1. Introduction:

1.1 Introduction:

Superoxide dismutase is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. Cigarette smoke contains abundant amount of oxidants and superoxide (O_2^-) anion from cigarette smoke may rich the vascular endothelium and can then react with nitric oxide (NO-) to form peroxynitrite anion, a highly reactive intermediate with strong cytotoxic potency. Thus the damaging free radicals in cigarette smoke may cause either direct arterial wall injury or protein peroxidation and activation of phagocyte platelet endothelial cell interaction.(PalanisamyPasupathi *etal*,.2009)

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defense, which may induce tissue injury. Oxidative stress can be assessed by measurement of reaction products of oxidative damage, like lipid peroxidation, DNA oxidation and protein oxidation. (Halliwell,.1996)

1.2 Rationale:

Cigarette smoking is a serious health problem and most important avoidable causes of death in world.

The leading causes of death from smoking are cardiovascular diseases (1.69 million deaths), chronic obstructive pulmonary disease (0.97 million deaths) and lung cancer (0.85 million deaths).

In Sudan, it has been estimated that the prevalence of cigarette smoking in the adult is 24% in 2009, according to a WHO report, published in 2010. Cigarette consumption is one of the main risk factors for a number of chronic diseases, including cancer, lung diseases, and cardiovascular diseases.

So, the SOD is considered as an antioxidant that enhance oxidative stress in smoking people.

This study is the first one done in Sudan, people must take care to the substance induced to their body because oxidant kill our cells and cause diseases.

1.3 Objectives:

1.3.1 General Objective:

To assess erythrocyte superoxide dismutase activity in Sudanese male cigarette smokers.

1.3.2 Specific Objective:

- To compare erythrocyte superoxide dismutase activity in cigarette smokers and non smokers.
- To correlate between erythrocyte superoxide dismutase activity and number of cigarette per day.
- To correlate between erythrocyte superoxide dismutase activity and duration of smoking.

2. Literature review

2.1 Smoking:

Cigarette smoke is a complex mixture of chemicals containing more than 4000different constituents. In the last 30-40 years, a large body of accumulated identifying the exact chemical composition of cigarette smokes both qualitatively and quantitatively. Some of the compounds identified include different pyridine alkaloids such as nicotine, ammonia, acrolein, phenols, acetaldehyde-nitrosamine; polycyclic aromatic hydrocarbons such as benzopyrine; combustion gases such as carbon monoxide, nitrogen oxides, hydrogen cyanide; trace metals, emitter radioactive elements such as polonium, radium, and thorium (Koul *etal*,.2001).

Two major phases were identified in cigarette smoke: a tar phase and a gas phase;both phases are rich in oxygen-centered, carbon-centered and nitrogen-centered free radicals as well as non-radical oxidants. From the analysis of each phase, it was estimated that a single cigarette puff contains approximately, 10^{x14} free radicals in the tar phase, and 10^{x15} radicals in the gas phase. These include various compounds, which are capable of causing an increase in the generation of various reactive oxygen species (ROS) like superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl (OH) and peroxyl (ROO) radicals. These reactive oxygen species in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (Kasap *etal*,.2007).

Evidence suggests that reactive oxygen species (ROS) may play important

roles in the pathogenesis in myocardial infarction. Following ischemia, ROS are produced during reperfusion phase. ROS are capable of reacting with unsaturated lipids and of initiating the self-perpetuating chain reactions of lipid peroxidation in the membranes (Anazawa *etal*,.2004).

Numerous reports have demonstrated the increased risk of coronary problems in smokers (Fagerstrom *etal*,.2002). Smoking is thought to have an influence on the prevalence of myocardial infarction by means of several mechanisms, including atherosclerotic injury, increase in platelet aggregation, and increase in the levels of adhesion molecules and fibrinogen and vasoconstriction (Zornoff *etal*,.2007).

Cigarette smoking leads to the uptake of many hazardous compounds. Such compounds or their metabolites may be electrophilic and thereby able to react with biological macromolecules, or they may give rise to oxidative stress by formation of reactive species or the initiation of radical chain reactions (Orhan *etal*,.2005).

2.2 Oxidative stress:

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS)

generated, e.g. O2− (superoxide radical), OH (hydroxyl radical) and H2O2 (hydrogen peroxide) (Kala Chandra *etal*,.2015).

