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The Effect of Fried Palm Oil Supplemented Diet on Enzymatic Antioxidant Status of Aging Mice

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Abstract: *There is increased evidence that reactive oxygen species (ROS) and their promoted oxidative damage are involved in a large number of pathologies, as well as in the aging process. ROS removal rate is mostly controlled by a variety of antioxidants. The extent of lipid peroxidation and antioxidants level, the way they are related to aging and whether they are affected by deep fried palm oil supplemented diet in aging mice was investigated. Standard laboratory methods were employed in the determination of Malondialdehyde (MDA), Catalase (CAT) and Superoxide Dismutase (SOD) activities in the plasma of the experimental animals. It was found that SOD activity in older mice not fed with fried palm oil supplemented diet was higher 24.75±1.96U/L than among younger mice 19.05±6.66 U/L; (P=0.091), and indication of increased production of superoxide radical with age. A significant (P=0.018) increase in SOD activity (43.05±14.91U/L) was observed in older mice fed with deep fried palm oil supplemented diet relative to 24.75±1.96U/L in older mice not fed with deep fried palm oil supplemented diet and 19.05±6.66U/L; (P=0.000) in younger mice, indicating a compensatory response to additional superoxide radical produced through the process of oxidative metabolism of deep fried palm oil supplemented diet. Expectedly, CAT activity increases with increased SOD activity in older mice not fed with deep fried palm oil supplemented diet 461.51±39.05U/L as against 440.10±29.20U/L in younger mice, although not to a statistically significant level (P=0.265). In contrast, a significant (P=0.008) decrease in CAT activity (366.29±63.56U/L) was observed in older mice fed with deep fried palm oil supplemented diet relative to older mice not fed with deep fried palm oil supplemented diet and younger mice (p=0.005). Increased MDA (102.53±1.02µM/L), a marker of lipid peroxidation due to free radicals was observed in older mice not fed with deep fried palm oil supplemented diet relative to 98.74±17.99µM/L in younger mice (p=0.652). However, the consequent increase in SOD activity in response to increased free radical production in older mice fed with deep fried palm oil supplemented diet helped modulate the extent of lipid peroxidation with consequent decrease (91.27±31.37µM/L; p=0.535) in MDA. Therefore, it can be concluded that consumption of deep fried palm oil supplemented diet increases superoxide radical formation, thus should be minimized, if possible avoided especially in ageing.*

Keywords: *Aging, oxidative damage, lipid peroxidation, Reactive oxygen species and Antioxidants.*

I. Introduction

Aging is a multi-factorial process involving morphological and biochemical changes in single cells and in the whole organism. It is the accumulation of damage to somatic cells, leading to cellular dysfunction, and culminates in organ dysfunction and n increased vulnerability to death [1]. However, successful aging refers to the changes due solely to the aging process, uncomplicated by damage from environment, lifestyle, or disease. The gain in survival includes both active and dependent years. The concept of the compression of morbidity i.e., that the time of dependency will decrease as the life expectancy increases, has been popularized but the mean prevalence of disability seems to be getting worse with time, thus decreasing the time of disability present challenges to both clinicians and scientists.

With aging, two phenomena occur, physiological decline and an increase in the prevalence of disease. Although these processes influence each other, physiologic decline do occur independent of disease. In healthy older adults, many physiologic fictions are maintained in the basal resting state, but decrements are seen in most organ systems and homeostatic mechanisms when these systems are challenged or stressed.

Several theories have been proposed as the scientific basis of aging and suggested possibilities include: (a) encodement of aging in DNA (made manifest in a manner similar to development), (b) progressive

breakdown in accuracy in protein (c) in higher organisms, "attack" of the immune system on self-antigens and (d) free radical reaction damage. This work is limited to the last-named possibility not only because accumulating evidence indicates that aging is largely due to free radical reaction damage but also because it shows promise of serving as a useful guide in the search for practical means of interventions that increases the mean life span and more importantly decreases disability.

