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THESIS

TURNOVER OF RED GRAPE EXTRACT AGAINST NICOTINE INDUCED OXIDATIVE STRESS IN THE LUNG TISSUE OF MALE ALBINO RAT WITH REFERENCE TO AGING

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Abstract

Grape (Vitis vinifera L.) is one of the world's largest fruit crops (Shaker, 2006). With nearly 70 million tones currently produced worldwide grapes are possibly the world's largest cultivated fruit, most of which are used in wine making, while the rest are consumed as table grapes or processed into raisins, juices, jams or other products. Of all grapes, cultivars of the Vitis vinifera L. species are the most important throughout world, but especially in Europe (Mazza, 1995**)**. There are dozens of other less important species of grapes that belong to Vitis genus (Chalker-Scott, 1999; Zhao, et al., 2010).

Grape is non-climatic fruit that grows on the perennial and deciduous woody vines of the genus Vitis. Grapes can be eaten raw or used for making jam, juice, jelly, vinegar, wine, grape seed extracts and grape seed oil. Anthocyanins and other pigment chemicals of the larger family of polyphenols in red grapes are responsible for the varying shades of purple in red wines (Waterhouse, 2002; Brouillard et al., 2003). Most grapes come from cultivars of Vitis vinifera According to the Food and Agriculture Organization (FAO), 75,866 square kilometres of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit.

Comparing diets among western countries, researchers have discovered that although the French tend to eat higher levels of animal fat, surprisingly the incidence of lung disease remains low in France, a phenomenon named the French Paradox thought to occur from protective benefits of regularly consuming red wine. Apart from potential benefits of alcohol itself, including reduced platelet aggregation and vasodilation (Providencia , 2006), polyphenols (e.g., resveratrol) mainly in the grape skin provide other suspected health benefits such as (Opie and Lecour, 2007).

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Thesis submitted to
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PREFACE

Since the beginning of human civilization, medicinal plants have always remained a part and parcel of human society to combat and treat different diseases. In particular, in the Indian systems of medicine Rigveda, Charak Samhita, and Sushruta Samhita such descriptions have been documented. According to estimates of the World Health Organization (WHO) nearly 75% of the world's population currently uses herbs and other traditional medicines to treat diseases of different natures. The grape has been well recognized worldwide for over 2,000 years as one among the edible sweet fruits and recognized for its wide spectrum of biological properties.

Grape is non-climatic fruit that grows on the perennial and deciduous woody vines of the genus Vitis. Grapes can be eaten raw or used for making jam, juice, jelly, vinegar, wine, grape seed extracts and grape seed oil. Anthocyanins and other pigment chemicals of the larger family of polyphenols in red grapes are responsible for the varying shades of purple in red wines. Most grapes come from cultivars of Vitis vinifera. According to the Food and Agriculture Organization (FAO), 75,866 square kilometres of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit.

India is well-known for its rich traditional systems of medicine. The distinguishing feature of the traditional Indian medicinal plants are rich sources of substances that have several therapeutic properties. Numerous studies have been documented describing the capable health benefits of red grape (vitis vinifera) consumption, including anti-oxidative, anti-carcinogenic, anti-inflammatory and anti- cardiovascular and antibacterial properties. Our studies on the Antioxidant properties of red grapes possesses the highest antioxidant potential and inhibit whole oxidative stress in rats, Also exhibit free radical scavenging effects as studied using biochemical assays.

Nicotine is a naturally occurring alkaloid found primarily in the members of the Solanaceae family, which includes tobacco, potato, tomato, green pepper, and eggplant. Nicotine was first isolated and determined to be the major constituent of

tobacco in 1828. In commercial tobaccos, the major alkaloid is nicotine, accounting for about 95 % of the total alkaloid content. Tobacco use is the leading cause of death in the world today. With 4.9 million tobacco-related deaths per year, no other consumer product is as dangerous or kills as many people as tobacco (WHO, An international treaty for tobacco control, 2003).

Nicotine is the principal alkaloid contained in tobacco and it is believed to be the primary reason for cigarette smoking in many people particularly as they derive satisfaction and pleasant sensation from inhaling nicotine. It is widely consumed through cigarette smoking and tobacco chewing in 30-40% of the world's population. Shaw et al., (2000) reported that ''one cigarette reduces your life by 11 minutes'. Nicotine is oxidized into its metabolite cotinine, which has a long half-life, and may play a significant role in vascular diseases as well as generates free radicals in tissues of rats and exerts oxidative tissue injury. Which could be prevents by antioxidants and free radical scavengers.

The lungs are the primary organs of respiration in humans and many other animals. In mammals and most other vertebrates, two lungs are located near the backbone on either side of the heart. Their function in the respiratory system is to extract oxygen from the atmosphere and transfer it into the bloodstream, and to release carbon dioxide from the bloodstream into the atmosphere, in a process of gas exchange. Respiration is driven by different muscular systems in different species. The animals use their musculoskeletal systems to support and foster breathing. In humans, the primary muscle that drives breathing is the diaphragm. The lungs also provide airflow that makes vocal sounds including human speech possible.

Aging "as the sum total of accumulated deleterious changes which appear during life span of an organism resulting in failure to withstand to the stress of the environment".

- Rockstein

With the aging process, the elastin component of the lung matrix decreases while the amount of type III collagen increases, (Murray, 1986) making the

pulmonary system more compliant. Because of this, during respiratory cycles alveolar units can become destabilized, leading to fluid/mucous retention and atelectasis. Alveolar basement membrane also thickens with aging (Niewoehner and Kleinerman, 1974), which reduces diffusion capacity and may result in poor gas exchange (i.e., increased alveolar-arterial oxygen gradient). Indeed, the arterial oxygen tension (PaO_2) decreases with aging independent of any superimposed disease processes such as chronic obstructive pulmonary disease (COPD), pulmonary hypertension or heart failure. Even among elderly individuals without any associated lung disease, the alveolar-arterial oxygen gradient is larger than that observed in the young, healthy population, making it imperative that prediction equations for oxygen tension take into account age-related changes to partial pressure of oxygen. Carbon dioxide tension, on the other hand, is not materially influenced by age.

Even in the absence of any material insult or injury to the airways, bronchiolar diameter diminishes significantly after age 40, largely in response to decreased elastin fiber deposition in the supporting connective tissues (Niewoehner and Kleinerman, 1974). Physiologically, this leads to a marked reduction in the "tethering" forces of the surrounding matrix to keep the adjacent airways patent. This can increase airway resistance and promote premature airway closure and as such, elderly individuals can demonstrate mild airflow obstruction at low lung volumes even in the absence of any disease. Since airway inflammation is not a prominent feature of age-related changes in the airways, corticosteroids and bronchodilators are largely ineffective in reversing mild airflow obstruction associated with aging.

The author is aware of the facts that more extensive and indepth studies are essential for a thorough understanding of carbohydrate metabolic profiles,oxidative and antioxidant defence mechanism in aging and changes in free radical metabolism by red grape extract treatment. However, the present findings may throw some light on the combination of nicotine and red grape extract induced modulation in countering the age associated changes in free radical production and defense.