2.3 Oxidative stress biomarkers:

All biomolecules can be damaged by ROS. Oxidative damage to lipids, proteins, nucleic acids, and carbohydrates can be deleterious and concomitant (Halliwell *etal*,.1999). The primary cellular target of oxidative stress depends on the cell type, the nature of the stress imposed, the site of generation, the proximity of ROS to a specific target, and the stress severity. The half-lives of ROS are usually short. Therefore, special techniques are necessary to detect ROS in vivo. So-called “oxidative stress biomarkers” can not only determine the extent of oxidative injury, but also indicate the source of the oxidant (Noiri *etal*,.2005). Oxidative stress biomarkers are important forpredicting the consequences of oxidation and for providing a basis for designing appropriate interventions to prevent or alleviate injury. Briefly, oxidative stress biomarkers are separable into two categories: (a) formation of modified molecules by ROS; and (b) consumption or induction of enzymes or antioxidants (Toyokuni *etal*,.1999) Measurement of these biomarkers in body fluids (e.g., blood, urine, cerebrospinal fluid, bronchoalveolar lavage fluid) or breath condensate (Kharitonov *etal*,.2002).

The first category (a) includes molecules that are generated in a reaction with ROS. Molecules are subjected to either scission, cross-linking or covalent modification in these reactions. Accordingly, the amount of these molecules is increased when ROS are generated. Some are rapidly removed or repaired, but others remain in intracellular or extracellular compartments for a long time. Major targets of ROS in the molecular components of the

cells are membrane lipids, proteins, nucleic acids, and carbohydrates. These markers are often measurable using stable adducts that are produced in vivo as a result of oxidative processes. Clinically applicable biomarkers include malondialdehyde-lysine, 4-hydroxy-2-nonenal-lysine, acrolein-lysine (markers of lipid peroxidation), 8-hydroxy-2'-deoxyguanosine (8-OHdG) (a marker of oxidative DNA damage), carboxymethyl-lysine, pentosidine (markers of glyco-oxidation), nitrotyrosine, nitrite/nitrate (markers of nitro-oxidation), bilirubin oxidative metabolites (BOM) (a marker of HO activity).The second category (b) consists of antioxidant enzymes and molecules that are associated with ROS metabolism. In most cases, these molecules are destroyed or modified and exhibit decreased activity or quantity after exposure to ROS. Conversely, they often show an overshooting response for a matter of hours, days, or weeks (Noiri *etal*,. 2005).

2.4 Antioxidant protection system:

To protect the cells and organ systems of the body against reactive oxygen species (ROS), humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals (Koleva *etal*,.2002).

These components include:

A. Endogenous Antioxidants

- Bilirubin

- Thiols, e.g., glutathione, lipoic acid, N-acetyl cysteine
- NADPH and NADH
- Ubiquinone (coenzyme Q10)
- Uric acid
- Enzymes:

-Copper/zinc and manganese-dependent superoxide dismutase.

-Iron-dependent catalase.

-Selenium-dependent glutathione peroxidase.

B.Dietary Antioxidants

- Vitamin C
- Vitamin E
- Beta carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein.
- Polyphenols, e.g., flavonoids, flavones, flavonol's, and Proanthocyanidins.

c. Metal Binding Proteins

- Albumin (copper)
- Ceruloplasmin (copper)
- Metallothionein (copper)

- Ferritin (iron)
- Myoglobin (iron)
- Transferrin (iron)

2.5 Superoxide dismutase (SOD) :

In 1967 biochemist Irwin Fridovitch of DukeUniversity and Joe McCord discovered the antioxidant enzyme SOD, which provides an important means of cellular defence against free radical damage. This breakthrough caused medical scientists to begin to look seriously at free radicals. In most cases the process is automatically controlled and the number of free radicals does not become dangerously high. Fortunately, the body has, throughout the course of millions of years of evaluation become accustomed to coping with free radicals and has evolved various schemes for doing this (Khal *etal*,.1986).

SOD (EC 1.15.1.1) is the antioxidant enzyme that catalysed the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPX reactions (Zheng *etal*,.2001).

2.5.1 Types of superoxide dismutase:

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) (Miller *etal*,.1995). SOD destroys O_2 by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates (Donovan *etal*,.1998).

