



Depletion of Antioxidants Vitamins and Oxidation of Low Density Lipoproteins observed among different workers with NIDDM Exposed to Vehicle Exhaust in the congested part of the CHENNAI city

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Abstract

This study was carried out among NIDDM patients who were exposed to traffic smoke more than 10hrs/day. We have measured the level of Vitamins, Vitamin C, Superoxide dismutase (SOD), Catalase, Oxidised LDL, Total thiols, Total cholesterol, plasma blood glucose, Urea, Creatinine and Urine sugar. It was found that the level of vitamin E, C, Catalase, total thiols was found to be depleted but the level of Oxidised LDL, LPO, LOH was found to be elevated. SOD was found to be slightly elevated, Common biochemical parameters plasma blood glucose, urea, creatinine was found to be increased. Most of the persons who participated in our study were NIDDM patients with varying degrees of severity of complications. The patients case histories were recorded which consist of the age, the hours of exposure to traffic smoke, the duration of diabetes in years. Sixty non-smoking males also participated as control. No Gross variation was observed in Lipid profile samples.

Keywords: NIDDM-Noninsulin dependent diabetes mellitus; SOD-Superoxide dismutase; LPO-Lipid peroxidation; LOH-Lipid hydro peroxides; TC-Total cholesterol; HDL-High density lipoprotein; LDL-Low density lipoprotein; VLDL-Very low density lipoprotein; Tgl-Triglycerides; ROS-Reactive oxygen species.

1. INTRODUCTION

Diesel engine, petrol engine exhaust contains a variety of toxic materials, they are Gaseous metallic chemical aggregates, Poly cyclic organic matter and particulates (Santo donato *et al.* 1978). The particulate fraction in automobile exhaust are carbonaceous in nature and cause major public health problems. The carbon particles from the diesel smoke get deposited and retained in the lungs, they are carriers of other mutagenic and carcinogenic emission products. The lead particle which is present in the diesel exhaust enters the blood by active smoking interfere with porphyrin synthesis and raises the level of Aminoleuvinic acid (Luckey *et al.* 1975). Free

radical and oxidant toxicity: Oxides of Nitrogen, Sulphur, Carbon monoxide, enters the living system and could produce active free radicals. Particulate matter which enters the living system enhances the development of macrophages resulting in an oxidative burst in the neutrophils releasing free radicals (Krinsky, 1982).

2. MATERIALS AND METHODS

The total number of persons enrolled in our study were 140 with NIDDM they were all males, their age group was between forty two to fifty two years, and their exposure to automobile emission was between 10-12 hours/day. The participants were traffic

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police constables, government workers, platform shop keepers, road side workers, with diabetic complications their fasting blood glucose was 172 ± 8 mg/dl and postprandial blood glucose was found to be 228 ± 4 mg/dl. Sixty non-smoking healthy males in the age group of thirty two to fifty two years participated as control, their exposure to diesel and auto mobile exhaust was within ninety minutes /day during their travel to the office. The patients case histories were recorded which consist of the age, the hours of exposure to traffic smoke, the duration of diabetes in years. The criteria adopted for diagnosis of diabetes was based on the recommendation of WHO study group on diabetes.

2.1 Blood Sampling

Blood sampling was carried out after 12 hours of overnight fasting by vein puncture. The blood thus obtained was discharged into two tubes. The tube which contain sodium fluoride as anticoagulant was used for the assay of glucose, urea, Creatinine. The tube which contained EDTA disodium salt 1mg/ml as anticoagulant was used for the remaining test ,Red cells were lysed their membranes were separated by the method of Dodge *et al.*, (1963). The red cell membrane were used for the assay of antioxidant enzymes Catalase and Superoxide dismutase (SOD).

The blood glucose estimation in plasma was done by the method of (Varley *et al.* 1984). Urea estimation in plasma was done by the method of (Marsh *et al.* 1965). Creatinine in plasma was estimated by the method of (Larson, 1972). Protein was measured by the method of (Lowry *et al.* 1951).

2.2 Assessment of traffic density

The number of fuel driven vehicles such as diesel engine vehicles and petrol engine vehicles passing at a point /hour were counted and classified accordingly as two stroke engines, four stroke engines, bikes, buses, Jeeps, Auto rickshaws, large trucks and vans.