ABBREVIATIONS:-

ADP	**:**	Adenosine diposhpate
AOS	**:**	Active oxygen species
AR	**:**	Analar grade
ATP	**:**	Adenosine triphosphate
B.C	**:**	Before Christ
CAT	**:**	Catalase
CDC	**:**	Centre for Disease Control
COPD	**:**	Chronic obstructive pulmonary disease
CUC	**:**	Cow urine concoction
DLCO	**:**	Diffusion capacity for carbon monoxide
DNA	**:**	Deoxy raibonucleic acid
EDTA	**:**	Ethylene diamine tetra acetic acid
FAA	**:**	Free amino acids
FAD	**:**	Flavin adenine dinucleotide
$FADH_2$	:	Flavin adenine dinucleotide (reduced)
FAO	**:**	Food and Agriculture Organization
FEVI	**:**	Forced expiratory volume in one second
FVC	**:**	Forced vital capacity
G-6-PDH	**:**	Glucose-6-Phosphodehydrogenase
GP_X	**:**	Glutathione peroxidase
GSH	**:**	Glutothine
GSSG	**:**	Glutahione disulfide
H_2O_2	**:**	Hydrogen peroxide
HDL	**:**	High density lipoprotein

HMP	**:**	Hexose monophosphate path way
HO_2^+	**:**	Hydroperoxl radical
ICDH	**:**	Isocitrate dehydrogenase
INT	**:**	2-P- iodophenyl 3-P- nitrophenyl tetrazolium chloride
IUB	**:**	International Union Biochemistry
LDL	**:**	Low density lipoprotein
MDH	**:**	Malate dehydrogenase
MEOS	**:**	Minsomal ethanol oxidation system
mRNA	**:**	Messenger RNA
NAD	**:**	Nicotinamide Adenine Dinucleotide
NADH	**:**	Nicotinamide Adenine Dinucleotide (reduced)
$NADP^+$	**:**	Nicotinamide Adenine Dinucleotide phosphate
NADPH	**:**	Nicotinamide Adenine Dinucleotide phosphate (reduced)
NNK	**:**	4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone (NNK)
NO	**:**	Nitric oxide
NOS	**:**	Nitric oxide synthase
NRT	**:**	Nicotine replacement therapy
NRT	**:**	Nicotine replacement therapy
Nt	**:**	Nicotine treatment
$^1O_2$	**:**	Singlet oxygen
$O_2^{\cdot-}$	**:**	Superoxide anion
$^{\cdot}OH$	**:**	Hydroxyl radical
OXLDL :	**:**	Oxidation low density lipoprotein
PAHs	**:**	Polycyclic aromatic hydrocarbons
PEP	**:**	Phophoenol pyruvate
PEPCK :	**:**	Phosphoenol pyruvate carboxy kinase

pH	**:**	Hydrogen ion concentration
RGEt	**:**	Redgrape extract treatment
ROM	**:**	Reactive oxygen intermediates
ROS	**:**	Reactine oxygen species
RRC	**:**	Functional residual capacity
RV	**:**	Residual volume
SD	**:**	Standard deviation
SDH	**:**	Succinate dehydrogenase
SOD	**:**	Superoxide dismutase
TBARS	**:**	Thiobarbituric acid reactive substances
TCA	**:**	Trichloro acetic acids
TLC	**:**	Total lung capacity
TTC	**:**	Triphenyl Tetrazolium Chloride
UK	**:**	United Kingdom
USA	**:**	Unite Sates of America
UV	**:**	Ultra violate
VC	**:**	Vital capacity
WHO	**:**	World Health Organization
XO	**:**	Xanthine Oxidase
ZTC	**:**	Zero time controls

CONTENTS

INTRODUCTION

RED GRAPE (Vitis vinifera L):

Grape (Vitis vinifera L.) is one of the world's largest fruit crops (Shaker, 2006). With nearly 70 million tones currently produced worldwide grapes are possibly the world's largest cultivated fruit, most of which are used in wine making, while the rest are consumed as table grapes or processed into raisins, juices, jams or other products. Of all grapes, cultivars of the Vitis vinifera L. species are the most important throughout world, but especially in Europe (Mazza, 1995**)**. There are dozens of other less important species of grapes that belong to Vitis genus (Chalker-Scott, 1999; Zhao, et al., 2010).

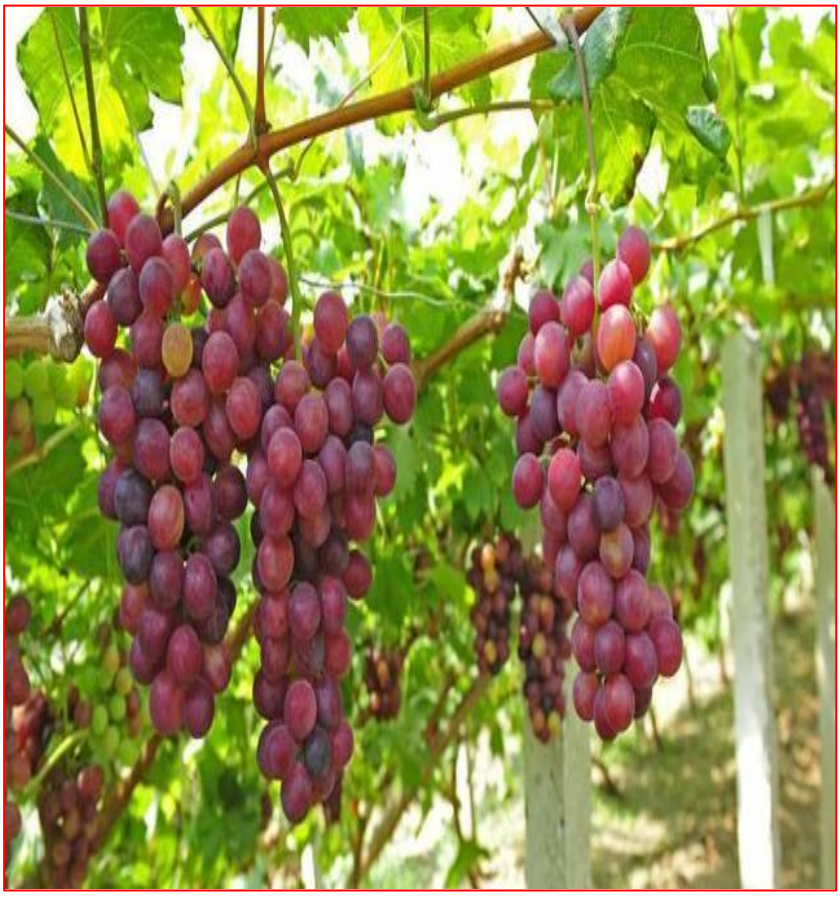
Grape is non-climatic fruit that grows on the perennial and deciduous woody vines of the genus Vitis. Grapes can be eaten raw or used for making jam, juice, jelly, vinegar, wine, grape seed extracts and grape seed oil. Anthocyanins and other pigment chemicals of the larger family of polyphenols in red grapes are responsible for the varying shades of purple in red wines (Waterhouse, 2002; Brouillard et al., 2003). Most grapes come from cultivars of Vitis vinifera According to the Food and Agriculture Organization (FAO), 75,866 square kilometres of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit.

Comparing diets among western countries, researchers have discovered that although the French tend to eat higher levels of animal fat, surprisingly the incidence of lung disease remains low in France, a phenomenon named the French Paradox thought to occur from protective benefits of regularly consuming red wine. Apart from potential benefits of alcohol itself, including reduced platelet aggregation and vasodilation (Providencia , 2006), polyphenols (e.g., resveratrol) mainly in the grape skin provide other suspected health benefits such as (Opie and Lecour, 2007).

