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Original Article

Age dependent variation in the antioxidant status and oxidative stress in rats fed diet containing coconut oil and sunflower oil

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ABSTRACT

ABSTRACT The aim of the study was to compare the effect of feeding coconut oil (CO) and sunflower oil (SO), fats customarily classified as saturated and polyunsaturated on oxidative stress in Sprague Dawley rats of different age groups. The rats were divided into six groups (I, II, III, IV, V&VI) based on their age (groups I & II-one month old, groups III & IV- 12 months old, groups V & VI-22 months old) and were provided with standard pellet diet along with 10% oil (groups I, III & V were fed with coconut oil, groups II, IV & VI were fed with sunflower oil). The concentration of lipid peroxidation products viz TBARS in the tissues and in RBC, hydroperoxides and conjugated dienes were found to be increased with age in rats fed SO compared to rats fed CO. The reduced glutathione content was found to decrease with respect to age, but rats fed CO showed higher glutathione content than rats fed SO in both the age groups. The activities of the antioxidant enzymes catalase, superoxide dismutase, were found to decrease with increasing age in rats fed SO. Glutathione peroxidase and Glutathione reductase decreased only in the aged rats. The in vitro LDL oxidation was also found to increase with age and depending on the nature of dietary fat. Thus we conclude that aging diminished total antioxidant capacity to an extent with both oil fed diets, but in a lower extent in rats fed coconut oil diet.

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1. Introduction

Free radical damage has long been believed to be a risk factor for the degenerative process which accompany ageing [1, 2]. Reactive oxygen species initiate a wide variety of oxidative reactions including peroxidation of lipids, proteins, nucleic acids and contribute to the process of ageing. Dietary factors are found to play important role in the etiology of different diseases and the most important one being the dietary fat [3]. Debate over the beneficial effects of saturated fatty acids versus unsaturated fatty acids has been a topic for research for the past decades. Reports indicated that PUFA rich oils tend to exert a hypocholesterolemic effect whereas saturated fatty acid rich oils tend to be hypercholesterolemic [4]. Dietary intake of PUFA was found to decrease the postprandial cholesterol and triacylglycerol levels in triacylglycerol rich lipoproteins [5], plasma [6,7,8] and hepatic triglycerides in rats[8]. The intake of PUFA have been reported to be beneficial in reducing risk of diseases such as coronary heart

disease and some cancers [9,10,11]. Conversely, PUFAs are highly susceptible to free radical-mediated lipid peroxidation [12,13,14], a process thought to be involved in the initiation and progression of atherosclerosis [15]. Saturated fatty acids undergo less peroxidation than their unsaturated counter parts. Diet rich in saturated fat was found to reduce microsomal lipid peroxidation by decreasing the availability of polyunsaturated fatty acids in substrate [16].

Coconut oil has been used by the people in the tropical regions as a source of food for many centuries. In Kerala, coconut oil is an indispensable ingredient for culinary purpose. The present study was carried out to investigate the antioxidant status and oxidative stress levels in rats of different age groups fed with saturated fat (coconut oil: CO) and unsaturated fat (sunflower oil: SO) diet.

2. Materials and Methods

2.1. Chemicals

All the chemicals used in this study were Analar Grade (AR) and obtained from the following scientific companies: Sigma-Aldrich (St. Louis, USA), Merck (Mumbai, India), Qualigens (Mumbai, India), Ranbaxy (New Delhi, India). Test oils (coconut oil and sunflower oil) were purchased from the local market.

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2.2. Animal Experiments

Male albino rats (Sprague- Dawley strain), Young (Y)-2months old (weight 102 ± 5 g), Middle aged (M)-12months old (weight 162 ± 5 g) and Aged (A)- 22 months (weight 310 ± 8 g) were housed individually in polypropylene cages having 6 rats in each group and maintained under temperature control room ($27 \pm 2^\circ\text{C}$) with 12:12h dark/light cycle. The rats were provided with standard pellet diet (Amrut rat feed, Pune, India) along with 10 % oil and water was provided ad libitum throughout the experimental period for 60 days. Food intake was assessed daily and the animals were weighed weekly. The experimental protocol was approved by the Animal Ethics Committee of the University of Kerala. The experimental groups were as follows: Group I -Young rats fed CO, Group II - Young rats fed SO, Group III- Middle aged rats fed CO, Group IV- Middle aged rats fed SO, Group V- Aged rats fed CO, Group VI- Aged rats fed SO. At the end of the experiment, the rats were deprived of food overnight and sacrificed by sodium pentathone injection, blood and tissues were removed, washed with ice cold saline and used for various biochemical analysis.

