Biological Sciences

- **Activation of autophagy protects D-galactose induced aging rat brain through mTOR/Akt/ CREB pathway**

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Introduction: Autophagy is catabolic process involved in continuous removal of toxic protein aggregates and cellular organelles to maintain the homeostasis and functional integrity of cells. The mechanistic understanding of autophagy-mediated neuroprotection during aging remains exclusive. Here, we investigated the potential role of rapamycin-induced activation of autophagy and mTOR/Akt/CREB pathway(s) in the neuroprotection of D-galactose-induced aging brain of rats.

Material and Methods: Adult male wistar rats were treated with D-galactose (500 mg/kg b.w., s.c., daily for 45 days) to establish the model of aging. Further, the effect of autophagy inducer-rapamycin (0.5 mg/kg b.w., oral, daily for 30 days) has been studied on D-galactose-induced oxidative stress, synaptic/neurotransmission/cognitive dysfunction.

Results: The administration of D-galactose impaired redox balance, induced synaptic/neurotransmission/ cognitive dysfunctions and suppressed pro-survival signaling in adult rats. Rapamycin administration caused a significant reduction of mTOR phosphorylation at Ser2481 and a significant increase in autophagy markers such as microtubule-associated protein-1 light chain-3 (LC3), Beclin-1, sequestosome-1/p62, unc-51-like kinase 1 (ULK1). In addition, rapamycin-induced activation of autophagy further activated p-Akt (Ser473), p-CREB (Ser183) expression in D-galactose treated rats. The activated autophagy markedly reversed D-galactose-induced impaired redox homeostasis. The activated autophagy also provided significant neuroprotection against D-galactose-induced synaptic dysfunction by increasing the expression of synapsin-I, synaptophysin and PSD95, neurotransmission dysfunction by increasing the levels of CHRM2, DAD2 receptor, NMDA receptor and AMPA receptor, and ultimately improved cognitive ability in rats.

Conclusion: Our study demonstrate that autophagy can be an integrated part of pro-survival (mTOR/Akt/ CREB) signaling that restores the oxidative defense mechanism(s) and maintains the integrity of synapse and neurotransmission in D-galactose induced rat model of aging.

- **Intra-generational PMN induces adolescent hyperactivity, impulsivity and impaired spatial learning suggesting accelerated ageing changes in rats**

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Introduction Early life protein malnutrition (PMN) is a major concern resulting in the structural and functional changes in the brain with increased predisposition to neuropathies during later life. This study was aimed to emulate the clinical conditions of protein malnourishment and investigate the resultant physical, behavioural and cognitive abilities along age advancement in F1 progeny.

Material and methods: A battery of neuro developmental reflex tests, locomotor [(open field, rotarod) and cognitive (elevated plus maze, morris water maze (MWM)] assays were performed during pre-weaning period and at 2, 3, 6, 12, 18 and 24 months of age.

Results: A decelerated growth curve with significantly restricted body and brain weight was noticed in LP F1, along with delayed apparition and poor performance in cliff avoidance and negative geotaxis reflexes supporting decreased risk assessment. Adolescent hyperactivity with loss of habituation in novel environment, increased exploration and low basal anxiety in open field was noticed with advancing age. LP F1 animals displayed increased impulsivity, aggressiveness with reduced forelimb grip strength mimicking

schizophrenia symptoms. Swim track analysis in MWM test revealed poor learning, impaired memory retention and integration, thus modelling signs of early onset dementia and accelerated ageing in LP animals.

Conclusions: This study concludes that early life PMN induces schizophrenia like repetitive and impulsive phenotype at one end and early onset dementia with accelerated ageing on other.

- **Novel, Noninvasive Biomarker for Parkinson's disease**

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Introduction - Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1% of all people over 60 years of age and is second most prevalent geriatric neurodegenerative disorder. In mid brain part of PD patient, the α-synuclein protein becomes misfolded and aberrant accumulation of amyloid occurs. Molecular chaperones are responsible for maintaining normal protein homeostasis within the cell by assisting protein folding, inhibiting protein aggregation, and modulating protein degradation pathway. Hsp70 (mortalin) can reduce the amount of misfolded, aggregated α-synuclein species in vivo and in vitro and protect it from synuclein-dependent toxicity.

Material and Methods – This study evaluates the expression level of Mortalin and α-syn in the serum of PD and compare it with healthy group. Blood sample were collected from 60 PD and 50 healthy elderly subjects. The concentration of Mortalin and α-syn in serum was measured by surface plasmon resonance technology.

Results – The significant higher expression of α-syn level was observed in PD (95% CI, 57.03–65.05) compare to control (95%CI, 57.64–56.83). However, there was no significant difference in the level of mortalin was observed in between PD (95% CI, 3.3– 4.01) and control group (95%CI, 3.49 – 4.14). This study confirms higher level of α-syn in PD blood. The cut off value, sensitivity and specificity obtained from ROC analysis revealed the accuracy to differentiate PD from control in respect to α-syn level.

Conclusion- α-syn level can be used as a blood based noninvasive biomarker and can have therapeutic interventions.

