux G, Vega FV. Resveratrol protects primary rat hepatocytes against oxidative stress damage: activation of the Nrf2 transcription factor and augmented activities of antioxidant enzymes.Eur J Pharmacol. 2008 ;591(1-3):66-72.
[20] Sugiura M, Koike H, Iijima M, Mori K, Hattori N, Katsuno M, Tanaka F, Sobue G. Clinicopathologic features of nonsystemic vasculitic neuropathy and microscopic polyangiitis-associated neuropathy: a comparative study.J Neurol Sci. 2006; 241(1-2):31-7.
[21] Surmeier DJ, Guzman JN, Sanchez-Padilla J, Schumacker PT. The role of calcium and mitochondrial oxidant stress in the loss of substantia nigra pars compacta dopaminergic neurons in Parkinson's disease. Neuroscience. 2011; 198:221-31.
[22] Wang Y, Sun Z. Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage.Hypertension. 2009; 54(4):810-7.
[23] Zhu Z, Yang C. [Changes of four common plant populations growth and their anti-oxidative enzymatic system in desertification process].Ying Yong Sheng Tai Xue Bao. 2004; 15(12):2261-6.