2.3. Antioxidant enzyme activities

Antioxidant enzyme activities: Activities of the antioxidant enzymes such as Catalase (EC1.11.1.6), Superoxide dismutase, (EC1.15.1.1), Glutathione peroxidase (EC1.11.1.9) and Glutathione reductase (EC.1.6.4.2) were determined by the methods of Maehly and Chance [17], Kakkar et al [18], Lawrence and Burk [19], David and Richard [20] respectively

2.4. Lipid peroxide levels

Malondialdehyde (MDA) in the tissues was estimated by the thiobarbituric acid method of Ohkawa et al [21]. Tissue homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.5). 1 ml of the homogenate was combined with 2 ml of 15% (w/v) TCA- 0.275% (w/v) TBA- 0.25N HCl reagent and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000g for 10 min and absorbance was read at 535 nm against a blank that contained no tissue homogenate. The extinction coefficient of Hydroperoxides (HP) and Conjugate dienes (CD) content were estimated as described by John and Steven [22]. Glutathione content was estimated by the method of Patterson & Lazarow [23].

2.5. Isolation of LDL

Isolation of LDL was carried out by the method of Bairaktari et al. [24] and Adler et al [25]. 3 ml of plasma was centrifuged at $d=1.006 \text{ kg/l}$ in an ultracentrifuge (Sorvall ultra 80) at 40,000 rpm using T -865 rotor at 14°C for 10h. After ultracentrifugation floating VLDL and chylomicrons were removed and LDL was separated by precipitation from the solution. 40 μl of 4% phosphotungstic acid in 1M NaOH was added, stirred and 10 μl of 2M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was added and centrifuged at 1500xg for 30 min at 4°C . The supernatant was discarded and the precipitated LDL was redissolved in 0.4ml of 0.5M Na_2CO_3 , kept in ice overnight and dialyzed against three changes of PBS for 12h.

2.6. In vitro LDL oxidation

LDL oxidation was determined by the method of Puhl et al. [26]. Isolated LDL (200 μg protein/ml) was incubated with 1.7mM copper sulfate to the reaction mixture. The thiobarbituric acid reactive substance (TBARS) was determined after 6h [21].

2.7. Erythrocyte membrane lipid peroxidation

Erythrocyte membranes from the experimental animals were isolated by hypotonic lysis in 7.6mM PO_4/l (pH 7.4) according to the procedure of Dodge *et al.* [27]. Membrane preparations were washed in the 7.6 mM PO_4/l until the supernatant was clear to remove hemoglobin and other cytoplasmic components. Aliquots were removed and TBARS were measured by the procedure of Ohkawa *et al* [21]. Protein was estimated by the method of Lowry *et al* [28].

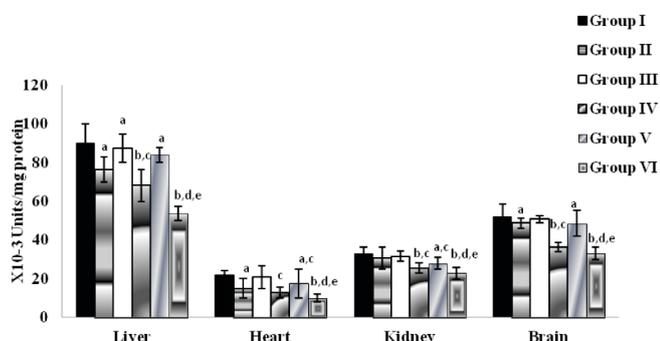
2.8. Statistical analysis

Results are expressed as mean \pm SD of six rats and evaluated using one way ANOVA. The difference among the mean for the groups were assessed using Duncan's post Hoc test to determine which mean values were significantly different at $p < 0.05$.

