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EFFECTS OF POLY-UNSATURATED FATTY ACIDS ON LEUKOCYTE TELOMERE LENGTH AND INFLAMMATORY MARKERS IN D-GALACTOSE TREATED WISTAR ALBINO MALE RATS

B. S. Sriram [1], V. Ravichandra [*1], Shailaja Moodithaya [2], Rajendra Holla [1] and N. Suchetha Kumari [3]

Department of Pharmacology [1], Department of Physiology [2], Department of Biochemistry [3], K. S. Hegde Medical Academy, Mangalore - 575018, Karnataka India.

Keywords:

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Correspondence to Author:
Ravichandra V.

Associate Professor,
Department of Pharmacology,
K. S. Hegde Medical Academy,
Mangalore - 575018, Karnataka India.

E-mail: ravi75chandra@yahoo.co.in

ABSTRACT: The present study aimed at analyzing the effects of PUFA in D-galactose treated Wistar albino male rats by determining leukocyte telomere length using real-time quantitative PCR, Il-6, and high sensitive C-RP levels estimation using ELISA. D-galactose is administered for 42 days orally (100 mg/kg), and on 43rd-day, blood is collected from tail vein and plasma is separated and subjected for IL-6 and high sensitive C-RP estimation using ELISA and determination of length of leukocyte telomere by real time qPCR. PUFA had no significant influence on length of leukocyte telomere and it decreased significantly IL-6 and high sensitive C-RP levels, indicating possible anti-aging effects by decreasing inflammation associated with aging process.

INTRODUCTION: Aging is a biological process associated with a decrease in physiological, physical, and cognitive functions with the risk of neurodegeneration, metabolic syndrome and cancer [1]. The most accepted theory is free radical theory of aging which states that excess free radicals cause adverse events and adds up to the process of aging and age-related pathological changes [2]. D-galactose which is a reducing sugar when administered oral or subcutaneous to rats increases oxidative stress in mitochondria, and is an aging model for screening antiaging drugs. This aging model mimics the aging of humans and is characterized by telomere loss, altered telomerase activity in hippocampus [3,] and also rise in thiobarbituric acid reacting substances (TBARS) and low super oxide dismutase level in various tissues [4]. Many evidences clarifies length of leukocyte telomere as one of the important biomarker in the process of aging [5], telomeres are present at the end of eukaryotic chromosomes as a continuous sequence of DNA and are essential for genomic stability and protective against DNA damage response [6].

Nutrition plays a key role in the prevention of aging onset diseases. PUFA is gaining attention as a supplement to combat aging. Eicosapentaenoic acid (EPA) and Docosa hexanoic acid(DHA)belongs to n-3 PUFA includes docosahexaenoic acid (DHA), which is present in avocado abundantly which are able to prevent or ameliorate many inflammatory conditions [7].

MATERIALS AND METHODS: D-galactose obtained from Sisco laboratories private limited India. IL -6 and High sensitive C-reactive protein ELISA kits and Telomere length estimation kits was purchased from Kaishen private limited, India.

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PUFA capsules (Docosa hexanoic acid + Eicosapentanoic acid) obtained from Novartis laboratory private limited India, all other materials were of analytical grade. Male rats of 4 months old (25-30 g) was used for study after obtaining permission from Institutional Animal Ethics Committee (115/1999) KSHEMA.

Wistar rats were housed in room temperature with free access to water and food. All procedures were approved by the Animal ethics committee and are in accordance with CPCSEA Guidelines.

Experimental Design:

Groups	Interventions
Control (C)	Distilled water in 5% carboxymethyl cellulose orally
Positive control (PC)	D-galactose in 5% CMC P.O left untreated
C + PUFA	Distilled water + PUFA in 5% CMC P.O
PC+PUFA	D-galactose + PUFA in 5% CMC P.O

Preparation of Solutions: D-galactose (100 mg/kg) taken in distilled water +5% CMC given orally for 42 days, PUFA capsules (100 mg/ kg) taken in 5% CMC and solution given orally from 15th day of D-galactose administration.