- **Variation in protein content in silken webs produced by young and old cellar spiders**

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Introduction: Spider's silk is one of the most versatile biomaterials ever known. It is a protein fibre spun by spiders. Owing to its remarkable properties, spider's silk is considered as a super fibre. There is a visible difference in the constituents and properties of silk proteins in the spider webs spun by young and old spiders. Aging is a universal and progressive phenomenon that affects all organisms with an amazing diversity in life spans and aging phenotypes. We have investigated the amount and quality of silk protein produced by spiders as a function of age.

Materials and methods: Spider web samples were collected from young and old spiders of the same spider species of family pholcidae. The web samples were checked for solubility in various solvents. Finally, it was dissolved in a constant boiling mixture of 6N HCl and 50% propionic acid (50:50 v/v). Protein content was estimated by Lowry's method and compared for the spider webs of the same spider species but at different ages (young and old).

Result: The protein content of the silk produced by young spiders was significantly higher than that produced by old members of the species**.**

Conclusion: Spider senescence, owing to the effect of lower intake of food or natural decline in metabolic processes, results in a visible difference in the silk property of webs. Studies are underway to analyze the amino acid content of silk obtained from young and old spiders.

- **Poly I:C induced developmental neuroinflammation impairs cognitive aspects at later life**

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Introduction Perinatal infections during critical periods of development may enhance the risk of a cluster of neurological and psychiatric disorders. Viral infections associated neuroinflammation is susceptible of debilitating behavioural & cognitive changes during adulthood and senility.

Materials and Methods Viral infection was established by injecting Poly I:C, a viral mimetic at a dose of 5 mg/kg body wt., intraperitonealy on postnatal day 7. Astrocytic changes were studied in hippocampus using specific signature markers, GFAP and S100ß on postnatal days 12, 15, 21, 30, 42, 84 and 180. Behavioural and cognitive studies were carried out at the age of 1, 3, 6, 12, 18 and 24 month, using optovarimex, elevated plus maze and Morris water maze.

Results : Results indicate the Poly I:C induced GFAP and S100ß upregulation in various hippocampal subfields indicating astrogliosis. GFAP+cells with hypertrophied cell body and thick ramified processes dominated the hippocampal cortex throughout the study. Western blot analysis also confirmed the GFAP upregulation. Co-localization of GFAP with Caspase-3 indicated massive astrocytic death. In addition, the neonatally challenged rats also presented hyperactivity, anxiety and impairment in learning and memory at early adolescence, adulthood and senility as compared to their age matched controls.

Conclusions: The results thus suggested that the prolonged and persistent activation of astrocytes during development and their caspase mediate continued cell death may lead to behavioural and cognitive impairment at later life.

- **Unbiased stereological estimates of the spiral ganglion neurons in the ageing human cochleae**

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Introduction- Constant exposure to occupational and environmental noise makes the cochlea susceptible to ageing changes. Age related hearing loss (presbycusis) is the most prevalent neurodegenerative condition of our aged population. Spiral ganglion neurons (SGNs) in the cochlea are the first order neuron of the auditory pathway and hence are vulnerable to noise induced insults leading to decreased ability to fight various chemical and environmental stresses that is part of the aging process. In this study we have quantified the SGNs in young and old adults using unbiased stereology to ascertain any changes with age.

Material & Methods- Twelve human cadaveric heads from young (21-30, n=6) and old (61-70, n=6) adults were obtained from the mortuary at All India Institute of Medical Sciences, New Delhi, after obtaining approval from institute ethics committee. Temporal bones were fixed, dissected, decalcified, cryoprotected and serially sectioned (30µm). Every 7th section was stained with cresyl violet. The total number of SGNs (Optical Fractionator), and SG volume (Cavalieri) were estimated with the StereoInvestigator software (Microbrightfield Inc. VT, USA).

Results - The mean volume of the SG was 2.19±0.7 and 1.77±0.40 mm^3 (p=0.2); and the mean number of neurons was 26296 ± 649.96 and 12532.83 ± 3175.82 ($p<0.001$) in young adult and old adults, respectively.

Conclusion - The number of SGNs decreases significantly with age, even though the total volume of the SG does not change. This may be a major cause of neural presbycusis.

- **Obesity-induced variations in total serum cholesterol concentrations of older adults at potential risk of cardiovascular diseases**

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Introduction: Pathophysiologic disturbances symptomatic of age-associated dysfunctions believably arise in late age marred with obesity. Majority of older adults with general preference for high calorie diet and sedentary life style surviving with stress of variable intensities in day-to-day life appear to be at high risk of metabolic disorders including cardiovascular complications irrespective of rural-urban divide and gender discrimination.

Materials and Methods: In the present study, haematologic values of Total Serum Cholesterol concentrations estimated quantitatively among men and women in the age range of 50 to 86 years (n=88) residing in Darbhanga, a commissionary town of Bihar (India) and its suburbs were analyzed in respect of their BMI-dependent obesity patterns.

Results: Higher mean concentration of chosen blood serum constituent was noticed in overweight/ obese group in contrast to normal weight group in both the sexes.