3. Results

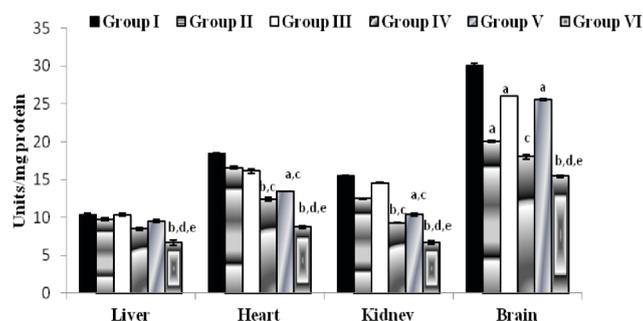
The activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) was found to diminish with age in both CO and SO fed rats (Fig 1 & 2). The decline in the CAT activity was highest in the liver of aged rats fed SO. When compared to rats fed SO, rats fed CO showed significant increase in the activity of CAT in all the age groups. Young and middle aged rats fed CO did not show any significant change in CAT activities in the heart, kidney and brain tissues. Rats of all the age groups fed CO did not show any significant decrease in the activity of SOD in the liver however, young and middle rats fed SO had a significant decline in the activity of SOD in the heart and kidney. The aged rats fed SO showed a greater decrease in the SOD activity in all the tissues compared to aged rats fed CO. The activities of both glutathione peroxidase (GPx) and glutathione reductase (GR) were found to be significantly lower in the aged rats fed SO (Table 1). The GPx activity in the heart of middle aged rats fed SO did not show any significant decrease. An increase in the activity of GR irrespective of the diet was observed in the middle aged rats compared to young rats. Rats fed CO did not show any significant change in the liver and brain. Feeding of SO and CO was found to increase the TBARS content (MDA) in the middle aged and aged rats in all the tissues measured (Fig 3). However the tissue concentration of TBARS was found to be greater in rats fed SO. The concentration of conjugated dienes and hydroperoxides were also found to increase with respect to age in both SO and CO fed groups (Table 2) and the changes were more in aged rats fed SO compared to aged rats fed CO. The glutathione content in all the tissues were significantly lowered during ageing, SO fed rats showed a lower glutathione content in all the aged groups compared to CO fed rats (Fig 4). The TBARS content in the erythrocyte membrane was found to be increased in rats fed SO (Fig 5). The rate of oxidized LDL formation was more in aged rats fed SO compared to the CO fed rats (Fig 6).

Figure 1. Activity of Catalase



Values are expressed as mean \pm SD of six rats in each group, a, significantly different from Group I, b significantly different from Group II, c significantly different from Group III, d significantly different from Group IV, e Significantly different from Group V.

Figure 2. Activity of Superoxide dismutase

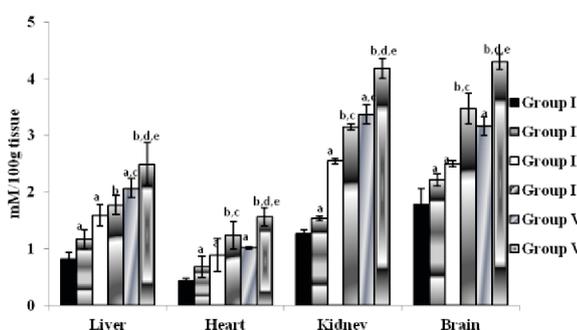


Values are expressed as mean \pm SD of six rats in each group, a, significantly different from Group I, b significantly different from Group II, c significantly different from Group III, d significantly different from Group IV, e Significantly different from Group V.