All types of SOD bind single charged anionssuch as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe-, Mn- or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibitedby N3-, CN-, and by F- (Halliwell,.1996)

- Mn-SOD is a homotetramer (96 kDa) containing one manganese atom per subunit those cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide (Halliwell & Gutteridge,.1999).The respiratory chain in mitochondria is a major source of oxygen radicals. Mn-SOD has been shown to be greatly induced and depressed by cytokines, but is only moderately influenced by oxidants (Halliwell & Gutteridge ,.1999). Inactivation of recombinant human mitochondrial Mn-SOD by peroxynitrite is caused by nitration of a specific tyrosine residue (Desmarchelier *etal*,.2000).
- Cu/Zn-SOD (SOD-1) is another type of enzymes that has been conserved throughout evolution. These enzymes have two identical subunits of about 32 kDa, although a monomeric structure can be found in a high protein concentration from E. coli (Halliwell &Gutteridge,.1989). Each subunit contains a metal cluster, the active site, constituted by a copper and a zinc atom bridged by a histamine residue (Gordon,.1990). Cu/Zn-SOD is believed to play a major role in the first line of antioxidant defence. Calves that were fed milk supplemented with 25 ppm Cu and 100 ppm Zn showed a stronger immune response and a higher SOD activity (Amado *etal*,.2007).

Other recent reports involving SOD knock-outs have revealed that Mn- SOD is essential for life whereas Cu/Zn-SOD is not. Cu/Zn-SOD knock-out mice appear normal and exhibit differences only after traumatic injury, whereas Mn-SOD knockouts do not survive past 3 weeks of age (Singleton & Rossi,.1965).

Among various human tissues Mn-SOD contents were roughly one-halfas large as the Cu/Zn-SOD contents (Singleton & Rossi,.1965).

- Extracellular superoxide dismutase (EC-SOD) is a secretory, tetrameric, copper and zinc containing glycoprotein; with a high affinity for certain glycosaminoglycans such as heparin and heparin sulphate. EC-SOD was found in the interstitial spaces oftissues and also in extracellular fluids, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid. EC-SOD is not induced by its substrate or by other oxidants and its regulation in mammalian tissues primarily occurs in a mannercoordinated by cytokines, rather than as a response of individual cells to oxidants (Velioglu *etal*,.1998).

2.5.2Biochemistry of superoxide dismutase :

SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin-forbidden. In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly (~105 $M^{-1}s^{-1}$ at pH 7). SOD is necessary because superoxide reacts

with sensitive and critical cellular targets. For example, it reacts with the NO radical, and makes toxic peroxynitrite(Smirnoff *etal*,.1993).

2.5.3 Physiology of superoxide dismutase:

Superoxide is one of the main reactive oxygen species in the cell. As a consequence, SOD serves a key antioxidant role. The physiological importance of SODs is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes. Mice lacking SOD2 die several days after birth, amid massive oxidative stress (Raychaudhuri & Deng,.2008). Mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma, (Elchuri *etal*,.2005). an acceleration of age-related muscle mass loss, an earlier incidence of cataracts and a reduced lifespan. Mice lacking SOD3 do not show any obvious defects and exhibit a normal lifespan, though they are more sensitive to hyperoxic injury (Sentman *etal*,.2006).

2.5.4 Role in disease:

Mutations in the first SOD enzyme (SOD1) can cause familial amyotrophic lateral sclerosis (ALS, a form of motor neuron disease) (Milani *etal*,.2011).

The most common mutation in the U.S. is A4V, while the most intensely studied is G93A. The other two isoforms of SOD have not been linked to any human diseases, however, in mice inactivation of SOD2 causes perinatal lethality (Groner *etal*,.1994) and inactivation of SOD1 causes hepatocellular carcinoma (Rujito *etal*,.2015) by a mechanism that is presently not understood, but not due to loss of enzymatic activity or a decrease in the conformational stability of the SOD1 protein. Overexpression of SOD1 has

been linked to the neural disorders seen in Down syndrome (Groner *etal*,.1994) In patients with thalassemia, SOD will increase as a form of compensation mechanism. However, in the chronic stage, SOD does not seem to be insufficient and tends to decrease due to the destruction of proteins from the massive reaction of oxidant-antioxidant (Rujito *etal*,.2015).