Conclusions: Hypercholesterolemia observed in overweight/obese older adults could be attributed to devastating obesity, in all probabilities, thought to be liable for exposing vulnerable older adults to age-related diseases including cardiovascular co-morbidities possibly with little impact of rural-

urban divide and gender differences in the given backdrop of observed tendency of non-adherence to controlled diet, regular exercise and zero stress in the ageing population, seemingly less caring to personal health.

- **Efficacy of quercetin as an anti-aging drug candidate**

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Introduction: Oxidative stress plays a central role in the process of biological aging and age associated diseases like Alzheimer, atherosclerosis, diabetes etc. The accumulated ROS and RNS lead to oxidative modification of biomolecules such as lipids, proteins and nucleic acids. Polyphenols such as quercetin, resveratrol, fisetin are ubiquitously distributed group of secondary metabolites found in plants which have antioxidant property. Quercetin is one of the most potent antioxidant against ROS, RNS and oxidative stress. The present study was undertaken to determine the effect of quercetin in young and old Wistar male rat.

Material & Methods: In present study, young (age 4 months) and old (age 24 months) rats were supplemented with quercetin (50 mg/kg b.w.) for 4 weeks. Biomarkers of oxidative stress in terms of Ferric reducing ability of plasma (FRAP), intracellular reduced glutathione (GSH), Lipid peroxidation (MDA), Plasma membrane redox system (PMRS), Advanced oxidation of protein products (AOPP) and osmotic fragility in erythrocytes and plasma were measured in control and experimental groups.

Results: After 4 weeks of supplementation, FRAP, GSH and PMRS activities were increased significantly ($p<0.05$) in both age groups compared to control. On the other hand, treated groups exhibited significant ($p<0.05$) reductions in MDA, AOPP, PCO and osmotic fragility level.

Conclusion: Our data provides evidence that quercetin administration restores antioxidant status and improves healthy aging. Long term interventions studies are being conducted in the lab.

- **Anti aging strategy based on dietary supplementation of whey protein to young and old Wistar rats**

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Introduction: Studies have shown that aging is associated with decreased cellular uptake of L-cysteine, an amino acid essential for glutathione biosynthesis. Whey protein, a liquid aspect of milk is an effective cysteine delivery system. The present study evaluates the antioxidant efficacy of dietary supplementation of whey protein in rats of different ages.

Material & Methods: In present study, young (age 4 months) and old (age 24 months) male Wistar rats were supplemented with whey protein concentrate (WPC) (300mg/kg b.w.) for 4 weeks. Biomarkers of oxidative stress: antioxidant capacity in terms of ferric reducing antioxidant potential (FRAP), lipid peroxidation (MDA), reduced glutathione (GSH), plasma membrane redox system (PMRS) and advanced oxidation protein products (AOPP) were measured in control and experimental groups.

Results: After 4 weeks of whey supplementation, FRAP and PMRS activities were significantly ($p< 0.01$) increased in both age groups compared to control. GSH increased significantly ($p< 0.01$) in old age group. MDA and AOPP decreased significantly ($p< 0.01$) in both age groups.

Conclusion: Whey protein supplementation offers a good strategy to counteract age dependent changes in redox status. The study also opens an area of anti aging research.

- **Downregulation of Glutamic Acid Decarboxylase (GAD1) Gene in Human Inferior Colliculus with ageing**

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Introduction: The inferior colliculus (IC) is an important nucleus of auditory pathway. GABA is the inhibitory neurotransmitter in IC, critically involved in temporal coding and spatial processing in IC. It is synthesised by glutamic acid decarboxylase (GAD), that is in turn a product of the GAD1 gene. Here we have studied the expression of GAD1in the human IC of different age groups.

Material & Methods: After obtaining ethical clearance, brainstems, containing the IC (n=27), were obtained and divided into three groups- young age (11-30 years), middle age (31-50 years) and old

age (>51 years). Primers for the genes GAD1, X-prolylaminopeptidase (XPNPEP1) and alanyl-tRNA synthetase (AARS) were designed; the gene of interest being GAD1 and the others as reference genes. After extraction of RNA, cDNA was synthesized (cDNA synthesis kit, Bio-Rad). Real time PCR using SYBR green was performed in triplicate. The relative expression was calculated by the ΔΔCt method. The relative expression was analysed using Kruskal-Wallis test followed by Dunn's pairwise comparison using SPSS software.

Results: With the young age group kept as normalising control, the relative expression in the middle group was 0.82 and in the old age group was 0.56. Expression of GAD1 in the older age group was significantly lower than that in the young age group (p=0.016).

Conclusions: The expression of the GAD1 and consequently GABA decreases with age and this may be one of the causes of increased neurotoxicity seen in the pathogenesis of presbycusis.