The free radical hypothesis of aging postulates that senescence is due to an accumulation of molecular oxidative damage, caused largely by oxidants that are produced as by-products of normal metabolic processes [2]. The human body is so endowed that chemicals and other harmful products formed under pathological and normal physiological conditions are usually eliminated by inbuilt normal endogenous systems. Free radicals and reactive oxygen species (ROS) are amongst the chemical groups normally formed as by-products or as intermediates in many of the biochemical reactions taking place in the body. Accumulation of these compounds in the body cause oxidative stress/damage and contributes to the aging process.

ROS is a family of free radicals generated from oxygen which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS include free radicals such as superoxide anion radicals (O^{2-}), hydroxyl radicals (OH^{-}), and singlet oxygen [3]. In 1987, Sawada and Carlson reported that superoxide radical formation increases with age. During the aging process, superoxide anion radicals are believed to be the major cause of oxidative damages of living tissues [4, 5, 6 and 7]. However, in 1997, Maxwell and Lip reported that a set of scavenging systems called antioxidants has been discovered. They protect and limit the potential threat of oxidative stress. Under normal conditions, the ROS and free radicals formed as bye products/ intermediates of biochemical reaction are removed by the aforementioned scavenger systems. Antioxidants are substances which when present in low concentration compared to oxidizable substrate significantly delay or inhibit the oxidation of that substrate [8, 9, 10 and 11]. In humans, the antioxidant system includes a number of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) amongst others. SOD catalyses the dismutation of superoxide radical to hydrogen peroxide. Although hydrogen peroxide is not a radical, it is rapidly converted by CAT to water. Thus, the susceptibility of an individual depends on the antioxidant status of the body.

Several exogenous sources of antioxidant including red palm oil are said to boost endogenous antioxidant capacities, and studies have shown that red palm oil has a wide range of protective properties against disease and aging as well as being modulator for cellular processes / functions by acting as scavengers of oxygen and peroxyl radicals [12 and 13]. However, it is a known fact that fats and oil when subjected to prolonged heating for frying are susceptible to a series of chemical-physical modification; and consequent adverse nutritive effects [14]. Therefore this work seeks to evaluate the effect of deep fired palm oil supplemented diet on the antioxidant capacity of plasma of naturally aging mice.

RATIONALE

The free radical hypothesis of aging postulates that senescence is due to an accumulation of molecular oxidative damage, caused largely by oxidants that are produced as by-products of normal metabolic processes [15]. A logical prediction based on this hypothesis is that the elevation of antioxidative defenses should delay aging and extend life span. Therefore this study is designed to investigate the effect of fried palm oil supplemented diet on antioxidant status of aging mice.

AIM

The aim of this study is to investigate the effect of fried palm oil supplemented diet on antioxidant status of aging mice.

OBJECT IVES

To supplement the diet of aging mice with fried palm oil.
To determine the Malondialdehyde (MDA) as an index of lipid peroxidation in the mice used.
To determine the activity of Superoxide Dismutase (SOD) as antioxidant in the mice used
To determine Catalase (CAT) activity as antioxidant in the mice used
To monitor the activities of SOD and CAT in the groups of aging mice used in view of understanding the role of fried palm oil diet in aging.

II. Materials And Methods

ANIMALS

Thirty six healthy adult mice weighing 26.5g (averagely) were used. The mice were obtained and kept in the Animal House Unit of Ladoke Akintola University of Technology. The mice were allowed to acclimatize for a period of two weeks.

DEEP FRIED PALM OIL PREPARATION

Fresh red palm oil was gotten from known source in Nkwo Market, Eke, Udi Local Government Area in Enugu State, Nigeria. Deep fried palm oil was prepared by frying fresh red palm oil above its smoke point - 235°C (ie temperatures at which visible smoke starts evolving). It was allowed to cool before being used to supplement the diet of the aging mice. 2.68µl of deep fried palm oil per gram feed per day was employed throughout the period of this experiment.