2.5.5 Pharmacological activity :

SOD has powerful ant inflammatory activity. For example, SOD is a highly effective experimental treatment of chronic inflammation in colitis. Treatment with SOD decreases reactive oxygen species generation and oxidative stress and, thus, inhibits endothelial activation and indicates that modulation of factors that govern adhesion molecule expression and leukocyte-endothelial interactions. Therefore, such antioxidants may be important new therapies for the treatment of inflammatory bowel disease (Seguí *etal*,.2004).

2.5.6 Cosmetic uses :

SOD may reduce free radical damage to skin for example, to reduce fibrosis following radiation for breast cancer. Studies of this kind must be regarded as tentative, however, as there were not adequate controls in the study including a lack of randomization, double-blinding, or placebo (Campana *etal*,.2004).

2.5.7 Commercial sources :

SOD is commercially obtained from bovine liver, horseradish, and cantaloupe and by fermenting certain bacteria, though it is found in most

living forms at diverse concentrations. For therapeutic purpose, SOD is usually injected locally. There is no evidence that ingestion of unprotected SOD or SOD-rich foods can have any physiological effects: as all ingested SOD is broken down into amino acids before being absorbed. However, ingestion of SOD bound to wheat proteins could improve its therapeutic activity, at least in theory (Romao,.2015).

3. Materials and methods

3.1 Study design :

Analytical case control study.

3.2 Study area :

The study was conducted in Khartoum states .

3.3 Study period :

The study was carried during the period from February to May 2015.

3.4 Study population :

Non hospitalized healthy Sudanese smoker individuals .

3.5 Selection criteria :

Inclusion criteria :

Cigarette smokers (more than 5 years) as test group and non smokers as control group, both test and control group were apparently healthy individual.

Exclusion criteria :

Smokers less than 5 year and individuals with clinical history of any chronic disease had been excluded.

3.6 Sample size :

The study sample size was 120 (60 smoker as test group and 60 non smoker as control) were enrolled in this study.

3.7 Sample collection :

After informed consent and use local antiseptic for skin (70%) ethanol, 3ml of venous blood was collected from each volunteer in this study using disposable plastic syringe. The venous blood poured in EDTA container.

3.8 Ethical consideration:

Objective of this study were explained to all participating in this study. Information were obtained from all participating in this study were kept as highly confidence, data and specimen result we're not be permitted.

3.9 Data collection :

Interview and questionnaire: interview with cigarette smoker and control were done to obtain the clinical data, and it was specifically designed to obtain information which help in either including or exclusion certain individual in or from study. See questionnaire sheet (Appendix I).

3.10 Biomedical measurement:

Superoxide dismutase activity was measured using spectrophotometer analyzer and reagent from fortress diagnostics.

Principle:

Superoxide dismutase (SOD) role is to accelerate the dismutation of the toxic superoxide radical (02•), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. Fortress method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride

(I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50%inhibition of the rate of reduction of INT under the conditions of the assay (Appendix II).

3.11 Data analysis :

Data was analyzed using SPSS (independent T-test and pearson correlation test).

3.12 Quality control :

The precision and accuracy of method use in study was cheeked and was analyzed.

4. Results:

Erythrocyte superoxide dismutase was measured in 60 smokers as test group and 60 non smokers as control group, during the period of February to May 2015.

Table (4.1): Comparison of superoxide dismutase activity in cigarette smokers and non smokers control group (mean ± SD: 907±134.47 versus 1159±93.44, P = 0.00).

Figure (4.1): A scatter plot shows negative moderate correlation between the level of superoxide dismutase and number of cigarette per day (r = -.044, P = 0.00).

Figure (4.2): A scatter plot shows significant weak negative correlation between the level of superoxide dismutase and duration of smoking (year) (r = -.25, P = 0.048)

Table (4.1) comparison of superoxide dismutase activity in cigarette smokers and non smokers control group:

Variable	**Smoker N=60**	**Non smoker N=60**	**p-value**
Superoxide dismutase activity mean(U/g Hb) ± SD	907.45 ± 134.476	1159.80 ± 93.447	0.00

The table shows the mean ± SD, and the probability (p).

T-test was used for comparison.

P-value ≤ 0.05 is considered significant.