- **Role of Stem Cells in Geriatrics**

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Ageing is a function of accumulation of subtle, irreversible cellular and molecular changes over time, leading to progressive decline in the regenerative and homeostatic potential that results into frailty, malfunctioning and eventual death. Such degenerative changes have been attributed to many factors, and one important among them is age-dependent changes and decline in the tissue/organ-specific stem cells, niches and microenvironments surrounding them along with the functional molecular cues that regulate their existence and activity. Repertoire of stem cells present in various organs steadily declines during ageing that is accompanied by global metabolic deterioration resulting from patho-physiological conditions and ageing. Stem cells serve as the cellular backup mechanism, continuously renewing themselves along with supplying cells required for renewal of tissues/organs so as to keep them intact, integrated and functional throughout life span of an individual. However, with the passage of time, stem cells start showing signs of replicative senescence and metabolic slowdown, thereby compromising on their self-renewal and proliferation, cutting down the supply of new cells/secretory molecules and growth factors to the organs. These progressive changes deprive the organ-specific niches of functional growth and molecules for renewal needed for functioning of the organs. The steady loss in the regenerative potential during ageing is primarily attributed to telomere shortening/attrition, decrease in the ratio of DNA repair to DNA damage, low level of ATP production owing to mitochondrial DNA damage, accumulation of damaged proteins, dysfunctional organelles and dysregulated gene expression etc. Thus correlation between decline in stem cell potential and ageing has been quite significant, and hence important to look at the multidimensional ageing process from the stem cells perspective, which could be helpful in future for improving the quality of life, and may extend it by many more years of quality life. The preventive approach in this direction could be maintaining the stem cell potential and the therapeutic approach could be agents restoring the stem cell potential. Further research in this direction could add health to old age.

- **Impact of embryonic deltamethrin exposure on the development of iron induced post traumatic epilepsy at adulthood in rat**

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Introduction: As a result of increasing evidence of post traumatic epilepsy and exposure of environmental stressors becoming a leading cause of neuropsychiatric disorders. Most studies have addressed these disorders either, in case of post traumatic epilepsy or in response to environmental stressors. Thus, the present work is an attempt to study the marked association between early gestational deltamethrin exposure and subsequent epileptogenesis in iron-induced model of posttraumatic epilepsy followed by antileptic effect of curcumin.

Material and Methods: Timed pregnant Wistar rats were exposed to deltamethrin (0.75mg/kg b.wt. intraperitoneally) from gestation day (GD) 7-15. At adulthood, these animals were injected with iron stereotaxically (5 µl, 100 mM dissolved in saline) to generate iron induced epilepsy. After the induction of epilepsy, electrophysiological recordings such as electroence-phalography and multiple unit activity in the hippocampus and cortex were recorded. Spatial learning and memory testing, dendritic arborisation, dendritic length and spine density were quantified.

Results: Deltamethrin exposed iron induced epileptic rats presented an increase in the epileptic firing along with MUA count both in the cortex and hippocampus, decreased dendritic arborisation, spine count and learning ability as compared to their age matched normal epileptic and saline treated rats. In addition, poor antileptic potency of curcumin treatment was found in the deltamethrin exposed iron induced epileptic rats.

Conclusion: Thus, it is proposed that the severity of epileptogensis increased in the deltamethrin exposed rats in terms of reduced dendritic arborisation and spine density, which further leads to deficits in learning and memory.

- **Studies on 2-Deoxyglucose as an Caloric restriction mimetic in Rat model**

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Introduction The most reliable strategy in attenuation of aging is caloric restriction. It refers to the reduction in caloric intake of malnutrition. CR extends life span in a remarkable range of organisms. However, despite the "magic" of CR, this intervention in humans may present itself with a number of health concerns, which may not impact the life of experimental animals. An alternative strategy is based on molecules that "mimic" biological effects of CR without significantly reduced food: caloric restriction mimetic (CRM). One such strategy to produce CR-like effects is by inhibiting glycolysis. The major candidate inhibitor is a glucose analogue 2-deoxy-D-glucose (2-DG).

Material & Methods: Male Wistar Rats were supplemented with a dose of 25 mg/kg 2-DG for 2 months. Oxidative stress markers such as total antioxidant potential (FRAP), lipid peroxidation (LPO), reduced glutathione (GSH), plasma membrane redox system (PMRS), Protein carbonyl (PCO), advanced glycation end products (AGE) and reactive oxygen species (ROS) were investigated.

Results: There was significant reduction in LPO, PCO, AGE and ROS in 2-DG supplemented rats compared to control. FRAP, GSH and PMRS were significantly ($p < 0.05$) enhanced in 2-DG treated group as compared to control.

Conclusions: Our data indicate that 2-DG could mimic the effects of CR by attenuating oxidative stress, thereby reducing frailty and improving health in rat model.

- **Acetylated histones H3K9/14ac and H4K12ac decrease in old mice and are upregulated upon dietary restriction**

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Introduction: Chromatin is a highly dynamic structure, and responds to the external milieu and regulates the uses of the DNA. One major way by which the chromatin is remodelled is through the modification of histones. In this present study, we investigated the change in the acetylated histone H3 at lysine 9/14 (H3K9/14ac) and histone H4 at lysine 12 (H4K12ac) in the liver and kidney tissues of BALB/c mice as a function of age, and if these changes could be modulated by dietary restriction. Results show that H3K9/14ac and H4K12ac decreased with age and both the proteins showed a similar trend in both the tissues studied. Upon dietary restriction (DR), the levels of the proteins are upregulated.

Materials and Methods: *Animals:* Young (2-), adult (9-) and old (24-month old) male albino (BALB/c) mice kept in a well ventilated room (12:12 hr light/dark cycle); fed with standard pellet (22.43% protein, 48% carbohydrate, and 4.22% fat) and free access to water.