EXPERIMENTAL DESIGN

The mice were randomly divided into three groups (n=12). Each group was cage separately. All mice were fed with commercial mice feed and distilled water ad libitum. The cages were cleaned daily and food/water changed daily. The commercial mice feed was obtained from TOPFEED, Nigeria.

Group I = Control 1: Normal feed and water daily and sacrificed after the two weeks acclimatization. It served as the younger control.

Group 2 = Control II: Normal feed and water daily throughout the experimental period.

Group 3= Experimental Group: Deep fried palm oil supplemented feed and water daily throughout the experimental period.

COLLECTION AND PREPARATION OF BLOOD SAMPLES

After the experimental period of three (3) months, the mice were sacrificed by humane killing of experimental animal. The blood samples were collected into ethylene diamine tetra acetic acid (EDTA). It was immediately spun at 4000rpm and the plasma was transferred into plain bottles and used for estimation of Catalase, Superoxide Dismutase and Malondialdehyde.

BIOCHEMICAL ANALYSIS

DETERMINATION OF CATALASE ACTIVITY IN PLASMA

Determination of catalase activity was based on the method of Sinha (1972).

Principle: The method is based on hydrogen peroxide determination remaining after decomposition and stoppage of the enzyme action on substrate with a mixture of potassium dichromate and glacial acetic acid.

Reagents: 80% glacial acetic acid was mixed with 5% potassium dichromate in ratio 3:1 according to the method of Sinha (1972)

Procedure: 0.04m1 of plasma was mixed with 0.5m1 of phospohate buffer, 0.2m1 hydrogen peroxide and 1ml dichromate-acetic acid mixture (ratio 3:1) in test tube. The mixture in each test tube was heated for 10 minutes in a boiling water bath to decompose the blue precipitate and produce a green solution which was measured at 570 nanometers in the spectrophotometer. Using the "standard curve" for hydrogen peroxide, the amount of hydrogen peroxide left in the solution when the enzyme was stopped with acetic acid was determined.

Enzyme activity was expressed in U/L. One unit of enzyme activity is the enzyme quantity that decomposed one micromole (unit) hydrogen peroxide micromole for one minute at 20°C and pH=7. The results were as stated in Table 2.

DETERMINATION OF SUPEROXIDE DISMUTASE ACTIVITY IN PLASMA

Superoxide Dismutase activity was assayed based on modified method Beauchamp and Fridovich (1971).

Principle: The method is based on the rate of SOD inhibition of photochemical reduction of NBT to formazan by superoxide radicals which is monitored spectrometrically at 520nm.

Procedure: The reaction mixture (3ml) was composed of 0.1 ml plasma and 13mM methionine, 0.075mM NBT, 0.002mM riboflavin in 50mM phosphate buffer (pH=7.8). The mixture in the tube was placed on a rotating tube holder in a light box for 7min. The absorbance was read at 520 nm with a spectrophotometer. SOD activity was calculated based on an extinction coefficient of 1.5 x $mol^{-1}cm^{-1}$ for formazan. The analysis was done in duplicate. The results were as stated in Table 2.

DETERMINATION OF MALONDIALDEHYDE CONCENTRATION IN PLASMA

Malondialdehyde was assayed based on the method Gutteridge and Wilkins (1982).

Principle: When sample under test is heated with TBA at a low pH, it gives a pink chromogen, allegedly a $(TBA)_2$—MDA adduct, which is measured pectrophotometrica1ly at a wavelength of 535 nm.