Table 1: Activity of Glutathione Peroxidase and Glutathione Reductase

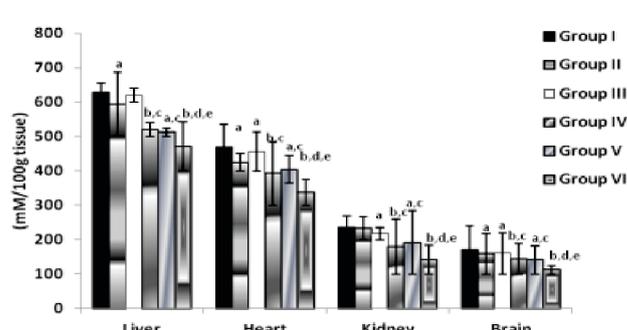
Groups	Glutathione Peroxidase								
	Liver		Heart		Kidney		Brain		Liver
	Heart	Kidney	Brain						
I	0.66 \pm 0.042	0.85 \pm 0.038	0.42 \pm 0.038	0.20 \pm 0.006	9.34 \pm 1.71	9.48 \pm 1.59	12.20 \pm 1.57	20.48 \pm 2.42	
II	0.62 \pm 0.048	0.85 \pm 0.043	0.39 \pm 0.022	0.21 \pm 0.013	9.24 \pm 1.54	9.2 \pm 2.22	10.60 \pm 1.24	20.02 \pm 1.87	
III	0.63 \pm 0.064	0.86 \pm 0.044	0.41 \pm 0.026	0.19 \pm 0.011 ^a	10.86 \pm 1.67	11.8 \pm 1.69 ^a	12.45 \pm 1.82	21.84 \pm 1.07	
IV	0.55 \pm 0.042 ^{b,c}	0.80 \pm 0.040	0.32 \pm 0.021 ^{b,c}	0.18 \pm 0.011 ^b	10.72 \pm 1.87	12.8 \pm 1.71 ^b	13.63 \pm 1.69 ^b	19.88 \pm 1.95	
V	0.63 \pm 0.025	0.78 \pm 0.059 ^{a,c}	0.39 \pm 0.017	0.17 \pm 0.038 ^{a,c}	10.22 \pm 1.65	10.38 \pm 0.87	9.9 \pm 1.42 ^{a,c}	19.13 \pm 1.35	
VI	0.45 \pm 0.038 ^{b,d,e}	0.67 \pm 0.032 ^{b,d,e}	0.28 \pm 0.04 ^{b,d,e}	0.15 \pm 0.019 ^{b,d,e}	7.39 \pm 0.9 ^{b,d,e}	8.68 \pm 0.53 ^d	6.62 \pm 1.03 ^{b,d,e}	15.82 \pm 1.41 ^{b,d,e}	

Figure 3. Concentration of tissue TBARS



Values are expressed as mean \pm SD of six rats in each group, a, significantly different from Group I, b significantly different from Group II, c significantly different from Group III, d significantly different from Group IV, e Significantly different from Group V.

Figure 4. Concentration of tissue GSH

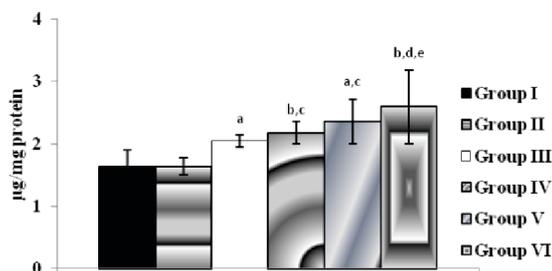


Values are expressed as mean \pm SD of six rats in each group. a significant compared to Group 1, b significant compared to Group II, c significant compared to Group III, d significant compared to Group IV, e significant compared to Group V.

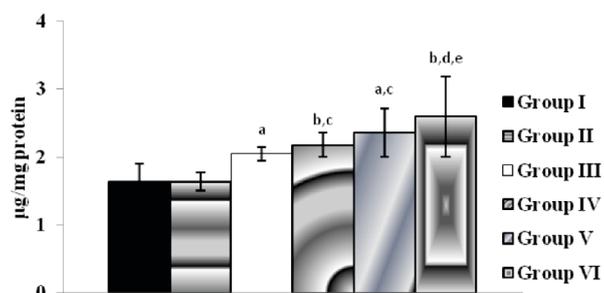
Table 2. Concentration of Hydroperoxides and Conjugated dienes