Dietary restriction regimen: Adult and old mice used for DR studies. Mice divided into two groups; *ad libitum* fed group (AL) had free access to food and water, and DR mice received feed on alternate days for a period of three months.

Tissue preparation: Mice sacrificed at a fixed time of day (11:00 h) by cervical dislocation. Liver and kidney tissue excised out immediately, washed in chilled normal saline (0.9%). 10% (w/v) homogenate prepared in ice-cold lysis buffer [10mMTris-HCl, pH 7.5, 0.25M sucrose, 0.5% triton X]; centrifuged at 2,000x g for 10 min at 2°C. Nuclear pellet used for histone extraction.

Western Blot analysis : Proteins subjected to 16% SDS. Blots incubated overnight at room temperature with primary antibodies diluted in 5% (w/v) skimmedmilk. H3 protein was used as a loading control. Densitometry analysis performed using Kodak Digital Science 1D Image Analysis Software, Version 3.0.

Results: In both the tissues studied the level of both H3K9/14ac and H4K12ac were reduced in the old (24-month) mice compared to the adult (9-month) mice. However, upon DR the protein level is increased significantly.

Conclusion: In conclusion, the regulation of H3K9/14ac and H4K12ac protein level is age dependent and there is an overall decline in the acetylation status at these particular sites of histones of old mice. The decreased acetylation can influence gene silencing, and by weakening the DNA-histone interaction also alter the global chromatin structure of the aged mice. DRreverts the histone modification changes observed in old mice and by increasing the acetylation may restore the chromatin assembly.

- **Alteration in redox balance in a rat model of Hutchinson Gilford Progeria Syndrome**

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Introduction: Hutchinson Gilford Progeria Syndrome (HGPS) is a genetic disorder with manifestations of premature aging phenotype in children leading to death, most often due to myocardial infarction at 13-14 of years of age on average. Dihydrotachysterol (DHT), is a vitamin D analog used in the treatment of hypocalcaemia associated with hypoparathyroidism in human. Chronic administration of DHT in rats induce premature aging and progeria like syndrome. The present study is aimed to assess the alteration in redox balance during dihydrotachysterol induced premature aging syndrome in rats and their relevance in relation to normal aging.

Materials and methods: Young Wistar rats were treated with Dihydrotachysterol (DHT), (50 µg in corn oil for thirty days) to induce experimental progeria. Studies on biomarkers of oxidative stress including direct assessment of intarcellular ROS in erythrocytes was performed and compared with young, middle aged and old control rats.

Result: Experimental progeria like syndrome was found to be associated with significantly higher levels of intracellular ROS, MDA, AOPP, and PMRS whereas GSH, and FRAP activity was significantly decreased when compared to young treated rats but these values were comparable with middle and old age rats.

Conclusion: The study concludes that accelerated aging accompanied during progeria like syndrome in rats is associated with redox imbalance in erythrocytes and blood plasma.

- **The effect of hormones supplementation with DHEA reverses immune senescence in post-traumatic epilepsy rat model.**

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Introduction: Cytokines are considered to play an important role in seizures: cytokines are also known to be involved in other neurological disorders. Pro inflammatory cytokines like IL-1, IL-6 and TNFα as well as inflammatory cytokines IL-10 are described in CNS and plasma of experimental models of seizures and in clinical case of epilepsy. In the present study we have seen the effect of DHEA treatment on post-traumatic epilepsy for various durations.

Materials and Methods: Male Wistar rats of age group 8-10 months were used for the study. Animals were made epileptic by the intra-cortical injection of $FeCl_3$.Treatment of DHEA (30mg/kg b.w./day) was given for 7, 14 and 21 days. After the completion of the experiment rats were sacrificed and the tissue was harvested for ELISA and Immunohistology.

Results: The protein expression of IL-1, IL-6 and TNF-α was increased in epileptic sample and decreased in DHEA treated samples. The levels of IL-10 were found to decrease in epileptic samples and were increased in DHEA treated samples. It was further confirmed with immunohistology.

Conclusion: Present study confirms that the hormones supplementation with DHEA would synergize reverse immune senescence.

- **Effect of ageing on skeletal muscle and muscle stem cells in correlation with calorie restriction: A morphometric analysis.**

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Introduction: Skeletal muscles have remarkable importance in human health. There are total of 640 skeletal muscles in the human body, of which ~38% accounts for total body mass for men and 30% for women. Ageing process leads to a progressive decline in muscle mass and strength. It is well known fact that more than 15% decline occurs in muscle strength per decade after attaining

50 years of age. Thus giving rise to fatal condition like frailty and sarcopenia. Presence of muscle stem cells (also known as satellite cells) opens the avenue for researches to explore the phenomenon of regeneration of skeletal muscle in aged population. Moreover, calorie restriction (CR) has proved to be significant in healthy ageing as well as longevity. CR displays a protective role against oxidative stress and reduces the formation of reactive oxygen species (ROC). Thus the effect of CR on skeletal muscle as wells as muscle stem cells (MuSCs) would provide better in sight to the understanding of muscle regeneration process with ageing.