2.3 Antioxidant enzymes assays:

The enzyme superoxide dismutase (SOD) was assayed in the haemolysate by the method of (Misra and Fridovich, 1972). Catalase was assayed in the red cell membrane by the method of (Sinha, 1972). Vitamin C was assayed by the method of (Tsan *et al.* 1982). Vitamin E was measured by the HPLC method of (McCormick *et al.* 1980). Oxidised LDL was measured by the commercially available competitive sandwich ELISA method as described by (Holvoet *et al.* 1998). Total thiols was measured by the method of (Koster *et al.* 1986). Vitamin E was assayed at Trivandrum fisheries institute by HPLC method. All

other biochemical experiments were carried at Ramanas clinic at venkatramana road near turn bull's road under the supervision of Mr Radhakrishnan former biochemist MMC

2.4 Assay of Oxidative damage:

The lipid hydroperoxides in the plasma was measured with the FOX II reagent as described by Zhen-Yue Jiang (1990). The lipid peroxidation product in the plasma was measured by the TBARS method as described by Buege and Aust (1978).

2.5 Lipid Profile:

Blood plasma total cholesterol was estimated by the enzymatic method as described by Tarbutton and Gunter (1974). HDL cholesterol was measured by enzymatic method as described by Lang and Schettler (1985). VLDL cholesterol was measured by using friedwald equation (1972), VLDL Cholesterol (mg/dl) = TGL/5 = VLDLcholesterol

LDL Cholesterol = Total cholesterol minus VLDLcholesterol+HDL cholesterol = LDLcholesterol.

All the biochemical kits were bought from Accurex India Limited. Throughout the experiment double distilled water was used

Table 1. General data on the subjects investigated

S. No.	Parameters	Control	NIDDM
1	Number Analysed	60(males)	140(males)
2	Age(Years)	32-52	42-52
3	Height(cm)	161±7	163±7
4	Weight(kgs)	64±7	65±9
5	BMI (kg/m ²)	24±3	25±3
6	NIDDM(Years)	NIL	4.5±1.5
7	Fasting plasma glucose(mg/dl)	82±6	172±8mg/dl *
8	Post prandial plasma Glucose(mg/dl)	142±8	228±4
9	Urea(mg/dl)	24±6	30±2
10	Creatinine(mg/dl)	0.6±0.2	1.0±0.2
11	Urine sugar (%)	NIL	100%
12	Urine Albumin (%)	NIL	80%
13	HbA _{1c} (%)	NIL	10.5±1.4
14	Protein(g/L)	6.0±0.8	7.0±1.4
15	Exposure to vehicle Exhaust(hrs)	90 minutes/day	10-12hours/day

Statistically different values among NIIDM From normal (healthy) volunteers at p<0.001 is denoted as *

Table 2. Status of Antioxidants, Vitamins, Oxidised LDL, Lipid peroxidation, Lipid hydro peroxides

S. No.	Parameters	Normal	NIDDM
1.	SOD, IU/mg Hb	2.9±0.5	3.2±0.5
2.	Catalase, amount of H ₂ O ₂ consumed/mg protein in the membrane	6.4±0.50	4.0±0.60*
3.	Total thiols, μ moleS/L	670±60.01	592±5.0*
4.	Vitamin E, μ moleS/L	27±2	20±2*
5.	Vitamin C, μ moles/L	64±6	40±6*
6.	Oxidised LDL U/L	93 U/L	118 U/L*
7.	(LPO)Lipid peroxidation, μmoles/L	1.9±0.3	7.4±0.5*
8.	(LOH)Lipid hydroperoxides μ moles/L	3.1±1.0	5.2±1.1

Statistically different values among NIDDM From normal (healthy) volunteers at $p < 0.001$ is denoted as *

Table 3. Assesment of vehicle density at busy road junctions

S. No.	Vehicles	Between 8am-1pm	2pm-7pm
1	Two wheelers+Three wheelers	1600	2000
2	Petrol driven vehicles with four stroke engine	600	820
3	Diesel driven vehicles with four stroke engine	810	1020
4	Large four wheelers,buses trucks and tankers	920	1100