	Groups			Glutathione Peroxidase				
	Liver	Heart	Kidney	Kidney	Brain	Brain	Liver	Liver
	Kidney	Brain						
I	12.55±1.5 ^o	47.08±2.56	1.62±0.19	14.07±1.56	37.08±3.01	12.47±3.23	9.38±2.41	32.93±1.92
II	16.54±2.04 ^a	51.35±1.71 ^a	2.34±0.37 ^a	15.32±1.50	50.35±2.29 ^a	15.03±1.511 ^a	11.38±1.38	38.02±2.01 ^a
III	20.89±2.35 ^a	53.01±2.16 ^a	2.66±0.16 ^a	16.42±1.34	53.34±1.56 ^a	20.83±2.73 ^a	18.12±1.02 ^a	41.34±3.48 ^a
IV	26.97±2.54 ^{b,c}	60.77±1.58 ^{b,c}	3.169±0.15 ^{b,c}	23.132±2.01 ^{b,c}	62.08±3.42 ^{b,c}	23.53±2.505 ^{b,c}	21.10±2.13 ^{b,c}	46.05±2.58 ^{b,c}
V	25.80±2.49 ^{a,c}	57.98±2.01 ^{a,c}	3.25±0.17 ^{a,c}	23.07±2.27 ^{a,c}	66.68±4.16 ^{a,c}	23.83±2.04 ^{a,c}	22.37±2.06 ^{a,c}	45.32±2.21 ^{a,c}
VI	30.18±1.71 ^{b,d,e}	63.45±1.84 ^{b,d,e}	3.82±0.24 ^{b,d,e}	29.58±1.93 ^{b,d,e}	76.70±2.65 ^{b,d,e}	27.33±4.46 ^{b,d,e}	26.13±2.09 ^{b,d,e}	50.45±2.71 ^{b,d,e}

Values are mean ± SD of 6 rats, a - significantly different from Group 1, b - significantly different from Group II, c - significantly different from Group III, d - significantly different from Group IV, e - significantly different from Group V. Hydroperoxides expressed as mM/100g tissue; Conjugate Dienes expressed as mM/100g tissue.

Figure 5. TBARS content of RBC membrane

Values are expressed as mean ± SD of six rats in each group. a significant compared to Group 1, b significant compared to Group II, c significant compared to Group III, d significant compared to Group IV, e significant compared to Group V.

Figure 6. In vitro oxidation susceptibility of LDL isolated from oil fed aged and young rats

Values are expressed as mean ± SD of six rats in each group, a, significantly different from Group I, b significantly different from Group II, c significantly different from Group III, d significantly different from Group IV, e significantly different from Group V.

4. Discussion

Oxidative stress is depicted as a possible ageing accelerating factor [29]. Dietary fats play a most important role which influences oxidative stress and antioxidant production. All organisms respond to oxidative stress by inducing a variety of defense systems [30,31,32]. In the present study the effects of two dietary oils viz CO and SO in oxidative stress and antioxidant status of rats of different age groups was investigated. Superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase constitute a mutually supportive team of defense against reactive oxygen species [33]. SOD, as an enzyme to remove superoxide anion provides a protection against lipid peroxidation of cell membrane [34]. The decrease in the activity of SOD will result in the increased production of superoxide anion which combines with hydrogen peroxide to form a hydroxyl radical [35] which damages the integrity of the membranes of mitochondria, endoplasmic reticulum and nucleus [36]. In our study feeding CO compared with SO was associated with significant difference in the levels of lipid peroxides and antioxidant status and the effects were more with aged rats fed SO. The activities of the key antioxidant enzymes catalase and superoxide dismutase was found to decrease with respect to age in rats fed SO. Reports states that higher incorporation of polyunsaturated fatty acids have been shown to be more susceptible to lipid peroxidation [37,38]. GSH is an endogenous antioxidant and is maintained in reduced state by glutathione peroxidase/glutathione reductase system. Our results indicate significant decrease in the glutathione content in SO fed aged rats, accompanied by increase in the concentration of malondialdehyde content in the tissues. Reports indicate that liver GSH was found to be either unchanged [39,40] or decreased [41] with respect to aging. The decrease in the glutathione content in our study is in agreement with the reports that inverse relationship exist between lipid peroxidation and glutathione content [42,43]. Glutathione peroxidase activities were lower with the SO fed rats while CO fed rats did not show any change in the

activity on ageing. GPx functions as a protection enzyme against peroxidative damage by catalyzing elimination of peroxides [44]. In vitro LDL oxidation was found to be increased with the age and dietary fat. The fatty acid composition of LDL is the prime important factor for LDL oxidation and it is reported that one of the first steps in the oxidative modification of LDL is the peroxidation of PUFA [45]. High proportion of PUFA confers greater susceptibility to oxidation while monounsaturated fatty acids protect against peroxidation [46,47]. Our results clearly indicate that the nature of dietary fat has significant effect on the antioxidant and oxidative changes and the effect was less with saturated fat like coconut oil when compared to unsaturated fat like sunflower oil.

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