Procedure: 2ml of glacial acetic acid and 2ml of 1% thiobarbituric acid were added to 0.2 ml of plasma. The tube was stoppered loosely and immersed in boiling water for 15min and swirled slightly at intervals; the mixture was cooled and centrifuged at 5000g at room temperature for 10min. The absorbance of the supernatant obtained was read at 532nm against the reagent blank. The values of MDA were calculated based on the molar extinction coefficient of the chromogen taken as 1.56 x $10^5$1/mol/cm, and expressed as moles of MDA formed per liter of plasma. The results were as stated in table 2.

III. Results

Table 1: Distribution Of Experimental Animal In Each Group At The End Of The Experimental

GROUP	N	Alive or Dead	Frequency	%
Younger Mice	12	Alive	9	75
		Dead	3	25
Older Mice	12	Alive	5	41.7

		Dead	5	58.3
Older Mice on Fried Palm Oil Supplemented Diet	12	Alive	11	91.7
		Dead	1	8.3

Table 2: Plasma Concentration Of Catalase, Superoxide Dismutase And Malondialedehyde Of Different Groups

Parameter	Group	N	Mean ±2SD
Catelase (CAT) U/L	Younger Mice	9	440.10±28.99
	Older Mice	5	461.51±39.05
	Older mice on fried palm oil supplemented Diet	11	366.29±63.56
Superoxide Dismutase (SOD) U/L	Younger Mice	9	19.05±6.66
	Older Mice	5	24.75±1.96
	Older mice on fried palm oil supplemented Diet	11	43.05±14.91
Malondialdehyde (MDA) µM/L	Younger Mice	9	43.05±17.99
	Older Mice	5	102.53±1.02
	Older mice on fried palm oil supplemented Diet	11	91.27±31.37

Table 3: Comparative Analysis Of Results Of The Various Analyses In Older Mice And Older Mice On Fried Palm Oil Supplemented Diet With Younger Mice

Parameter	Group	N	Mean ±2SD	P-value
` Catelase (CAT) U/L	Younger Mice	9	440.10±28.99	-
	Older Mice	5	461.51±39.05	0.265
	Older mice on fried palm oil supplemented Diet	11	366.29±63.56	0.005
Superoxide Dismutase (SOD) U/L	Younger Mice	9	19.05±6.66	-
	Older Mice	5	24.75±1.96	0.091
	Older mice on fried palm oil supplemented Diet	11	43.05±14.91	0.000
Malondialdehyde (MDA) µM/L	Younger Mice	9	98.74±17.99	-
	Older Mice	5	102.53±1.02	0.652
	Older mice on fried palm oil supplemented Diet	11	91.27±31.37	0.535

Note: The mean difference is significant at the 0.05 level
≤0.05 = Significant
≤0.01 = Highly Significant
>0.05 = Not Significant

Table 4: Comparative Analysis Of Results Of The Various Analyses In Older Mice With Older Mice On Fried Palm Oil Supplemented Diet

Parameter	Group	N	Mean ±2SD	P-value
Catelase (CAT) U/L	Older Mice	5	461.51±39.05	-
	Older mice on fried palm oil supplemented Diet	11	366.29±63.56	0.008
Superoxide Dismutase (SOD) U/L	Older Mice	5	24.75±1.96	-
	Older mice on fried palm oil supplemented Diet	11	43.05±14.91	0.018
Malondialdehyde (MDA) µM/L	Older Mice	5	102.53±1.02	-
	Older mice on fried palm oil supplemented Diet	11	91.27±31.37	0.444

Note: The mean difference is significant at the 0.05 level
≤0.05 = Significant
≤0.01 = Highly Significant
>0.05 = Not Significant