Table 4. Distribution of Totalcholesterol,Lipoprotein(mg/dl) in NIDDM and in Normals

S.No	Contents	Total cholesterol (Tc)(mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
1	Controls	210±19	55±5	105±12	48±12
2	NIDDM	206±6	39±1.5	120±3	60±10

3. RESULTS

Table 1 shows the General data on the subjects investigated, all the subjects investigated were males both in the control as well as in the NIDDM. The fasting blood glucose in NIDDM who were exposed to traffic pollution was found to be 172±8mg/dl in plasma and in post prandial state it was found to be 228±4mg/dl in plasma, further their HbA_{1c} levels were elevated above 10%, this shows that the patients were in poor glycaemic control. The urea level was found to be 24±6mg/dl in control and

in NIDDM it was found to be 30±2mg/dl. The level of Creatinine in control was found to be 0.8±0.2mg/dl and in NIDDM it was found to be 1.0±0.2mg/dl in plasma.

The presence of urine albumin among NIDDM subjects exposed to traffic exhaust was found to be 80% and urine sugar was found to be 100% ,the presence of urine albumin and urine sugar among NIDDM indicates the patients have developed renal complications due to oxidative stress. There was a little increase in body mass index(BMI) when

compared to normals. The level of protein was found to be increased among **NIDDM** ($7.0 \pm 1.4 \text{g/L}$) when compared to normals ($6.0 \pm 0.8 \text{g/L}$), this increase in protein level indicates that there is a derangement in protein metabolism among **NIDDM**. There is no gross variation observed in height and weight measurements.

Table 2 Shows the oxidative stress parameters in terms of Superoxide Dismutase (SOD), Catalase, Total thiols, Vitamin E, Vitamin C, Oxidised LDL, Lipid peroxidation (LPO) and Lipid hydro peroxides (LOH) in both normal and in **NIDDM** subjects exposed to traffic smoke. From the table it can be seen that the level of SOD was found to be slightly increased ($3.2 \pm 0.5 \text{ IU/mgHb}$) among **NIDDM** when compared to healthy normal ($2.9 \pm 0.5 \text{ IU/mgHb}$). But the level of Vitamin E and C was found to be depleted significantly among **NIDDM** subjects exposed to traffic smoke when compared to healthy normals. The level of Vitamin E in **NIDDM** was found to be depleted ($20 \pm 2 \mu \text{ moles/L}$) when compared to normals ($27 \pm 2 \mu \text{ moles/L}$). The level of vitamin C in **NIDDM** was found to be ($40 \pm 6 \mu \text{ moles/L}$) when compared to healthy controls ($64 \pm 6 \mu \text{ moles/L}$), this is shown in Figure 2. There is also a depletion in catalase activity was noted among **NIDDM** subjects exposed to traffic smokes ($4.0 \pm 0.6 \text{ mmoles of H}_2\text{O}_2 \text{ consumed/mg protein in the membrane}$) and in normals it was found to be ($6.4 \pm 0.50 \text{ mmoles of H}_2\text{O}_2 \text{ consumed/mg protein in the membrane}$), this is shown in Figure 1.

From table 2 it can be observed that the level of lipid peroxidation was found to be increased to six fold among **NIDDM** exposed to traffic smoke ($7.4 \pm 0.5 \mu \text{ moles/L}$) when compared to normals ($1.9 \pm 0.3 \mu \text{ moles/L}$) and also the level of lipid hydro peroxides was found to be increased to a greater extent among **NIDDM** exposed to traffic smoke ($5.2 \pm 1.1 \mu \text{ moles/L}$), the comparison is shown in Fig. 4. The level of Oxidised LDL was found to be elevated among **NIDDM** workers exposed to traffic exhaust 118 U/L when compared to normals 93 U/L .

The level of plasma total thiols was found to be severely depleted among **NIDDM** when compared to normal, the comparison of plasma total Thiols with Oxidised LDL is clearly shown in Fig. 3

Table 3 shows the Assessment of vehicle density at busy road junctions it was found that, two wheelers, three wheelers, petrol and diesel driven four stroke engines, large four wheelers and buses were found to be drastically increased at traffic junctions during peak hours and also between 2pm and 7pm.