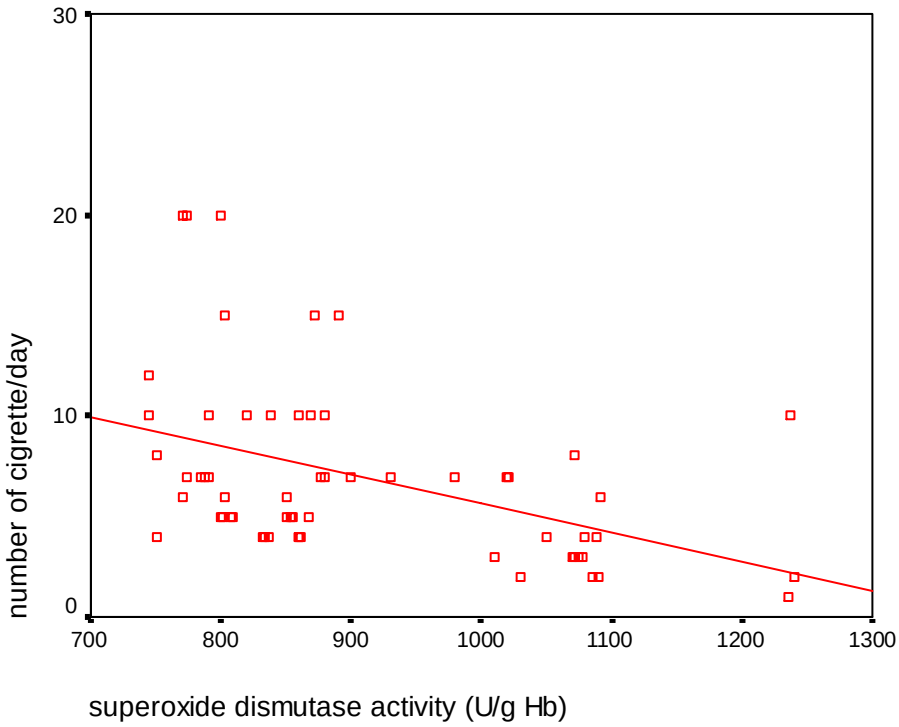

Figure (4.1): correlation between the level of superoxide dismutase and number of cigarette per day (r = -.044, P = 0.00).

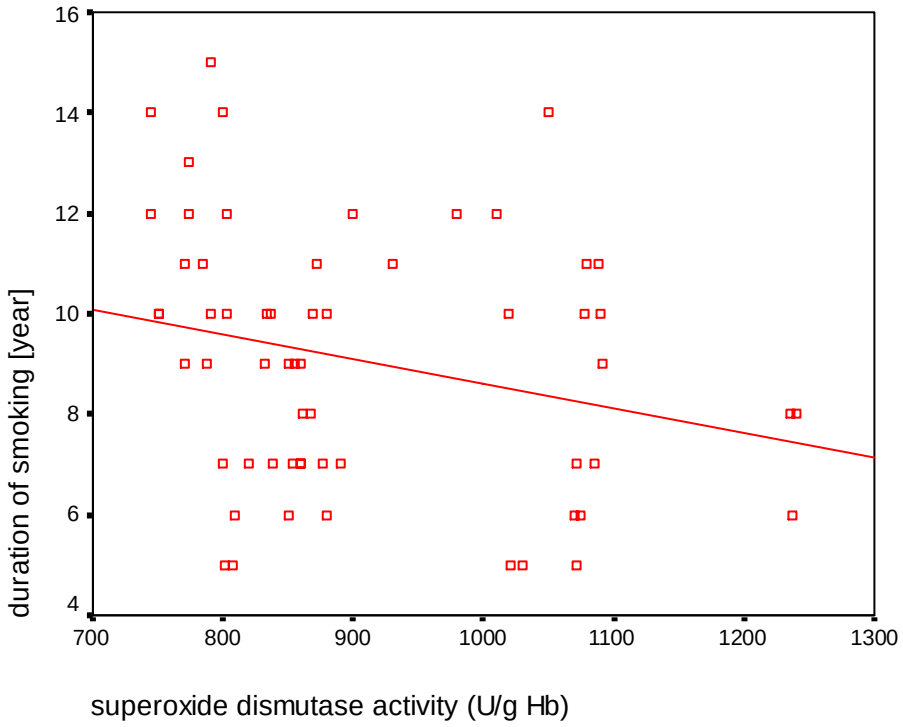

Figure (4.2): correlation between the level of superoxide dismutase and duration of smoking (year) (r = -.25, P = 0.048).