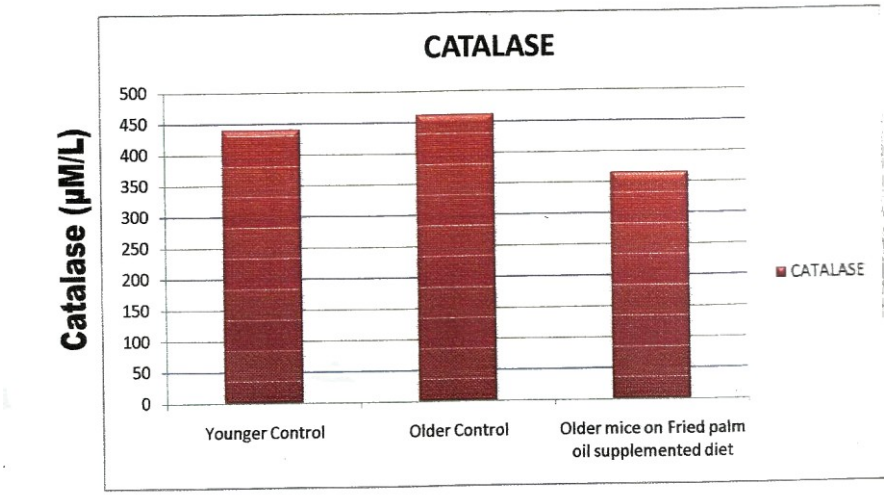

Figure 1: Plasma Catelase Activity of different experimental groups

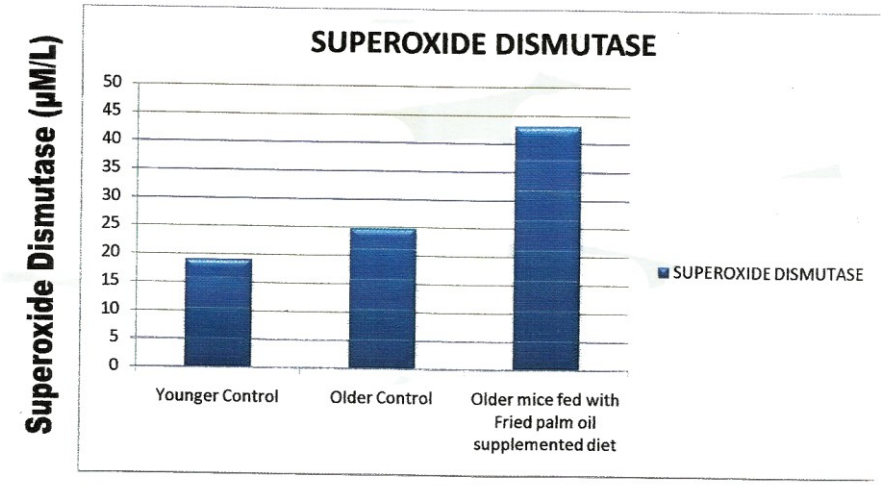

Figure 2 :Plasma Superoxide Dismutase Activity of different experimental groups

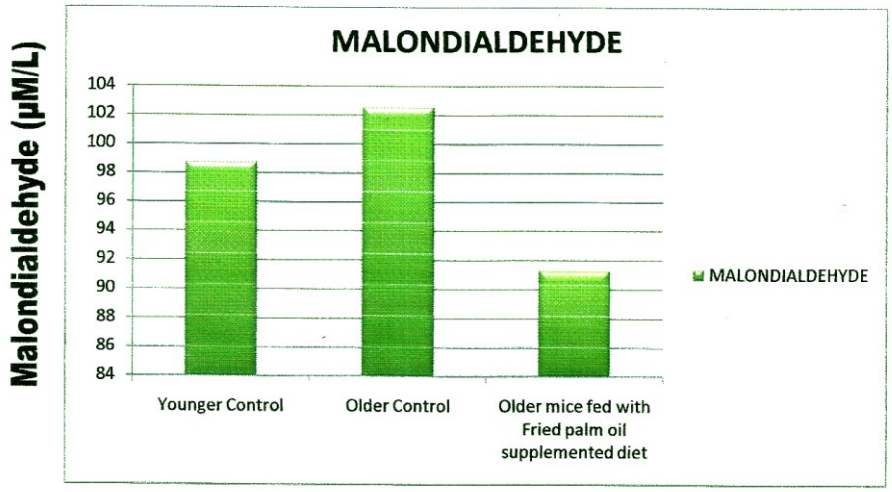

Figure :3 Plasma Malondialdehyde Levels of different experimental groups

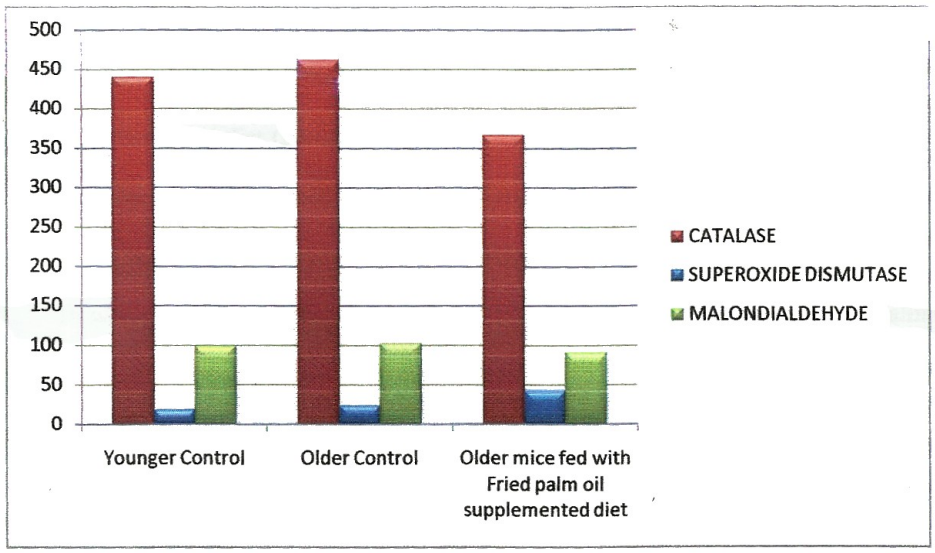

Figure:4 Plasma Catalase and Superoxide Dismutase Activities, and Malondialdehyde Levels of different experimental groups

The enzyme activity of SOD and Catalase as well as the plasma level of MDA of the three different experimental groups are presented in Tables 2, 3 and 4. There was no significant (P=0.091) mean difference between the mean SOD activity of younger mice (group 1) and the mean SOD activity of older mice not fed with deep fried palm oil supplemented diet (group 2). There was a significant (P=0.018) mean different between the mean SOD activity of older mice not fed with deep fried palm oil supplemented diet (group 2) and the mean SOD activity of group 3 i.e. older mice fed with deep fried palm oil supplemented diet.

At P=0.265, no significant mean difference existed between the mean catalase activity in younger mice (group 1) and the mean catalase activity of older mice not fed with deep fried palm oil supplemented diet (group 2). The mean catalase activities of older mice not fed with deep fried palm oil supplemented diet (group 2) and that of the older mice (group 3) fed with deep fried palm oil supplemented diet showed a significant (P=0.008) mean difference.

However, the mean plasma level of MDA in older mice not fed with deep fried palm oil supplemented diet (group 2) showed no significant (P=0.652) mean difference when compared with the mean MDA level in plasma of (group 1) younger mice. Also there was no significant (P=0.444) mean difference between the mean plasma MDA concentrations of older mice not fed with deep fried palm oil supplemented diet (group 2) and that of the older mice (group 3) fed with deep fried palm oil supplemented diet.

IV. Discussion

Free radical and reactive oxygen species (ROS) are amongst various harmful and toxic chemical compounds formed as bye/intermediate product of normal biochemical reactions. They are chemical compounds which contain unpaired electron, spinning on the peripheral layer around the nucleus; and cause damage to other molecules by extracting electrons from them in order to attain stability. Studies have shown that free radical production increases with age [18] and so far, various age-related changes have been investigated in lipid peroxidation and in antioxidant enzyme capacity.