Table 4 shows the distribution of cholesterol and lipoprotein among **NIDDM** and controls. The total cholesterol, LDL and VLDL distribution in **NIDDM** subjects is increased when compared to controls. But the level of HDL cholesterol is decreased.

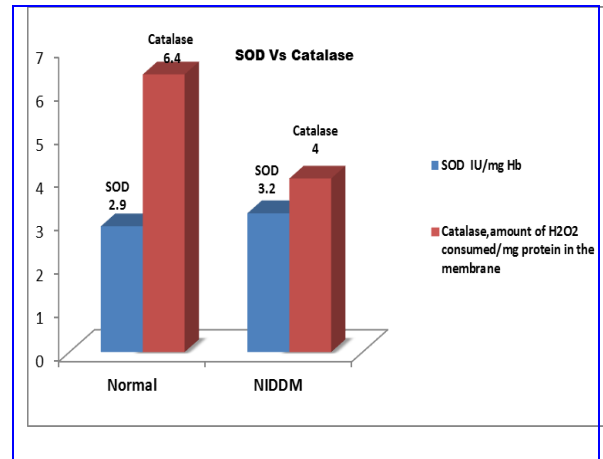


Fig. 1: Comparison of Catalase and SOD among NIDDM and Normal

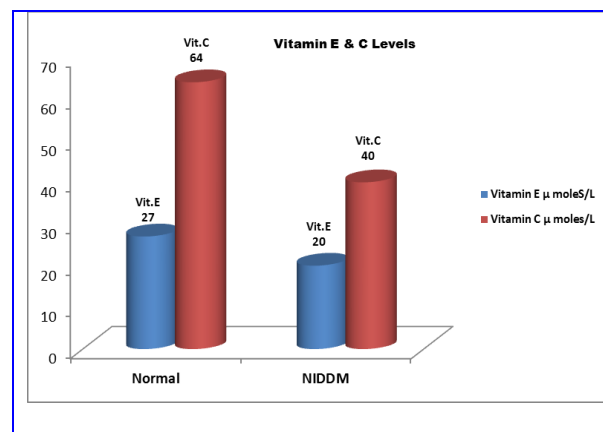


Fig. 2: Comparison of Vitamin E and C among NIDDM and Normals.

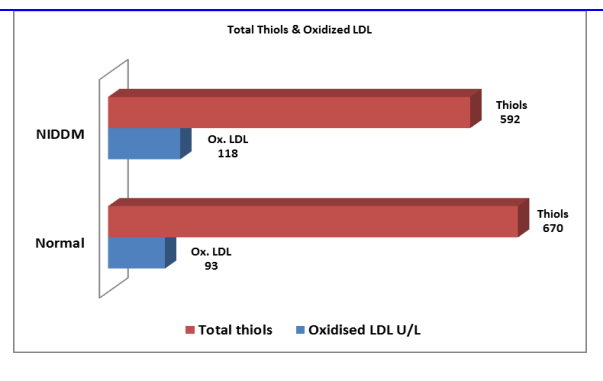


Fig. 3: Comparison of Total Thiols and Oxidized LDL among NIDDM and Normals.

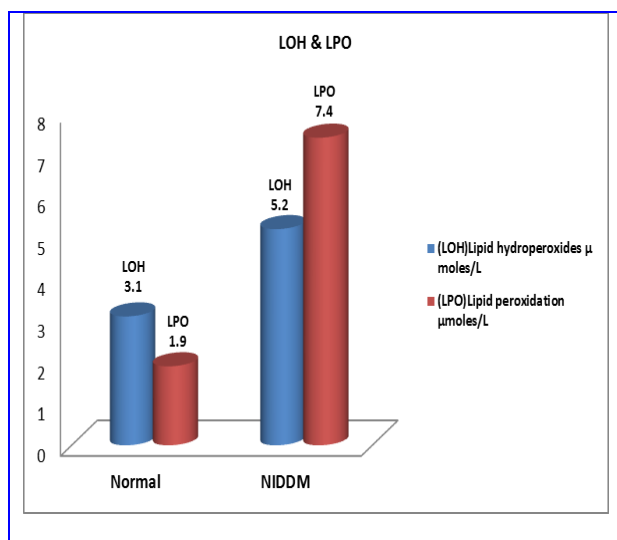
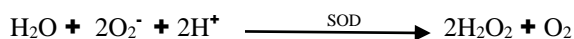


Fig. 4: Comparison of LOH and LPO among NIDDM and Normals.