- Alteration of molecular mechanisms in blood vessels, reducing susceptibility to vascular damage.
- Decreased activity of angiotensin, a systemic hormone causing blood vessel constriction that would elevate blood pressure.
- Increased production of the vasodilator hormone, nitric oxide (endothelium-derived relaxing factor).

Red grape (Vitis vinifera.L) presumably originates from Western Asia, from the area between the Caspian Sea region and Asia Minor, where also its cultivation seems to have begun (Bombardelli,1995; Johnson, 1989). These archeological finds, up to this day the oldest documentation for the use of grape vine products in human culture, were dated to 3500-2900 B.C. (McGovern, 1995). In progress of spreading west, towards Europe, grape vine reached, almost simultaneously with the westward extension, the cultivation of vine spread towards East over Iran, Pakistan and India so that it reached China at the end of the second century B.C. (Bombardelli, 1995). Today, Vitis vinifera has reached all continents but is successfully cultivated only in temperate climate regions with warm and dry summers and relatively mild winters with sufficient rain (Bombardelli, 1995).

Since the beginning of human civilization, medicinal plants have always remained a part and parcel of human society to combat and treat different diseases. In particular, in the Indian systems of medicine Rigveda, Charak Samhita, and Sushruta Samhita such descriptions have been documented. According to estimates of the World Health Organization (WHO) nearly 75% of the world's population currently uses herbs and other traditional medicines to treat diseases of different natures (Kuruvilla, 2002). The grape has been well recognized worldwide for over 2,000 years as one among the edible sweet fruits and recognized for its wide spectrum of biological properties (Grapes Vitis spp, 2008).



Redgrape

Taxonomic position of Red grape

Group	:	Thalamifloreae,
Order	:	Rhamnales,
Family	:	Vitaceae,
Genus	:	*Vitis*,
Species	:	vinifera.

Red grapes- Properties:

Consumption of grape flavonoids has been shown to confer antioxidant protection, inhibit platelet activity, reduce thrombus formation and lead to the concentration of inflammatory biomarkers (Castilla et al., 2006; O'Byrne et al., 2002). This effect may be considered to be beneficial for the prevention of cardiovascular disease (Castilla et al., 2006). In vitro studies showed that grape juice has significant antioxidant activity and can inhibit oxidation of low density lipoprotein (LDL) (Castilla et al., 2006; O'Byrne et al., 2002). Also, red grape juice (RGj) supplementation improves the lipoprotein profile by decreasing plasma concentrations of LDL and increasing those of high density lipoprotein (HDL) and greatly reducing the plasma concentrations of oxidized LDL (Castilla et al., 2006). On the other hand, red grape juice consumption is associated with significantly lower cholesterol/HDL as atherogenic index, which is commonly used as the best lipid parameter for determining human heart disease risk (Vinson et al., 2001).

In addition to their antioxidant activity, polyphenols also possess many different biological properties that may contribute to their cardioprotective effects, including the ability to inhibit platelet activity and thrombosis (Demrow et al.,1995; Freedman et al.,2001) and the potential to reduce plasma lipids. In vivo studies showed that administration of dealcoholized red wine (Vinson, 2001), grape seed procyanidins (Del Bas, 2005), lowered plasma cholesterol in laboratory animals with diet-induced hypercholesterolemic. Recently, reduction of cholesterol LDL plasma concentration was reported in women consuming a lyophilized grape powder (Zern et al., 2005) or red wine (Naissides et al., 2006).

Oxidative stress is a hallmark of various health problems. Resveratrol (3,5,40-trans-trihydroxystilbene) is a natural phytoalexin abundantly found in grapes and red wine, which has potent antioxidant property.

Red grape- Health benefits:

Doctors do not recommend excessive consumption of red wine, but one glass a day for women and two for men may confer health benefits (Alcohol, Harvard School of Public Health)· Compounds such as resveratrol (a polyphenol antioxidant) have been discovered in grapes and these have been positively linked to fighting cancer, heart disease, egenerative nerve disease and other ailments, synthesized by many plants, resveratrol apparently serves antifungal (Diagram-1) and other defensive properties.

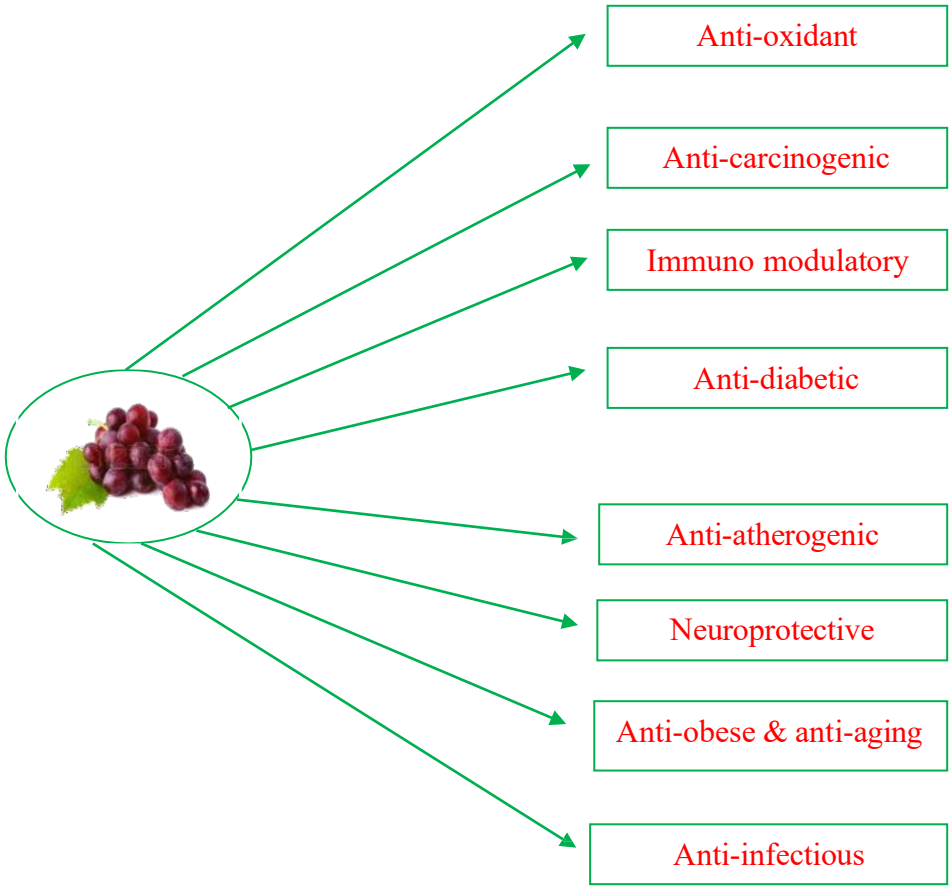


Diagram-1: Red grape-Health benefits

Dietary resveratrol has been shown to modulate the metabolism of lipids and to inhibit oxidation of low-density lipoproteins and aggregation of platelets (Chan, and Delucchi, 2000). Resveratrol is found in wide amounts among grape varieties, primarily in their skins and seeds which, in muscadine grapes, have about one hundred times higher concentration than pulp (Le Blanc, 2005). Red wine offers health benefits more so than white because many beneficial compounds are present in grape skin, and only red wine is fermented with skins. The amount of fermentation time a wine spends in contact with grape skins is an important determinant of its resveratrol content (Gu et al., 1999). Ordinary non-muscadine red wine contains between 0.2 and 5.8 mg/L (Gu et al., 1999), depending on the grape variety, because it is fermented with the skins, allowing the wine to absorb the resveratrol. By contrast, a white wine contains lower phenolic contents because it is fermented after removal of skins.