Free radical induced damage is thought to be responsible, at least in part, for the degenerative effects of ageing [19]. Many investigators believed that free radical damage to cellular molecules and organelles is the primary cause of ageing of the organism [20]. Cellular defense mechanism against free radicals involves a series of enzyme linked reactions which remove toxic radicals and repair radical induced damage.

[21] proposed that enzymes scavenging active oxygen species are key factors in determining the longevity of animals and [22] pointed to correlations between the levels of these enzymes and the maximum life span potential of different species.

Several exogenous sources of antioxidant including red palm oil are said to boost endogenous antioxidant capacities, and studies have shown that red palm oil has a wide range of protective properties against disease and aging as well as being modulator for cellular processes / functions by acting as scavengers of oxygen and peroxyl radicals [23]. However, it is a known fact that fats and oils when subjected to prolonged heating for frying are subjected to a series of physicochemical modification, with consequent adverse nutritive

effects [24]. It is therefore necessary to evaluate the effect of various diets on these endogenous/enzymatic antioxidants.

In the present study, the effect of deep fried oil supplemented diet on catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation in ageing mice was investigated. It was found that SOD activity in older mice not fed with fried palm oil supplemented diet was higher 24.75±1.96 U/L than among younger mice 19.05±6.66U/L;(P=0.091). These may be due to increasing production of superoxide radical whose formation is said to increase with age as reported by [25]. However, there a significant (P=0.0l8) increase in SOD activity of 43.05±14.91U/L was observed in older mice fed with deep fried oil supplemented diet as against 24.75±1.96U/L in older mice not fed with deep fried oil supplemented diet. This may be a concomitant response to bye/intermediate product (free radical) of oxidative metabolism of deep fried oil supplemented diet.

SOD catalyses the dismutation of superoxide radical to hydrogen peroxide with simultaneous conversion of hydrogen peroxide (H_2O_2) to water and free oxygen by catalase. Expectedly catalase activity increased with increased SOD activity in older mice not fed with deep fried palm oil supplemented diet 461.51±39.05U/L as against 440.10±29.20U/L in younger mice (P=0.265). In contrast, a significant (P=0.008) decrease of CAT activity was observed in older mice fed with deep fried oil supplemented diet (366.29±63.56U/L) relative to older mice not fed with deep fried oil supplemented diet (461.51±39.05U/L). This decrease may be adduced to glutathione assisted neutralization of the excessive H_2O_2 turnover from SOD, thus less of the H_2O_2 is available for CAT to act upon.

It has been stated earlier that during the ageing process, tissues are damaged to some extent due to oxidative processes primarily caused by reactive oxygen species. One of the main target substrate for these oxygen radicals include polyunsaturated fatty acids in membrane phospholipids whose modification result in disorganization of cell framework and function [26]. The consequence of these reactions (peroxidation process) is the appearance of malondialdehyde - end product of lipid peroxidation in blood. Biochemical detection of this malondialdehyde (MDA) gave useful information on membrane lipid peroxidations. However, an increase in MDA of 102.53±l.02µM/L in older mice not fed with deep fried palm oil supplemented diet was observed relative to 98.74±17.99µM/L in younger mice (P=0.652). In contrast, a decrease in MDA of 91.27±31.37µM/L in older mice on deep fried oil supplemented diet relative to older mice not fed with deep fried palm oil supplemented diet 102.53±1.02µM/L; (P=0.444) was observed. This may be adduced to the increased SOD activity (observed in this group), which is believed to reduce the extent of lipid peroxidation.

V. Conclusion

As earlier stated, the need to evaluate the effect of some diet especially in ageing cannot be overemphasized. This work on deep fried oil would perhaps give additional information. However, it can be concluded that consumption of deep fried oil supplemented diet increases superoxide radical formation, thus should be minimized, if possible avoided especially in ageing

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