Plasma IL -6 Estimation and Plasma High Sensitive C-RP Estimation: The plasma levels of IL-6 and High sensitive C-RP are estimated using commercially available Rat ELISA kits according to instructions provided in the manual. (Kaishen Pvt. Ltd. India).

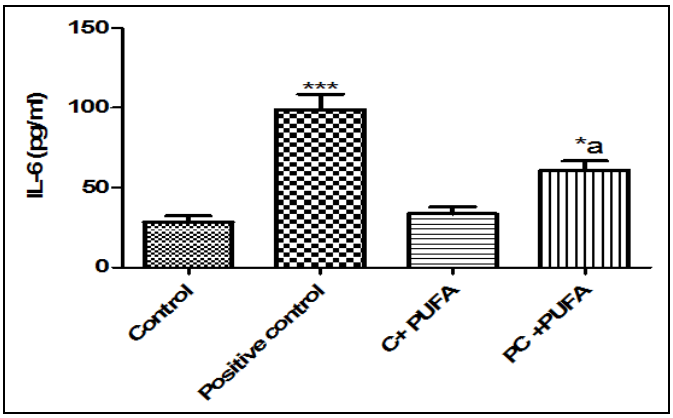
Length of Leukocyte Telomere Estimation using Real-Time Quantitative PCR (rt QPCR): The length of leukocyte telomere in the plasma obtained from PCR was calculated using Standard edition optical system and software by establishing standard curves for each gene.

Relative expression of the target gene in relation to beta-globin gene and samples were determined by the comparative Ct method [8, 9, 10].

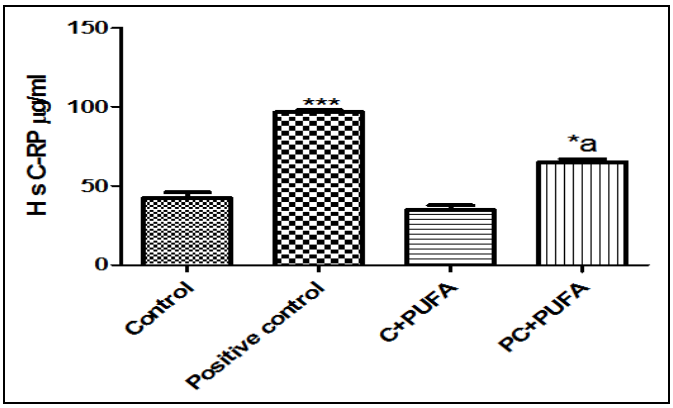
DNA per reaction was used to construct the standard curve as instructed in the manual.

Statistical Analysis: Graph pad prism version 5.0. is used for statistical analysis. One way ANOVA followed by Dunnett's multiple comparisons as post hoc test with a p-value less than 0.05 is considered significant.

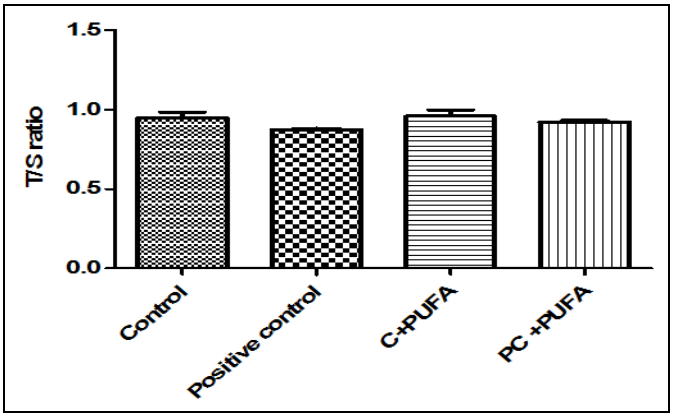
RESULTS:



GRAPH 1: SHOWING RESULTS OF PLASMA IL-6 LEVELS IN GROUPS. N= 6 rats in each group, Values are expressed as Mean ± SD, One way ANOVA followed by Tukey's test is used, ***p = 0.001 compared to control, *[a]p = 0.05 compared to control and positive control. p < 0.05 is considered as significant.