4. DISCUSSION

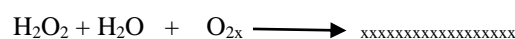
The level of fasting and postprandial glucose was increased to a greater extent among **NIDDM** subjects when compared to normals, the glycosylated haemoglobin was above 10% this indicates the patients were left in poor glycaemic control. There is also excretion of urine albumin, urine sugar was noted among **NIDDM** exposed to vehicle exhaust, this indicates the patients might develop renal complications. The depletion of Antioxidants and vitamins among **NIDDM** Exposed to traffic smoke can be discussed under the following

Antioxidants are compounds that protects the living system against the potentially harmful processes or reactions that can cause excessive oxidations. (Halliwell and Gutteridge, 1989). In our study we have observed there is a slight elevation in SOD (super oxide dismutase) activity combined with a decrease in catalase activity. High SOD activity was reported in chronic and acute leukemia (Schwaiger *et al.*, 1989). The SOD catalyses the following reactions



There is an increase in antioxidant activity was noted, when the tissue is damaged, the antioxidant enzymes are abnormally elevated. (Kokoglu *et al.*, 1989). Methyl viologen and in vivo oxygen free radical generator caused a strong increase in manganese super oxide dismutase (MnSOD) in anaerobic cultures (Hassan, Fridovich 1977). SOD is an inducible enzyme and it is induced in cells and tissues to fight against superoxide concentrations. In

our study the slight increase in SOD activity might be due to exposure of patients to superoxide radicals, which are present in petrol and diesel exhaust. Increase in SOD activity along with decrease in catalase activity was reported by (Rister and Baehner, 1976). Catalase is everywhere distributed in tissues of all species it is a haem protein, it acts on a substrate hydrogen peroxide (H_2O_2). The activity of catalase was controlled by the concentration of hydrogen peroxide. Catalase catalyses the following reactions.



In our present observation there is a decrease in the activity of catalase was observed in the red cell membrane. (Rister and Baehner, 1976) have reported the catalase inactivation in the bovine liver by oxygen free radical and hydrogen and hydrogen peroxide.

In the presence of SOD the catalase was inactivated to a lower extent but when the concentration of SOD falls, the catalase was inactivated to a greater extent this was proved in a biphasic experiment by (Kono and Frido Vich, 1982). In our study the slight increase in SOD level and decrease in catalase activity might be due to exposure of **NIDDM subjects** to vehicle exhaust. In our study oxidative stress parameters both lipid peroxidation and lipid hydroperoxides was elevated to a greater extent. The level of lipid peroxidation was increased to six fold when compared to normals but the level of lipid hydroperoxides was increased to double fold, this indicates the severity of complications.

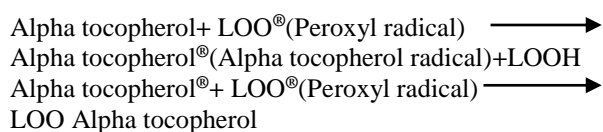
We would like to discuss the elevation in lipid peroxidation and lipid hydroperoxides under the following. Reactive oxygen species results due to uncontrolled hyperglycaemia. (Brown Lee, 2001). (Wolff and Dean, 1987) and (Mullarkey *et al.*, 1990) stated the theories of oxidation and glycation relationship, in the presence of trace of metal ions simple monosaccharides like glucose and fructose autooxidise and generate superoxide radicals, hydrogen peroxide and hydroxyl radicals. In uncontrolled hyperglycaemia a different situation arises, the excess glucose activates several major biochemical pathways, these include Advanced glycation end products (AGEs) and receptors for advanced glycation end products (AGE) (RAGE) (Brown Lee, 1995), Protein kinase C (PKC) (Koya *et al.*, 1998), and polyol pathway (Stevens *et al.*, 2000). Uncontrolled hyperglycaemia also leads to activation of additional biochemical pathway including the stress activated signalling pathways of nuclear factor-