Anthocyanins tend to be the main polyphenolics in red grapes, whereas flavan- 3-ols (e.g., catechins) are the more abundant phenolic in white varieties (Cantos et al., 2002). Total phenolic content, an index of dietary antioxidant strength, is higher in red varieties due almost entirely to anthocyanin density in red grape skin compared to absence of anthocyanins in white grape skin (Cantos et al., 2002). It is these anthocyanins that are attracting the efforts of scientists to define their properties for human health. Phenolic content of grape skin varies with cultivar, soil composition, climate, geographic origin, and cultivation practices or exposure to diseases, such as fungal infections.

Several epidemiological studies have indicated that regular intake of red wine, vegetables, fruit, and green tea, is associated with a decreased global mortality due to a reduced number of cancer and coronary diseases (Hertog, et al., 1993; Renaud and de Lorgeril, 1992). The protective effect has been attributable, at least in part, to polyphenols Hertog et al., 1995; Knekt et al., 1996). Indeed, grape products such as red wine contain high levels of polyphenols, which are predominantly found in skins, seeds and stems. The protective effect of red wine polyphenols on the vascular system is thought to include their ability to prevent oxidation of low-density lipoproteins (Frankel et al., 1993; Stein et al.,, 1999), platelet aggregation and adhesion (Freedman et al., 2001, Wollny et al., 1999, and smooth muscle cell migration and proliferation (Iijima et al., 2000; Iijima et al., 2002).

Flavonoids and other plant phenolics have been reported to have multiple biological effects, including antioxidant activity (Frankel et al., 1993, 1995), anti- inflammatory action (Moroney et al., 1988) inhibition of platelet aggregation (Kanner et al., 1994) and antimicrobial activity (Renaud and Longeril, 1992). Grapes, including the skin and seeds, are rich sources of monomer phenolic compounds such as (+) –catechins, (+) – epicatechins, dimeric, trimeric and tetrameric procyanidins, which act as antimutagenic and antiviral agents (Jayaprakasha et al., 2001).

A number of studies have demonstrated that the polyphenolic (flavonoid) compounds derived from grape products can improve endothelial function and increase endothelial nitric oxide (NO) production (Folts et al., 2002). Human studies measuring increases in flow-mediated dilation of the brachial artery have shown that the consumption of grape juice (Stein et al., 1999) and red wine can improve endothelial function in vivo.

Grape polyphenolics have been shown to inhibit LDL oxidation and the initial onset of atherosclerosis (Vinson et al., 1998; Blanco-Colio et al., 2000). In an in vitro study, a flavonoid-rich grape extract dose dependently inhibited several markers of Cu^{2+}-induced LDL oxidation including the lag time to formation of conjugated dienes and thiobarbituric acid reacting substances (TBARS), (Viana et al.,1996). Grape extracts may also spare the endogenous enzymes responsible for protecting against oxidative damage. These in vitro and animal studies suggest that human consumption of grape extracts may offer protection against oxidative stress.

Grape polyphenols and Cardiovascular risk:

Flavonoids may contribute to the cardioprotective effects of grape products, as suggested by several studies associating increased flavonoid intake with reduced risk of coronary events (Knekt et al., 1996; Rimm et al., 1996). Most acute coronary syndromes are caused by platelet adhesion, aggregation, and thrombus formation in areas of ruptured atheromatous plaques (Falk, 1983; Willerson et al., 1989). Supplementation with red wine or alcohol-free red wine prolongs bleeding times and reduces platelet adhesion, and infusion of a nitric oxide synthase (NOS) inhibitor prevents these effects (Wollny et al., 1999).

Grapes contain a wide variety of polyphenol compounds, including flavonoids, phenolic acids, and resveratrol. There is extensive epidemiological evidence suggesting that dietary intake of these compounds reduces cardiovascular mortality (Hertog et al., 1995). Numerous studies in vitro as well as in animals and humans demonstrate beneficial effects of grape polyphenols on traditional cardiovascular risk factors. Population-based studies have observed markedly lower cardiovascular disease mortality in cohorts with higher consumption of relevant flavonoids, including flavonols, flavones, and flavan-3-ols (Arts et al., 2001; Knekt et al.,1996; Hertog et al., 1995; Hirvonen et al.,2001). In a study of 34,489 postmenopausal American women, dietary intake of foods containing flavanones and anthocyanidins was associated with decreased cardiovascular and all-cause mortality (Mink et al., 2007). In that study, consumption of red wine was specifically associated with decreased risk of coronary heart disease.

Grapefruit juice inhibits CYP_2A_6, as evidenced by inhibition of coumarin metabolism in people (Runkel et al., 1997). Grapefruit juice has been shown to inhibit the metabolism of nicotine to cotinine in nonsmokers, who were given nicotine orally, with evidence of a greater effect with larger doses of grapefruit juice (Hukkanen et al., 2006). Grapefruit juice also increased renal clearance of nicotine and cotinine by an unknown mechanism. Grapefruit juice had no significant effect on overall exposure to nicotine (area under the plasma concentration–time curve) because the effects of slowed metabolism were offset by the effects on increased renal clearance.

Whether the effects of grapefruit juice on nicotine levels in users of tobacco are significant has not been investigated. Consumption of watercress enhances the formation of nicotine glucuronide, cotinine glucuronide, and 3–hydroxycotinine glucuronide in smokers (Hecht et al., 1999b). Watercress has no effect on the excretion of nicotine, cotinine, and 3–hydroxycotinine in smokers. Thus, watercress may induce some UGT enzymes involved in nicotine metabolism, but has no effect on CYP_2A_6-mediated nicotine metabolism.

Bioactivity of Phenolic compounds from grape:

Recently, growing interests on phenolic compounds from grapes have focused on their biological activities linking to human health benefits, such as antioxidant, cardioprotective, anticancer, antiinflammation, antiaging and antimicrobial properties.

Grape fruit contains various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Polyphenols are the most important phytochemicals in grape because they possess many biological activities and health- promoting benefits (Shrikhande, 2000; Wada et al., 2007). The phenolic compounds mainly include anthocyanins, flavanols, flavonols, stilbenes (resveratrol) and phenolic acids (Dopico-Garcia et al., 2008; Spacil et al., 2008). Anthocyanins are pigments, and mainly exist in grape skins. The reported evidences of beneficial health effects of phenolic compounds include inhibiting some degenerative diseases, such as cardiovascular diseases (Shanmuganayagam et al., 2007; Tsanga, et al., 2005), and certain types of cancers (God et al., 2007; Jung et al.,2006), reducing plasma oxidation stress and slowing aging (Meyer et al.,1997; Sato et al.,1996). Phenolic compounds are also regarded as preservatives against microbes and oxidation for food (Rodriguez-Vaquero et al., 2007; Rhodes et al., 2006).