Material and Methods: With the objective to analyze the effect of calorie restriction with ageing on muscle stem cells and skeletal muscle morphometry, we chose C57BL/6 mice as a model group for the study. Young control, Calorie Restricted, and aged mice group were used. Animals were sacrificed (n=2 each), Tibialis Anterior muscle were isolated, isopentane cooled liquid nitrogen freezing was done, followed by cryostat sectioning. Hematoxylin & Eosin (H&E) staining and immunofluorescence assay (IFA) were performed along with morphometric analysis for all the model groups.

Results: H&E staining clearly showed the relevance of calorie restriction on morphology of the myocytes. Morphometric analysis revealed significant decrease in area of the myocytes with age and CR. MuSCs population analyzed through IFA showed decreased population in aged models as compared to young whereas population of cells maintained in CR groups.

Discussion: Initial leads provided by experimental results shows Calorie Restriction helps in maintaining MuSCs population as well as myocyte morphology which declines with age leading to muscle atrophy. Furthermore if grip strength analysis is performed it would better support this information.

- **Characterization of bone marrow and mesenchymal stem cells for bone marrow transplantation in mouse**

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One of the reasons of the decline of all physiological functions during aging in mammals may be partly due to depletion of stem cells in the bone marrow and other tissues. Therefore the overall regeneration and rejuvenation capacity of the body is greatly compromised in the old age. Bone marrow houses large amount of stem cells. Bone marrow is the soft lymphoid organ that produces various kinds of blood cells and cells of the immune system. Damaged bone marrow can lead to different types of diseases of blood, immune system and cancer. The purpose of this study regeneration of bone marrow cells after irradiation by transplantation of freshly isolated bone marrow cells in mouse. Cell cycle analysis by FACS and colony forming unit (CFU) assay were conducted to analyze the bone marrow regeneration. Cell cycle analysis results showed 24% recovery of G1-phase cells in the bone marrow of transplanted mouse, but the recovery was not significant. However, colony forming unit assay showed significant (32-34%) recovery of the bone marrow potential in the transplanted mouse. The bone marrow cells were cultured to generate mesenchymal stem cells (MSCs). MSCs were analyzed by morphology and immune-phenotyping of cell surface markers, e.g. CD29, Sca-1, CD44 (positive markers) and CD11b, CD34, CD45 (negative markers) as well as localization of transcription factors (e.g. IRF-1 and IRF-2) by immune-fluorescence. This Study may be taken further towards bone marrow derived stem cell therapy for recovery from radiation-induced damage and age-related diseases.

- **Sestrin as a blood-based marker for mild cognitive impairment and Alzheimer's disease**

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Introduction: Alzheimer's disease (AD) is characterized by misfolded proteins of amyloid-ß (Aß) and tau proteins along with decreased aggregate clearance that eventually leads to oxidative stress and neuronal loss. Sestrins (sesn) are highly conserved proteins that play an important neuroprotective role, in part as a consequence of their antioxidative capacity, which prevents reactive oxygen species (ROS) formation.

Material & Methods: In this study, the concentrations of sesn1 and sesn2 in the serum of 41 AD patients; 27 mild cognitive impairment (MCI) and 60 elderly controls, was evaluated using surface plasmon resonance and confirmed by western blot. Moreover, the mRNA level of sestrins in all the study groups was determined by real rime polymerase chain reaction.

Results: A significant elevation of serum sesn2 protein and mRNA levels was observed in the

AD group compared to MCI and elderly control groups. Analtered level of serum sesn2 was also found between MCI and control group. ROC analysis showed highly sensitive, selective threshold values for sens2 in the differentiation of AD, MCI and controls. No significant difference in sesn1 level was observed among the study groups.

Conclusions: This study highlights the important role of sesn2 in the progression of AD, indicating its potential utility as a protein marker in this devastating disease. The identification of novel biomarkers would help in the detection of disease possibly before the symptoms onset and also for analyzing the effectiveness of any future clinical trials.

- **Evaluation of serum levels of FOXO3a and Sirtuin3 proteins in older cognitively impaired patients**

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder results in the death of nerve cells, deterioration of cognitive function and eventual death from complications. Thus, it is a great challenge to establish reliable markers to diagnose and monitor disease progression in AD and other forms of dementia. It is well documented that impaired glucose metabolism or mitochondrial dysfunction is one of the major pathological changes observed in various neurodegenerative diseases including AD.FOXO3a and Sirtuin3 play an important role in this pathway and are such potential markers to study neurodegenerative changes.

Material & Methods: Sixty one subjects were enrolled for the study according to inclusion and exclusion criteria. They were divided into 2 groups (Cognitively impaired patients and Control older subjects). Serum levels of FOXO3a and Sirtuin3proteins were estimated by Surface Plasmon Resonance and Western blot techniques. The statistical analysis was done with unpaired t test by Stata9 and graph pad prism5.

Results: Serum FOXO3a levels (mean + SD) in both groups were as following: Control older subjects –2.382 ± 0.304 ng/µl; Cognitively impaired patients –1.769 ± 0.427 ng/µl. Serum Sirtuin3 levels (mean + SD) in both groups were as following: Control older subjects –5.121 ± 0.771 ng/µl; Cognitively impaired patients – 3.747 ± 1.029 ng/µl. The serum concentration of FOXO3a and Sirtuin3 declined steadily and significantly ($p < 0.0001$) with the disease progression.