kB(NF-kB)NH₂-TERMINAL Jun kinases/stress activated protein kinases(JNK/SAPK), p38 mitogen activated protein kinases and hexoseamine.(Barnes *et al.*,1997),(Kyriakis and Avruch,1996),(Marshall *et al.*,1991). Due to increased Reactive oxygen species or Reactive Nitrogen species oxidative stress results.The reactive oxygen species (ROS) include superoxide and hydroxyl radicals,uncharged species H₂O₂(Hydrogen peroxide).(Rosen *et al.*,2001). Reactive oxygen species (ROS) directly oxidise lipids , membranes and causes lipid peroxidation , lipid hydroperoxides formation,it also affect DNA and protein. ROS are responsible to play a major role in the pathogenesis of late diabetic complications (Nishikawa *et al.*,2000).

In our study the elevation in Reactive oxygen species (ROS) among **NIDDM** patients might be due to chronic exposure to diesel and petrol exhaust which can trigger the elevation in lipid peroxidation and lipid hydroperoxide formation. We have also observed elevation in oxidised LDL levels.Oxidised LDL is elevated under the following conditions a)Uncontrolled hyperglycaemia b)Obesity. (Wolff and Dean ,1989) stated the theories of oxidation and glycation relationship.In the presence of trace of metal ions simple monosaccharides like glucose and fructose Autooxidise and generate superoxide radicals,hydrogen peroxide and hydroxyl radicals.Thus when lipoproteins in high glucose medium undergoes oxidation,which could affect both the apolipoproteins and the lipid core of the particle.Glyoxidised lipoproteins have greater atherogenic potential. Elevation of Oxidised LDL is also seen in a)Atherogenesis b)endothelial injury c)Expression of Adhesion molecules d)Leukocyte retention and recruitment as well as foam cell and thrombus formation (Stein Berg *et al.*,1989). The level of vitamins E and C was significantly reduced among **NIDDM** exposed to diesel and vehicle exhaust.The depletion of vitamin E and C is accompanied with the depletion of catalase activity. Ascorbic acid or vitamin C undergoes oxidation and reduction. Ascorbic acid protects the cell from cellular damage, oxygen toxicity,and also lipid peroxidation caused by free radicals.(Procter and Reynold, 1984)

Tocopherol or Vitamin E effectively inhibits lipid peroxidation (Burton and Ingold, 1981).

Alpha tocopherol catalyses the following reactions



The alphatocopherol radical thus formed reacts with another peroxy radical to form a stable adduct(Matsumoto *et al.*,1986)

In our studyt the elevation in lipid peroxide and lipid hydroperoxide radical might be the cause of depletion of vitamin E among **NIDDM** subjects exposed to diesel and petrol vehicle exhaust.

The anti-atherogenic mechanism of vitamin E and C on LDL oxidation was discussed very clearly by (Anita *et al.* 2000). It was proved in their observation that the positive synergistic action of Vitamin E and C in protecting LDL on oxidation.In our observation, both Vitamin E and C was found to be very much depleted among **NIDDM** subjects when compared to healthy controls.This depletion in Vitamin E and C level might due to chronic exposure of **NIDDM** subjects to petrol and diesel exhaust which contain more free radicals.

4. CONCLUSION

Depletion in Vitamins and antioxidants still worsens the **NIDDM** subjects on exposure to vehicle exhaust and smoking.The **NIDDM** subjects should be administered with antioxidants and vitamins to combat the free radicals damage.The administration of vitamin E and C to a certain extent lower oxidised LDL levels.

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REFERENCES

- Anitra, C., Carr, Ben Zhan Zhu, and Balz Frei., Potential Antiatherogenic Mechanism of Ascorbate (Vitamin C)and α - Tocopherol(Vitamin E)., *Cir Res*, 87, 349-354 (2000).
- Barnes P. J., Karin M, Nuclear Factor- Kappa B :a pivotal transcription factor in chronic inflammatory disease,*N Eng. J.Med*, 336, 1066-1071 (1997).
- Brown Lee M., Advanced protein Glycation in diabetes and Ageing., *Ann. Rev. Med* 46, 223-234 (1995).