Given the oxidative hypothesis of atherosclerosis, a potentially important property of grape-derived polyphenols is the ability to inhibit LDL oxidation. In the key initial step in atherogenesis, oxidized LDL is taken up by macrophages in an unregulated manner to form foam cells. Oxidized LDL also promotes atherosclerosis by altering endothelial function, stimulating platelet activation, and inducing a proinflammatory state in the vascular wall (Diaz et al., 1997). In vitro studies have demonstrated that grape-derived flavonoids and resveratrol limit ex vivo LDL oxidation (Frankel et al., 1993). Red wine has been found to be more potent than white wine or pure ethanol in this regard (Vinson et al., 2001). These treatments were associated with protection against LDL oxidation. Vinson et al., (2001) demonstrated reduced aortic atherosclerosis in hamsters supplemented with polyphenol-rich beverages.

Zern et al., (2003) observed a reduction in cholesterol accumulation in the aortas of ovariectomized guinea pigs fed a lyophilized grape preparation. Interestingly, Stocker et al., (2004) observed reduced atherosclerosis but no decrease in LDL oxidation within the arterial wall following treatment with dealcoholized red wine in mice, suggesting that mechanisms other than LDL protection may also be important. To translate these mechanistic findings to humans, Stein et al., (1999) demonstrated a reduction in the susceptibility of LDL to copper-mediated oxidation following consumption of purple grape juice for 2 week in patients with coronary artery disease.

Red wine consumption in healthy subjects also reduced urinary levels of prostogland in F2-α, a marker of systemic lipid peroxidation (Pignatelli et al., 2006). A similar effect on urinary isoprostane concentrations were observed in pre and postmenopausal women following treatment with lyophilized grape powder for 4 week (Zern et al., 2005). At the present time, no study, to our knowledge, has shown a relation between polyphenol consumption and reduced atherosclerosis in humans. It remains to be determined whether inhibition of LDL oxidation is a clinically relevant mechanism in humans.

NICOTINE:-

Nicotine is a naturally occurring alkaloid found primarily in the members of the Solanaceae family (Nicotiana tobacco), which includes tobacco, potato, tomato, green pepper, and eggplant. Nicotine was first isolated and determined to be the major constituent of tobacco in 1828 (Schevelbein, 1982). In commercial tobaccos, the major alkaloid is nicotine, accounting for about 95 % of the total alkaloid content (Jacob et al., 1993). Tobacco use is the leading cause of death in the world today. With 4.9 million tobacco-related deaths per year, no other consumer product is as dangerous or kills as many people as tobacco (WHO, An international treaty for tobacco control, 2003).

Cigarette smoke is a complex mixture of more than 4700 chemical compounds including free radicals and oxidants. Toxicity exhibited by cigarette smoke may be due to combined action of these compounds inducing many cellular processes mediated through reactive oxygen species (ROS). Major Player probably nicotine as it is present in tobacco, in higher concentrations. The compounds that induce intracellular oxidative stress recognized as the important agents involved in the damage of biological molecules. Experiments using animal and cell culture model systems suggested that moderately higher concentrations of some forms of ROS like NO and H_2O_2 can act as signal transducing agents.

Men who smoke cigarette have been shown to have immature sperm, low sperm count, sperm with multiple heads (Weisberg, 1985) and decreased sperm motility and penetrability through cervical. Cigarette smoking is known to have deleterious effects on visceral tissues in women as epidemiologic studies have clearly indicated that women who

smoke suffer a lowered fertility (Rosevear et al., 1992; Hughes et al., 1996; Kaffman et al., 1980; and Weisberg, (1985) also reported early menopause among smokers. These toxic effects of cigarette smoking are often associated with the nicotine content of cigarettes. However, it is now well known that nicotine is not the only toxic substance in cigarette. Cigarette also contains other toxic, carcinogenic, mutagenic, growth retardative and immunosuppressive compounds such as polycyclic aromatic hydrocarbons, cyanide, carbon-monoxide, lead, cadmium nitric oxide and nitric dioxide (Halliwell, 1993a,b). Also the American tobacco industry under pressure from the American congress, released a top-secret list of other 599 chemicals it adds to cigarette, which among hundreds of others include ammonia and insecticides (Brodie, 1994). It has also been emphasized that cigarette smoking is not always synonymous with nicotine administration (Oyebola and Adetuyibi, 1977; Alada, 2001).

Nicotine is the principal alkaloid contained in tobacco and it is believed to be the primary reason for cigarette smoking in many people particularly as they derive satisfaction and pleasant sensation from inhaling nicotine (Benowitz et al., 1982). It is widely consumed through cigarette smoking and tobacco chewing in 30-40% of the world's population. In Nigeria, nicotine is an important constituent of cow urine concoction (CUC), a local panacea for the treatment of convulsion among the Yorubas (Familusi and Sinnette, 1977). Available evidence showed that nicotine affects many biological activities.

For instance, it causes bronchitis and interferes with alveoli formation causing development of emphysema-like lesion and cancer of the lungs (Maritnez et al., 1992). Cigarette and tobacco have been shown extensively to affect offspring of mothers exposed to smoking. For example, intrauterine growth retardation has been observed in babies of smokers (Seller and Brait, 1995), so also are low birth weight (Bardy et al, 1993), increase incidence of respiratory tract infection (Fergusson et al.,1981), reduced forced expiratory flow (Hanrahan et al., 1992), asthma (Weitzman et al., 1990), prenatal and neonatal death or mortality (Walsh, 1994) and reduction in uterine blood flow in both rats and humans (Birnbaun et al., 1994: Economides and Braithwaite, 1994).

Nicotine also induces oxidative stress both in vivo and in vitro that causes a peroxidant/antioxidant imbalance in blood cells, blood plasma and tissues (Suleyman et al., 2002). Oxidative stress generates free radicals that attack on the membrane lipids

resulting in the formation of malondialdehyde (MDA), which causes peroxidative tissue damage (Srinivasan and Pugalendi, 2000). Animals studies have shown significantly higher liver and serum levels of MDA, conjugated dienes, hydroperoxides, and free fatty acids in rats induced by cigarette smoke (Ashakumary and Vijayammal, 1996; Zhang et al., 2001). Smokers incur a sustained free radical load that increases their ascorbic acid (vitamin C) (Helen and Vijayammal, 1997) and a- tocopherol (vitamin E) (Tsuchiya et al., 2002) requirement. Supplementation with ascorbic acid and α-tocopherol is considered safe and ease, because these are susceptible to dietary manipulation (Byers and Perry, 1992).

Absorption of Nicotine:

Absorption of nicotine across biological membranes depends on pH. Nicotine is distilled from burning tobacco and carried proximally on tar droplets (also called particulate matter), which are inhaled. Nicotine is a weak base with a pKa of 8.0. In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes. The pH of smoke from flue-cured tobaccos, found in most cigarettes, is acidic (pH 5.5–6.0). At this pH, nicotine is primarily ionized.

As a consequence, there is little buccal absorption of nicotine from flue-cured tobacco smoke, even when it is held in the mouth (Gori et al., 1986). Smoke from air-cured tobaccos, the predominant tobacco used in pipes, cigars, and some European cigarettes, is more alkaline (pH 6.5 or higher) and, considerable nicotine is unionized. Smoke from these products is well absorbed through the mouth (Armitage et al., 1978). It has recently been proposed that the pH of cigarette smoke particulate matter is higher than previously thought, and thus, a larger portion of nicotine would be in the unionized form, facilitating rapid pulmonary absorption (Pankow, 2001).