Conclusion: This study shows inverse relation of Alzheimer's disease with human serum FOXO3a and Sirtuin3 concentration.

- **High fat diet induced hyperlipidemia and hypercholesterolemia: an age-dependent study**

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Introduction: Hyperlipidemia and hypercholesterolemia are risk factors involved in the development of cardiovascular diseases (CVD). Aging equally contributes to these conditions. Diets rich in total saturated fat and cholesterol have been found to be associated with hyper lipidemia and enhanced ROS production. In the present investigation, we have evaluated the effect of high fat diet in rat at different ages i.e. 4 months and 24 months old for the assessment of possible vulnerability towards oxidative stress, hyperlipidemia and hypercholesterolemia.

Material & Methods: Young(4 months) and old (24 months) male Wistar rats were treated with high fat diet (HFD) (suspension (w/v) of 0.5% cholesterol, 3% coconut oil and 0.25% cholic acid for thirty days) to induce experimental hyperlipidemic. Studies on biomarkers of oxidative stress including ROS, protein carbonyl (PCO) level, plasma membrane redox system (PMRS) level, oxidation of plasma protein (AOPP) level, paraoxonase-1 (PON1) and lipid peroxidation (LPO) level were performed.

Result: Experimental hyperlipidemia was found to be associated with significantly higher levels of MDA, AOPP, and PMRS, LPO whereas GSH, PON 1 and FRAP activity was significantly decreased when compared to old aged treated rats but these values were comparable with young and control aged rats.

Conclusion: The study concludes that accelerated aging accompanied during using high fat diet in rats is associated with redox imbalance in erythrocytes and plasma.

- **Ameliorative effect of fisetin on D-galactose induced accelerated aging rat model**

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Introduction: Oxidative stress plays a central role in the process of biological aging. RBC is a reliable model for the study of oxidative stress due to the high cellular concentration of oxygen and haemoglobin. Antioxidant supplementation has been proved to be an ameliorator of oxidative stress. Fisetin a bioactive flavonol molecule found in number of vegetables and fruits. The aim of the present study is to investigate potential anti-aging effects of fisetin *in vivo* in an accelerated senescence aging rat model.

Material and Methods: Rats were divided into six groups as follows: young control, fisetin (10mg/kg b.w. orally), young D-gal treated (500mg/ kg b.w. SC), D-gal with fisetin, old control and old control with fisetin. The antioxidant status in the aging rat was measured by determining the activities of Ferric reducing ability of plasma (FRAP), Reactive Oxygen species (ROS), reduced glutathione (GSH), lipid peroxidation (MDA) Plasma membrane redox system (PMRS) and protein carbonyl (PCO) in erythrocytes and plasma.

Results: Compared with control group, fisetin supplementation significantly (<0.01) enhanced the activities of FRAP, PMRS and GSH whereas ROS, MDA, PCO content decreased in the aging rat model.

Conclusion: The data provides evidence that fisetin has an anti-aging effect *in vivo*. Long term supplementation studies are underway.

- **Delineating the role of *Bacopamonnieri* extract (CDRI 08) on the early onset of cognitive aging markers during type II diabetes in mice**

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Introduction: Increased life span in the recent decades has lead to a global swell in geriatric disorders in human society. DM II is one of the most prominent disease in elderly persons which may accelerate the onset of cognitive aging. The present study is focused on how DM II accelerates the onset of aging and aging-like symptomsalong with the possible contribution of *Bacopamonnieri* extract (CDRI 08) on age-related cognitive changes.

Methods: DM II mice were generated by ipadministration of 100 mg/kg b.w. STZin neonatal mice and occurrence of aging markers in hippocampus were assessed by lipofuscin accumulations. Alongside this spatial memory alteration was assessed by escape latency test by Morris water maze. Finally to exploring the possible mechanism of impaired memory expression of AMPA receptor and its traffickers (PICK 1 and Stargazin),western blotting (translational level) and RT-PCR (transcript level) were performed in the tissue lysate of hippocampus .

Results: The lipofuscin accumulation was significantly high in diabetic mice and CDRI 08 (100 mg/kg b.w.) elapse back the perturbed brain conditions towards normal condition. The reduced spatial memory induced by diabetes was recovered by CDRI 08 administration. The altered expression of GluR2, PICK 1, Stargazin were normalised by CDRI 08 treatment at both transcript level and translational level.

Conclusion: The present study, thus, demonstrates the Brahmi extract has the potential to check the early onset of cognitive aging in diabetic mouse.