GRAPH 2: SHOWING RESULTS OF PLASMA HIGH SENSITIVE C-RP LEVELS IN GROUPS. N= 6 rats in each group, Values are expressed as Mean ± SD, One way ANOVA followed by Tukey's test is used, ***p = 0.001 compared to control. *[a] p = 0.05 compared to control and positive control. p < 0.05 is considered as significant.



GRAPH 3: SHOWING RESULTS OF LENGTH OF LEUKOCYTE TELOMERE IN GROUPS. N= 6 rats in each group, Values are expressed as Mean ± SD, One way ANOVA followed by Tukey's test is used, ***p = 0.001 compared to control, p < 0.05 is considered as significant.

PUFA Caused Decrease in IL-6 and High Sensitive C-RP Levels: Graphs 1 and 2 show the anti inflammatory effects of PUFA by decreasing plasma IL-6 and High sensitive C-RP levels in D-galactose treated Wistar albino rats.

PUFA has no Significant Effect on Telomere Length: From **Graph 3** it is evident that PUFA had no significant impact on length of leukocyte telomere in treated and non treated groups.

DISCUSSION: In this study, the effect of PUFA on D-galactose induced aged male rats was evaluated using ELISA estimation of IL-6 and High sensitive C-RP levels in rat plasma. We demonstrated that PUFA due to its anti-inflammatory properties, has significant anti-aging effects.

Several studies has shown that Inflammation is one of the main cause for aging, since senescent cells release inflammatory markers and cause disruption of healthy functioning of normal cells leading to the cascade of aging process [11, 12, 13]. In the present study PUFA significantly reduced plasma IL-6 and C- RP levels, this could be due to its role in improving antioxidant levels and also in balancing omega 6: omega 3 ratio, suggesting its potential role in ameliorating age related inflammations.

Telomeres are present at the end of chromosomes which are essential for genomic stability and is helpful in preventing the DNA damage response [14] although many evidences has pointed the role of length of leukocyte telomere in aging and age related diseases which is attributed as one of the important hallmark in aging [15]. Naturally telomere length shortens as we age and excessive shortening is prevented by telomerase enzyme; thus homeostasis between telomere length and telomerase helps in maintaining division of cells appropriately, if fails leads to the development of cancer [16, 17, 18]. On the contrary several cohorts suggesting the correlation between telomere length and aging; some studies also state that leukocyte telomere length may not serve as a true biomarker in aging since several infections tend to decrease telomere length like CMV and other infections.

Thus, CMV seropositivity and other cofounders must be ruled out before estimating telomere length. Recently a Newcastle study of 85+ showed no significant change in leukocyte telomere length and aging [19].

Interestingly in the present study, the length of leukocyte telomere was not significantly changed in any groups and also in the positive control; this could be due to the route of D-galactose administered since oral route administration has unpredictable bioavailability and also the duration of an experiment could also have been influenced since leukocyte telomere length is dependent on several factors [20]. Thus, it has to be ruled out in future studies.

Thus, in the present study PUFA significantly reduced inflammation associated with aging in D-galactose treated Wistar albino rats and could be useful as a potential anti-aging drug. Further molecular studies and clinical trials are required to know its mode of action and efficacy.

CONCLUSION: The study concludes that, there is a significant decrease in levels of IL-6 and High sensitive C-RP, the inflammatory markers in D-galactose treated Wistar albino rats with PUFA supplementation compared to group without PUFA supplementation, indicating anti inflammaging effects. However, study showed that there is no significant difference in length of leukocyte telomere between D-galactose treated Wistar albino rats with and without PUFA supplementation.

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AUTHORS CONTRIBUTION: First author (S.B.S) initiated and conducted the research. The second authors (R.V and S.M) conducted animal care and prepared the manuscript. (R.H and S.C) helped in the Estimation of IL-6 and High sensitive C-RP levels.

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