Nicotine absorption can occur through the oral cavity, skin, lung, urinary bladder, and gastrointestinal tract. Absorption of nicotine across biological membranes depends on pH (Yildiz, 2004). In its ionised state, such as in acidic environments, nicotine does not rapidly cross membranes. The respiratory absorption of nicotine is 60 % to 80% (Health Council of the Netherlands, 2004). The rapid absorption of nicotine from cigarette smoke through the lung occurs because of the huge surface area of the alveoli and because of dissolution of nicotine at physiological pH (approximately 7.4), which facilitates transfer across cell membranes. Absorption through the alveoli is also

dependent on the nicotine concentration in the smoke. Nicotine is poorly absorbed from the stomach due to the acidity of the gastric fluid, but is well absorbed in the small intestine, which has a more alkaline pH and a large surface area (Yildiz, 2004). Nicotine base can be absorbed through the skin, and there have been cases of poisoning after skin contact with pesticides containing nicotine (Saxena et al., 1985; Benowitz et al., 1987). Likewise, there is evidence of cutaneous absorption of and toxicity from nicotine in tobacco field workers (Health Council of the Netherlands, 2004).

Concentrations of nicotine in the blood rise gradually with the use of smokeless tobacco and plateau at about 30 min, with levels persisting and declining only slowly over 2 h or more (Benowitz et al., 1988). Various formulations of nicotine replacement therapy (NRT), such as nicotine gum, transdermal patch, nasal spray, inhaler, sublingual tablets, and lozenges, are buffered to alkaline pH to facilitate absorption of nicotine through cell membranes. Absorption of nicotine from all NRTs is slower and the increase in nicotine blood levels is more gradual than from smoking. This slow increase in blood and especially in brain levels results in low abuse liability of NRTs (West et al., 2000). Only nasal spray provides a rapid delivery of nicotine that is closer to the rate of nicotine delivery achieved with smoking (Gourlay and Benowitz, 1997; Guthrie et al., 1999). The absolute dose of nicotine absorbed systemically from nicotine gum is much less than the nicotine content of the gum, in part, because considerable nicotine is swallowed with subsequent first-pass metabolism (Benowitz et al., 1987).

Distribution of Nicotine in body tissues:-

After absorption, nicotine enters the bloodstream where, at pH 7.4, it is about 69% ionized and 31% unionized. Binding to plasma proteins is less than 5% (Benowitz et al., 1982a). The drug is distributed extensively to body tissues with a steady state volume of distribution averaging 2.6 L/Kg. Based on human autopsy samples from smokers, the highest affinity for nicotine is in the liver, kidney, spleen, and lung and lowest in adipose tissue. In skeletal muscle, concentrations of nicotine and cotinine are close to that of whole blood. Nicotine binds to brain tissues with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers (Breese et al., 1997). Increase in the binding is caused by a higher number of nicotinic cholinergic receptors

in the brain of the smokers. Nicotine accumulates markedly in gastric juice and saliva (Lindell et al., 1996). Gastric juice/plasma and saliva/plasma concentration ratios are 61 and 11 with transdermal nicotine administration, and 53 and 87 with smoking, respectively (Lindell et al., 1996). Accumulation is caused by ion-trapping of nicotine in gastric juice and saliva. Nicotine also accumulates in breast milk (milk/plasma ratio 2.9) (Dahlstrom et al., 1990). Nicotine crosses the placental barrier easily, and there is evidence for accumulation of nicotine in fetal serum and amnionic fluid in slightly higher concentrations than in maternal serum (Dempsey and Benowitz, 2001).

The pattern of tissue uptake, examined in tissues of rabbits by measuring concentrations of nicotine in various tissues after 24-hr constant i.v. infusion of nicotine, showed that spleen, liver, lungs, and brain have high affinity for nicotine, whereas the affinity of adipose tissue is relatively low (Benowitz, 1986). Nicotine readily crosses the placenta and the foetuses of mothers who smoke are exposed to higher nicotine concentrations than their mothers (Hellstrom-Lindahl et al., 2002).

Human pharmacokinetics of Nicotine and Cotinine

	Nicotine	**Cotinine**
Half-life	120 min	18 hr
Volume of distribution	180 L	88 L
Total clearance	1,300 mL/min	72 mL/min
Renal clearance	200 mL/min	12 mL/min
Non renal clearance	1,100 mL/min	60 mL/min

Source: Average values based on data from Benowitz, et al., (1982) and Benowitz,et al., (1983).

Steady state distribution of nicotine

Tissue	Tissue to blood ratio
Blood	1.0
Brain	3.0
Heart	3.7
Muscle	2.0
Adipose	0.5
Kidney	21.6
Liver	3.7
Lung	2.0
Gastrointestinal	3.5

Source:Benowitz, N.L. Human pharmacology of nicotine. In: Cappell, et al. (eds.) Research dvances in Alcohol and Drug Problems, Volume 9. New York: Plenum Press, 1986b.

Nicotine metabolism:-

It has been demonstrated that nicotine is excreted through urine, faeces, bile, saliva, gastric juice, sweat, and breast fluid (Balabanova et al., 1992; Seaton et al., 1993). When 14C-nicotine is given to an animal, it has been shown that about 55 % of the radioactivity is excreted in the urine. However, only 1 % of the radioactivity was observed in the form of unchanged nicotine. This result demonstrates that nicotine is excreted following extensive metabolism. Nicotine disappears rapidly from the blood, with a half-life of 2 h to 3 h in humans (Health Council of the Netherlands, 2004).

In another study, researchers reported that the daily nicotine intake was 18 % higher in persons with increased nicotine excretion and concluded that the rate of elimination of nicotine affects the rate of consumption (Benowitz and Jacob, 1985). The rate of nicotine excretion is also influenced by the pH of the urine. When the pH of the urine is made alkaline, the proportion of uncharged nicotine increases and reabsorption of nicotine occurs and as a result, less nicotine is excreted (Becket et al., 1965).

In most mammalian species, nicotine is rapidly and extensively metabolized, primarily in the liver (Kyerematen and Vesell, 1991). The major metabolic pathways of nicotine in mammals are C-oxidation and N-oxidation, i.e. cotinine and nicotine-1′- N-oxide formation, respectively. Cotinine formation from nicotine is a two-step reaction in mammals (Diagram-2).