- **A long noncoding RNA from the rat genome shows tissue-specific and age-related differential expression**

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Approximately, 2% of the mammalian genome functions for coding all the proteins while the rest is non-protein coding in nature but mostly transcribed into noncoding RNAs. We have recently reported a novel long noncoding RNA, named as LINC–RSAS (long intergenic non-protein coding repeat-rich sense-antisense transcript). LINC-RSAS showed tissue-specific, differential expression and localization in brain (hippocampus, cortex, and cerebellum) and testis during aging of the rat. Its expression increased from immature stage (4 weeks) to adulthood (16 weeks)and declined in old age (70 weeks) in the brain and testes of the rats as judged by RT-PCR. In situ RNA hybridization showed both differential and age-related expression patterns similar to above. Over-expression of LINC-

RSAS in HeLa cells mostly showed cytoplasmic RNA-granules. Thus age-dependent expression of LINC-RSAS may underline functional significance of the intergenic, repeat-rich long noncoding RNA during aging.

- **Changes in aging rat retina due to oral administration of iron sulphate**

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Introduction: Iron is a vitalcomponent in cellular metabolic processes. In the retina, it is used in oxidative phosphorylation, membrane biogenesis and retinol isomerization. When in excess, it causesoxidative stress and age related macular degeneration. It is unknown the ways in which the retinal iron content is regulated and the role of iron regulatory proteins in it.

Material and methods: Male Wistarrats were fed with oral $FeSO_4$ (500 mg/kg bw/week) starting from 2 months of age until 18 months. Retinal changes were examined by light- and transmission electron microscopy (LM, TEM) and expression of transferrin-1, transferrin receptor-1, ferritin and ferroportin-1, by immunohistochemistry (IHC) and Western blotting (WB) at 6, 12 and 18 months of age (N=40/ group; IAEC approval No.: 692/2012). The animals were anesthetized, their eyes enucleated, fixed and processed for LM, TEM and IHC. Retinal proteins were extracted in RIPA buffer for WB.

Results: LM and TEM showed damage in inner-and outer nuclear layer (INL/ONL), necrosis of INL and mitochondrial alterations in photoreceptor inner segments. Compared to age-matched controls, IHC and WB revealed increased expression of transferrin-1 and ferritin, and decreased levels of transferrin receptor-1 and ferroportin-1 in Müller cells, capillaries, outer plexiform layer and inner segments of treated retinas at 18 month of age.

C**onclusions**: Iron accumulation with aging causes retinal cellular necrosis. Müller cells appear to be involved in iron homeostasis via upregulation of transferrin-1 and ferritin, and down regulation of transferrin receptor-1 and ferroportin-1 (supported by CSIR, New Delhi, 37/1593/13/EMR-II, TCN).

- **Novel, Noninvasive Biomarker for Parkinson's disease**

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Introduction - Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1% of all people over 60 years of age and is second most prevalent geriatric neurodegenerative disorder. In mid brain part of PD patient, the α-synuclein protein becomes misfolded and aberrant accumulation of amyloid occurs. Molecular chaperones are responsible for maintaining normal protein homeostasis within the cell by assisting protein folding, inhibiting protein aggregation, and modulating protein degradation pathway. Hsp70 (mortalin) can reduce the amount of misfolded, aggregated α-synuclein species in vivo and in vitro and protect it from synuclein-dependent toxicity.

Material & Methods –This study evaluates the expression level of Mortalin and α-syn in the serum of PD and compare it with healthy group. Blood sample were collected from 60 PD and 50 healthy elderly subjects. The concentration of Mortalin and α-syn in serum was measured by surface plasmon resonance technology.

Results –The significant higher expression of α-syn level was observed in PD (95% CI, 57.03–65.05) compare to control (95% CI, 57.64–56.83). However, there was no significant difference in the level of mortalin was observed in between PD (95% CI, 3.3– 4.01) and control group (95% CI, 3.49 – 4.14). This study confirms higher level of α-syn in PD blood. The cut off value, sensitivity and specificity obtained from ROC analysis revealed the accuracy to differentiate PD from control in respect to α-syn level.

Conclusion-α-syn level can be used as a blood based noninvasive biomarkerand can havetherapeutic interventions.

- **Generation of Parkinson's disease mouse model for understanding cognitive decline**

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Parkinson's disease (PD), an age-dependent progressive neurodegenerative disease is characterized by motor abnormalities, however, the PD patients are also known to suffer from multitudes of non-motor disorders such decline in learning, memory and cognition. For understanding the molecular mechanisms of the cognitive dysfunction, mice model has been used. For understanding the precise molecular mechanisms of PD-induced cognitive impairments, the PD mouse model was developed and validated. Swiss strain mice were subcutaneously injected with various doses of rotenone (0.5-2.5 mg/kg BW in DMSO medium)for three weeks while control mice were treated only with DMSO. PD and control mice were examined for motor disabilities using Treadscan Clever Sys instrument and cognitive impairment was examined by Morris water maze test. For the biochemical validation, expression of α- Synuclein and dopamine receptor (DR2), the biochemical hallmarks of PD in midbrain was studied by Western blotting and Reverse transcriptase PCR. Our results reveal that 2.0 mg/kg BW dose of rotenone was the suitable dose for producing the motor as well as cognitive decline in PD mice. Our gene expression data revealed that above dose of rotenone was able to up regulate the level of α-Synuclein and down regulation of the DR2. Our results suggest that rotenone is a suitable drug for developing the PD model which can further be used for studying the underlying molecular mechanisms of age- associated cognitive decline in PD and its treatment.