- doi:10.1146/annurev.med.46.1.223
- Brown Lee, M., Biochemistry and Molecular cell biology of diabetic complications., Nature, 414, 813-820(2001).
doi:10.1038/414813a
- Buege J. A. and Aust S. D., Microsomal lipid peroxidation, Methods in Enzymol., 52, 302-310 (1978).
doi:10.1016/S0076-6879(78)52032-6
- Burton, G. W., Ingold K. U., Auto oxidation of Biological molecules: The antioxidant activity of Vitamin E and related chain breaking phenolic antioxidants invitro., J. Am. Chem. Soc., 103 (21), 6472-6477 (1981).
doi:10.1021/ja00411a035
- Dodge, J. T., Mitchell G., and Hanahan D.J., The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes., Arch Biochem Biophys, 100, 119-130 (1963).
doi:10.1016/0003-9861(63)90042-0
- Friedewald, W. T., Levy R. I., Fredrickson DS., Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clinical Chemistry, 18 (6), 499-502(1972).
- Halliwell B., Gutteridge, J. M. C., Free Radicals in Biology and Medicine., Oxford Clarendon press 2nd Edition, Society for Experimental biology and Medicine., 206, 248-254(1989).
- Hasan, H. M. and Fridovich I., Regulation of the synthesis of Superoxide dismutase in Escherichia Coli, Induction by Methyl Viologen., The journal of Biological chemistry., 252, 7667-7672(1977).
- Hol Voet P., Vanhaecke J., Janssens S., Van de Werf P., Collen D., Oxidised LDL and Malondialdehyde modified LDL in patient with acute coronary syndrome and stable coronary artery disease, Circulation, 98, 1487-1494 (1998).
doi:10.1161/01.CIR.98.15.1487
- Kokoglu, E., Aktuglu, G., and Belce A., Leukocyte superoxide dismutase levels in acute and chronic Leukemias., Leukemia Res., 13, 457-458(1989).
doi:10.1016/0145-2126(89)90166-5
- Koster, J. F., Biemond, P., Swaak A. J., Intracellular and Extracellular sulphhydryl levels in Rheumatoid arthritis, Ann Rheu Dis, 45, 44-46(1986).
doi:10.1136/ard.45.1.44
- Koya, D., King, G. L., Protein kinase C activation and the development of diabetic complications., Diabetes, 47, 859-866(1998).
doi:10.2337/diabetes.47.6.859
- Krinsky, N. I., Deneke, S. M., The interaction of oxygen and oxygen radicals with carotenoids., JNCI, 68, 205-210, (1982).
- Kyriakis JM., Avruch J., Sounding the alarm : Protein kinase cascades activated by stress and inflammation, J Biol chem, 271, 24313-24316 (1996).
doi:10.1074/jbc.271.40.24313
- Lang P. D. and Schettler, G., In Schettler G and R Gross Arteriosclerosis, Grundlageon-Diagnostik-Therapie, Deutscher Arzte verlag GmbH, cologne, W. Germany (1985).
- Larson K, Creatinine assay by a reaction kinetic principle, Clin.chim.Acta:41, 209-211 (1972).
doi:10.1016/0009-8981(72)90513-X
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randal, R. J., Protein measurement with folin phenols reagent., J. Biol. Chem., 193, 265-275 (1951).
- Luckey, T. D., Venugopal B. and Hutcheson., Heavy metal Toxicity and Hormonology, Academic press New York, 9, 650-654(1975)
- Marsh, M. H., Fungerhut, B. and Miller, H. et al., Automated and manual direct method for the determination of blood urea, Clin. chem., 11, 624-627(1965).
- Marshall, S., Garvey, W. T., Traxinger, R. R., New insight into the metabolic regulations of insulin action and insulin resistance role of glucose and aminoacids, FASEB.J, 5, 3031-3036 (1991).
- Matsumoto, S., Matsuo, M., litaka, Y. and Niki E., Oxidation of Vitamin E model compound 2,2,5,7,8-Pentamethylene chroman-6-01, with tetrabutyl peroxy radical., Journal of the Chemical Societ. Chemical Communication, 14, 1076-1077 (1986).
doi:10.1039/c39860001076
- McCormick, D. B. and Wright, L. D., Vitamins and coenzymes, In methods in Enzymology, McCormick DB, Wright LD, Eds New York Academic., (1980).
- Misra and Fridowich I., The role of superoxide anion in the Autooxidation of Epinephrine and a simple assay for superoxide dismutase., J. Biolchem, 247, 3170-3175(1972).
- Mullarkey, C. J., Edelstein D. and Brown Lee M., Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in Diabetes., Biochem. Biophys. Res. Commun., 173, 932-939 (1990).
doi:10.1016/S0006-291X(05)80875-7
- Procter H., Reynold ES., Free radicals and disease in man., Physiol Chem Phys, 16, 175-195 (1984).
- Rister and Baehner, The Alteration of Superoxide Dismutase, Catalase, Glutathione peroxidase, and NAD(P)H Cytochrome c Reductase in Guinea pig polymorpho nuclar leukocytes and Alveolar