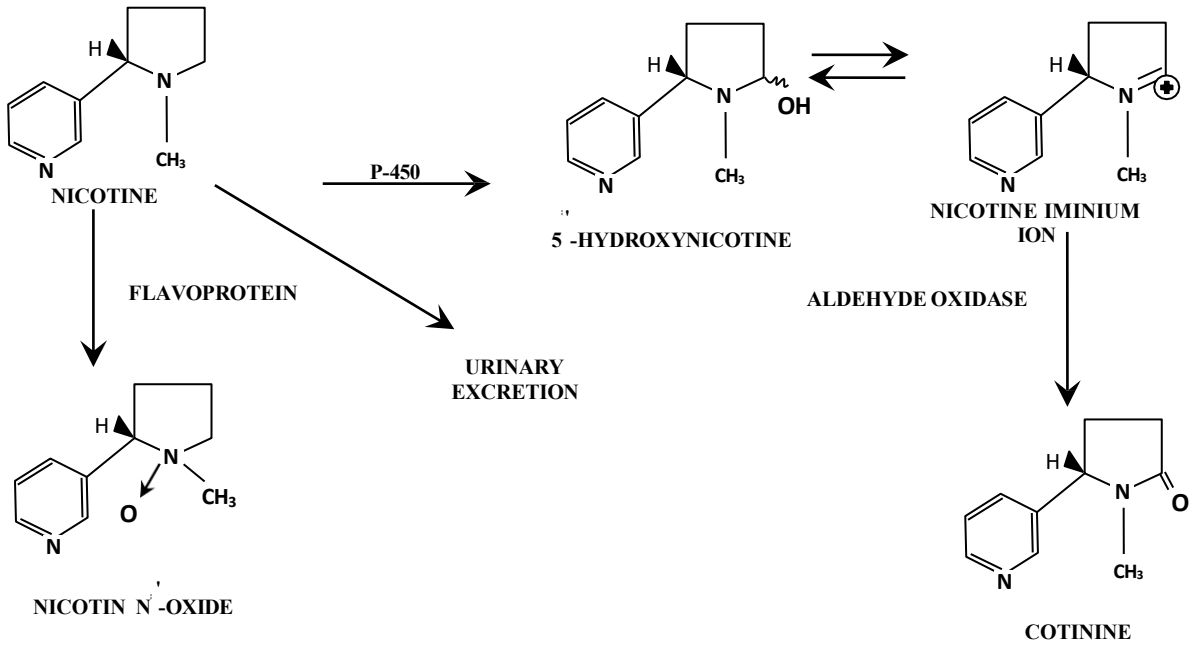


Diagram:2. Major Pathways of nicotine metabolism.
Source : Peterson, L.A. et al., (1987).

The first step is the conversion of nicotine to nicotine-$\Delta^{1'(5')}$-iminium ion by CYP. The second step, the conversion of the iminium ion to cotinine, is mediated by cytosolic aldehyde oxidase (KyerematenandVesell, 1991). About 4% of nicotine is converted to nicotine-1′-N-oxide, which is largely (if not entirely) excreted in urine without further metabolism (Jacob et al., 1988). In rats, nicotine is excreted as cotinine and nicotine-1′-N-oxide, both at about 10% (Kyerematen et al., 1988). It was reported that CYP catalyzes the formation of cotinine from nicotine and FMO catalyzes the formation of nicotine-1′-N-oxide in mammals (Cashman et al., 1992). Therefore, nicotine is a good probe to simultaneously estimate changes in CYP and FMO after liver injury. A variety of methods for the determination of nicotine and its metabolites in biological samples have been reported in the literature (Schepers and Walk, 1988; Voncken et al., 1989).

Nicotine – Stress:

Nicotine is known to induced oxidative stress and depletes antioxidant defense mechanisms; produced reduction in glutathione peroxidase in circulation, lung, liver and kidney of nicotine-treated animals (Yildiz, 2004; Muthukumaran et al., 2008). Nicotine also increases both free fatty acid release from the liver and the hepatic synthesis of very low-density lipoproteins; also maternal nicotine exposure induced oxidative stress and causes histopathological changes in the lung and liver of lactating offspring (El-Sokkary et al., 2007).

Nicotine has been reported to induce oxidative stress both in vivo and in vitro (Suleyman et al., 2002). The oxidative stress increases in smokers and in patients with chronic obstructive pulmonary disease (COPD). It is known that smoking and chronic bronchitis are both associated with increased numbers of activated neutrophils and macrophages in the airspaces, which release more $O_2^{\cdot -}$ than those from healthy controls (Ludwig and Hoidal, 1982). And a correlation between $O_2^{\cdot -}$ release by peripheral blood neutrophils and bronchial hyperreactivity in patients with COPD exists, suggesting a role for ROS in the pathogenesis of the airway abnormalities in COPD. A major site of free radical attack is in polyunsaturated fatty acids in cell membranes producing lipid peroxidation. The end products of lipid peroxidations such as malondialdehyde, ethane and pentane were significantly increased in smokers (Petruzzelli et al., 1990).

Oxidative stress may result in overproduction of oxygen free-radical precursors and/ or decreased efficiency of the antioxidant system (Baynes, 1991). The oxygen free-radical generation is associated with auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides (Strain et al., 1991). There are various endogenous defense mechanisms against free radicals, such as the enzymes SOD, GSH-Px and CAT, whose activities eliminate superoxide, hydrogen peroxide and hydroxyl radicals (Mark Percival, 1998).

LUNG:

The lungs are the primary organs of respiration in humans and many other animals. In mammals and most other vertebrates, two lungs are located near the backbone on

either side of the heart (Diagram-3). Their function in the respiratory system is to extract oxygen from the atmosphere and transfer it into the bloodstream, and to release carbon dioxide from the bloodstream into the atmosphere, in a process of gas exchange. Respiration is driven by different muscular systems in different species. The animals use their musculoskeletal systems to support and foster breathing. In humans, the primary muscle that drives breathing is the diaphragm. The lungs also provide airflow that makes vocal sounds including human speech possible.