5. Discussion, Conclusion and Recommendation

5.1 Discussion:

Cigarette smoking is a serious health problem and most important avoidable causes of death in world. The risk of disease increases with increasing intensity and duration of smoking. In this study the level of superoxide dismutase was found to be significantly decreased in cigarette smokers compared with non smokers (907±134.47 versus 1159±93.44). Justification of this study results that SOD is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. Cigarette smoke contains abundant amount of oxidants and superoxide (O_2^-) anion from cigarette smoke may rich the vascular endothelium and can then react with nitric oxide (NO-) to form peroxynitrite anion, a highly reactive intermediate with strong cytotoxic potency. Thus the damaging free radicals in cigarette smoke may cause either direct arterial wall injury or protein peroxidation and activation of phagocyte platelet endothelial cell interaction.

This finding within the same line with those obtained by (Mahendra *etal*,.2014) which found decreased in SOD activity in smokers compare with control (714.25±72.42 versus 952.58±92.25).

(Palanisamy asupathi *etal*,.2009) which found decreased in SOD activity in smokers compare with control(2.71±0.29 versus 4.30±0.40) , and (HeidarTavilani *etal*,.2012) which also found that SOD activity in smokers is 1.26 ±0.24 compare with 144.2±15.2 in control.

Also study result showed negative moderate correlation between the level of superoxide dismutase and number of cigarette per day(r = -.044, P = 0.00).

decrease enzyme activity due to increase number of cigarette. also showed significant weak negative correlation between the level of superoxide dismutase and duration of smoking (r = -.25, P = 0.048)increase smoking duration lead to decrease superoxide dismutase activity. No recent studies support this finding.

5.2 Conclusion:

From this study it is concluded that:

1 .The level of superoxide dismutase is significantly decreased in cigarette smokers.

2 .There is significant negative correlation between the level of superoxide dismutase and number of cigarette per day.

3 .There is significant negative correlation between the level of superoxide dismutase and duration of smoking (year).

5.3 Recommendation:

1. Periodic follow up of the enzyme because smoking is consider as risk factor.

2. Using of SOD enzyme as a biomedical marker for oxidative stress.

References

- Amado LL, Jaramillo MD, Rocha AM, Ferreira JLR, Garcia LM, Ramos PB et al. (2007) A new method to evaluate total antioxidant capacity against reactive oxygen and nitrogen species (RONS) in aquatic organisms. Comp. Biochem. Physiol. Part A,148: S75-76.
- Anazawa, T., Dimayuga, P.C., Li, H., Tani, S., Bradfield, J., Chyu, K.Y., Kaul, S., Shah, P.K., Cercek, B., Thromb. Vasc. Biol. 2004, 24: 1652-1658.
- Campana F, Zervoudis S, Perdereau B, Gez E, Fourquet A, Badiu C, Tsakiris G, Koulaloglou S (2004). "Topical superoxide dismutase reduces post-irradiation breast cancer fibrosis". Journal of Cellular and Molecular Medicine 8 (1): 109–16.
- Desmarchelier C, Ciccia G, Cussio J (2000)Recent advances in the search for antioxidant activity in South American plants.In: Atta-ur-Rahman editor. Studies in Natural Products Chemistry, Vol. 22, pp. 343-367
- Donovan JL, Meyer AS, Waterhouse AL (1998) Phenolic composition and antioxidant activity of prunesand prune juice (Prunusdomestica). J. Agric. Food Chem.46: 1247-1752.
- Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, Epstein CJ, Huang TT (Jan 2005). "CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life". Oncogene 24 (3): 367–80.
- Fagerstrom, K., Drugs. 2002, 2: 1-9.
- Goodarzi , Respir Care 2012;57(12):2090–2094.