- Macrophags during Hyperoxia, *J Clin Invest*, Nov, 58 (5), 1174-1184 (1976).
- Rosen P., Nawroth, P. P., King G., Moller W., Tritschler R. J., Packer L., The Role of oxidative stress in the onset and progression of diabetes and its complications a summary of Congress Series sponsored by UNESCO-MCBN, The American Diabetes Association and the German Diabetes society. *Diabetes Metab Res Rev.*, 17, 189-212, (2001).
doi:10.1002/dmrr.196
- Rosen P., Nawroth, P. P., King G., Moller W., Tritschler, R. J., Packer, L., The Role of oxidative stress in the onset and progression of diabetes and its complications a summary of Congress Series sponsored by UNESCO-MCBN, The American Diabetes Association and the German Diabetes society. *Diabetes Metab Res Rev.*, 17, 189-212 (2001).
doi:10.1002/dmrr.196
- Schwaiger, H, Weirich, H. G, Brunner, Rass P, Hirsch-Kauffmann M., Groner, Y, Schweiger, M., Radiation sensitivity of Down's syndrome fibroblasts might be due to overexpressed Cu/Zn-superoxide dismutase (EC 1.15.1.1). *Eur. J. Cell Biol*, triangle park NC (1978).
- Sinha, A. K. Colorimetric assay of catalase., *Anal Biochem.*, vol 47, 389-394 (1972).
doi:10.1016/0003-2697(72)90132-7
- Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., Witztum, J. L., Beyond cholesterol modification of low- density lipoprotein that increases its atherogenicity, *New Engl J Med*, 320, 915-924 (1989).
- Stevens, M. J., Obrosova, I., Feldman, E. L. and Greene D. A., The sorbitol- osmotic and sorbitol –redox hypothesis, In *Diabetes Mellitus :A Fundamental and clinical Text.*, LeRoith D., Taylor SI., Olef Sky JM., Eds. Philadelphia Lippincott Williams & Wilkins 2000, 972-983 (2000).
- Tsan, Z. L., Chin, N., Kiser, M. D., Bigler WN., Specific spectrophotometry of Ascorbic acid in serum or plasma by use of ascorbic acid oxidase., *Clin Chem*: 28, 2225-2228 (1982).
- Varley, H., Gowenlock, A. H. and Bell, M., *Practical Clinical Biochemistry* Fifth edition, Heinemann Professional Publishing Ltd., Wang, J and Yang, G, Colorimetric detection of glycosylated haemoglobin in humans, 5, 385-405 (1984),
- Wolff, S. P. and Dean, R. T., Glucose Autooxidation and protein modification the potential role of "Auto oxidative glycosylation" in diabetes mellitus., *Bio chem. J.*, 245, 243-250 (1987).
- Zhen-Yue Jiang, Alison, C. S., Woolard, D. and Wolff, S. P., Hydrogen peroxide production during experimental protein glycation, *Febs Lett.*, 268, 69-71 (1990).
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