- Gordon M (1990) The mechanism of antioxidation action in vitro. In: Hudson BJF editor. Food Antioxidants. London: Elsevier. pp. 1-18.
- Groner Y, Elroy-Stein O, Avraham KB, Schickler M, Knobler H, Minc-Golomb D, Bar-Peled O, Yarom R, Rotshenker S (1994). "Cell damage by excess CuZnSOD and Down's syndrome". Biomedicine & Pharmacotherapy = Biomédecine&Pharmacothérapie 48 (5-6): 231–40.
- Halliwell B (1996) Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radical Res. 25: 57-74.
- Halliwell B, Gutteridge JMC (1989). Free radicals in biology and medicine ,Clarendon Press: Oxford.
- Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine, ed 2,Clarendon Press, Oxford, 1999.
- HeidarTavilani , EbrahimNadi , JamshidKarimi , and Mohammad Taghi ,Respir Care 2012;57(12):2090–2094.
- Kala Chandra, Ali Syed Salman*, AbidMohd., RajpootSweety, Khan Najam Ali.2015, Protection Against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of Costusspeciosus Rhizome ,International Journal of Pharmacognosy and Phytochemical Research 2015; 7(2); 383-389.
- Kasap, S., Gonenc, A., Sener, D.E., Hisar, I., J. Clin. Biochem. Nut. 2007, 41: 50-57.
- Khal R, danHilderbrant AG (1986) Methodology for studying antioxidant activity and mechanism of action of antioxidant. Food Chem Toxicol.24: 1007-1014.

- Kharitonov, S.A.; Barnes, P.J. Biomarkers, 2002, 7: 1-32.
- Koleva II, Van Beek TA, Linssen JPH, de Groot A. danEvstatieva LN (2002) Screening of plant extract for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 13: 8-17 .
- Koul, A., Bhatia, V., Bansal, M.P., BMC 2001, 2: 14-18.
- MahendraD.BikkadSandeep H. GhugeSachin D. Somwanshi And Sachin B. Ingle(2014) International Journal of Basic and Applied Medical Sciences, Vol. 4 (1) January-April, pp. 1-6/ Bikkadetal.
- Milani P, Gagliardi S, Cova E, Cereda C (2011). "SOD1 Transcriptional and Posttranscriptional Regulation and Its Potential Implications in ALS".
- Miller NJ, Diplock AT, dan Rice-Evans CA (1995) Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage.J. Agric. Food Chem. 43: 1794-1801.
- Noiri, E.; Tsukahara, H. J. Investig. Med.,2005, 53, 167-175.
- Orhan, H., Evelo, C.T.A., Sahin, G., J. Biochm. Mol. Toxicol. 2005, 19, 226-233.
- PalanisamyPasupathi , G.Saravanan and J. Farook , Pharm. Sci. & Res. Vol.1(2), 2009, 55-62.
- Raychaudhuri SS, Deng XW (2008). "The Role of Superoxide Dismutase in Combating Oxidative Stress in Higher Plants". The Botanical Review 66 (1): 89–98.
- Romao S (Mar 2015). "Therapeutic value of oral supplementation with melon superoxide dismutase and wheat gliadin combination". Nutrition 31 (3): 430–6.

- Rujito L, Mulatsih S, Sofro AS (May 2015). "Status of Superoxide Dismutase in Transfusion Dependent Thalassaemia". North American Journal of Medical Sciences 7 (5): 194–8.
- Seguí J, Gironella M, Sans M, Granell S, Gil F, Gimeno M, Coronel P, Piqué JM, Panés J (Sep 2004). "Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine". Journal of Leukocyte Biology 76 (3): 537–44.
- Sentman ML, Granström M, Jakobson H, Reaume A, Basu S, Marklund SL (Mar 2006). "Phenotypes of mice lacking extracellular superoxide dismutase and copper- and zinc-containing superoxide dismutase". The Journal of Biological Chemistry 281 (11): 6904–9.
- Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdicphosphotungestic acid reagents. Am. J. Enol. Viticult. 16: 144-158.
- Smirnoff, Nicholas (1993). "Tansley Review No. 52 The role of active oxygen in the response of plants to water deficit and desiccation". Plant Phytology 125.
- Toyokuni, S. Pathol. Int.,1999, 49, 91-102.
- Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Agric. Food Chem. 46: 4113-4117.
- Zheng W, dan Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J. Agric.Food Chem. 49(11): 5165-5170.

- Zornoff, L.A.M., Duarte, D.R., Minicucci, M.F., Azevedo, P.S., Matsubara, B.B., Matsubara, L.S., Campana, A.O., Paiva, S.A.R., Arq. Bras. Cardiol. 2007, 89, 151-157.