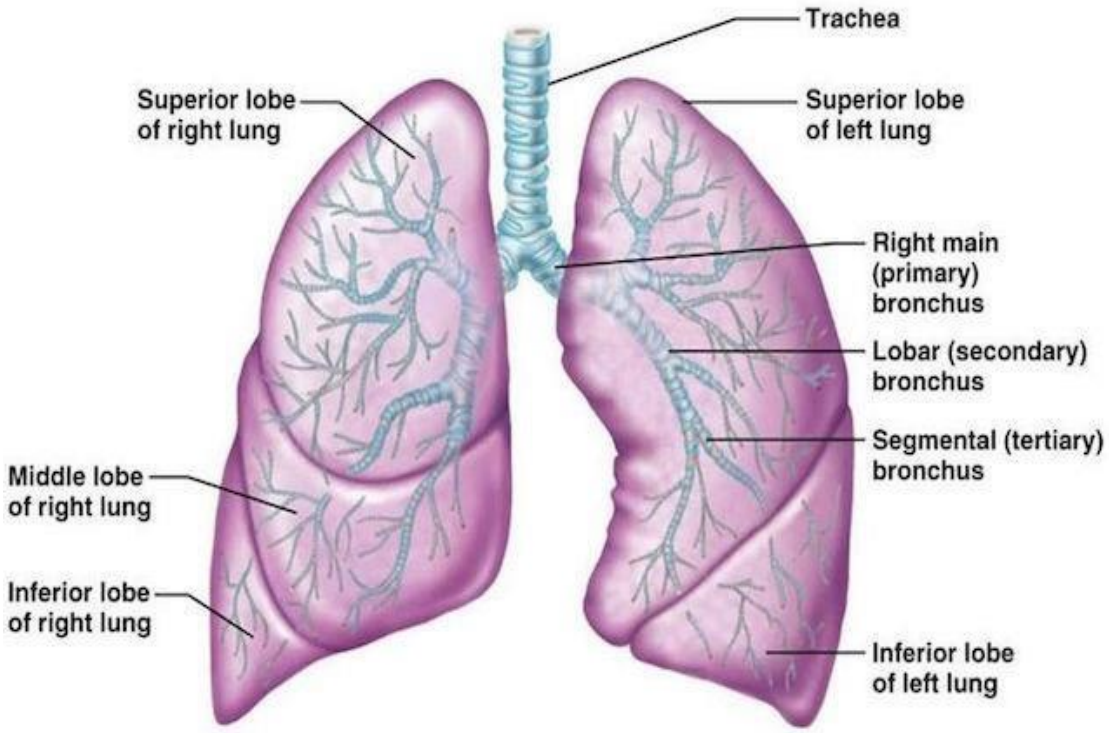


Diagram-3: Human Lungs

Humans have two lungs, a right lung and a left lung. They are situated within the thoracic cavity of the chest. The right lung is bigger than the left, which shares space in the chest with the heart. The lungs together weigh approximately 1.3 kilograms (Albino rat Lung weight approximately 1.5 Grams), and the right is heavier. The lungs are part of the lower respiratory tract that begins at the trachea and branches into the bronchi and bronchioles and which receive air breathed in via the conducting zone. These divide until air reaches microscopic alveoli, where the process of gas exchange takes place. Together, the lungs contain approximately 2,400 kilometres (1,500 mi) of airways and 300 to 500 million alveoli. The lungs are enclosed within a sac called the pleural sac which allows

the inner and outer walls to slide over each other whilst breathing takes place, without much friction. This sac encloses each lung and also divides each lung into sections called lobes. The right lung has three lobes and the left has two. The lobes are further divided into bronchopulmonary segments and lobules. The lungs have a unique blood supply, receiving deoxygenated blood sent from the heart for the purposes of receiving oxygen (the pulmonary circulation) and a separate supply of oxygenated blood (the bronchial circulation).

Lung function:

Lung function can be divided into three categories (Spirometry to assess the dynamic flow rates). Forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV-1/FVC ratio. The dynamic flow rates are dependant on lung volumes. The static lung volumes include total lung capacity (TLC), vital capacity (VC), residual volume (RV), and functional residual capacity (FRC). The gas exchange across alveolar capillary membrane is measured using diffusion capacity for carbon monoxide (DLCO). Pulmonary function tests are reported as % predicted compared with individuals of same age, sex, and height. The lungs undergo a phase of growth and maturation during the first two ecades of life and achieve maximal lung function around age 20 years in females and 25 years in males. Lung function remains steady with very minimal change from age 20 to 35 years and starts declining thereafter.

The decline in pulmonary function tests depends on peak lung function achieved during adulthood, the duration of the plateau phase, and rate of lung function decline. Studies on lung function are done either to establish the reference values for the pulmonary function laboratories or to determine the age-related decline (Knudson, 1981; Janssens et al., 1999; Zeleznik, 2003). The variability in similar physiologic measurements is much greater among healthy older individuals compared with younger individuals, making it problematical to establish a “normal” range for the older adults.

Environmental exposures, nutritional deficiencies, or childhood infection common to individuals born within a defined time period that may not be present in successive generations explains why younger individuals now have a higher lung function compared with 50 years ago. The cumulative cohort effect is estimated to be 5ml/yr. This means that

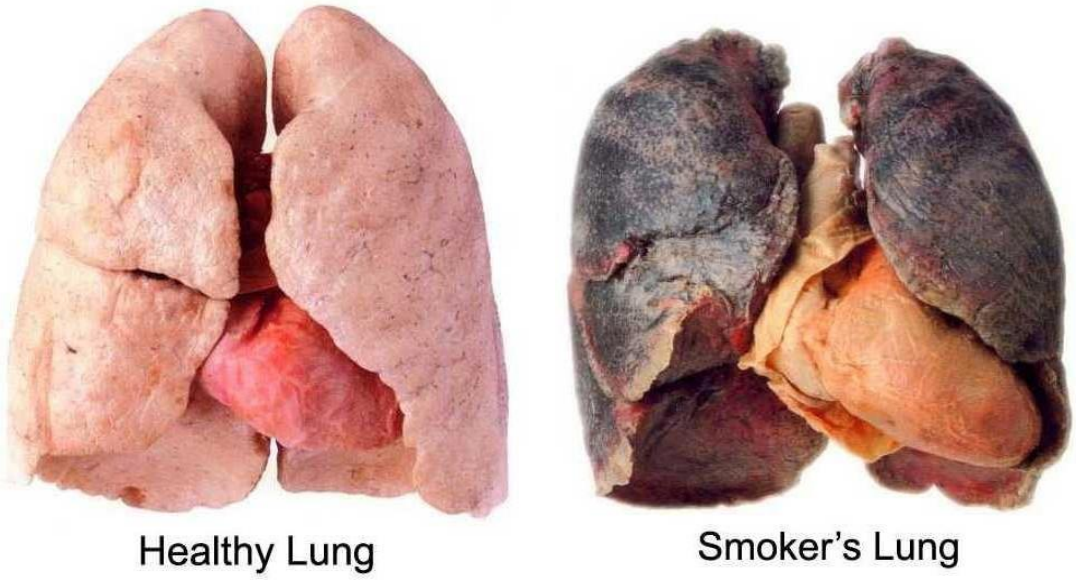
each former 25 year old generation had a vital capacity which is lower by 125 ml compared with the present generation (Xu et al.,1995). On the contrary, period effects include changes in techniques, equipment, and "learning effect" from repeated measures that can all improve the lung function measurement on subsequent studies. Studies estimating the period effect on lung function showed an average FEV1 increase by 250 ml in men and 219 ml in women during two different survey periods (from 1973–1978 and 1985–1990) (Xu et al.,1995). Most cross-sectional studies showed linear decline in FEV1 with age and, underestimated the rate of decline. Longitudinal studies showed a nonlinear decline with age.

The estimated rate of decline in FEV1 is 25–30 ml/yr starting at age 35–40 years and can double to 60ml/yr after age 70 years. Lung volumes depend on body size, especially height. Total lung capacity (TLC) corrected for age remains unchanged throughout life. Functional residual capacity and residual volume increase with age, resulting in a lower vital capacity. Gas exchange in the lungs occurs across the alveolar capillary membrane. It is measured by diffusing capacity of carbon monoxide (DLCO). The DLCO is dependant upon lung volume (TLC) and alveolar ventilation. Diffusion across the alveolar–capillary interface is directly proportional to the alveolar surface and inversely proportional to the alveolar–capillary membrane thickness. Stam et al., (1994) studied effect of age on diffusion capacity in 55 healthy subjects (age ≥70 yrs, n=3) and showed a decline in DLCO with age corrected for alveolar volume. This suggests alteration in the alveolar–capillary membrane as the potential mechanism, though not proven.

Nicotine-Carcinogens and Lung Cancer:

Lung cancer continues to be the leading cause of cancer death in both men and women. Worldwide, lung cancer kills over one million people each year (World Cancer Research Fund/American Institute for Cancer Research Food, nutrition and the prevention of Cancer, 1997). Extensive prospective epidemiologic data clearly establish cigarette smoking as the major cause of lung cancer (Blot et al., 1996). It is estimated that about 90% of male lung cancer deaths and 75%–80% of female lung cancer deaths in the United States each year are caused by smoking. Reducing the health consequences of smoking; 25 years of progress. Washington (DC) U.S. Govt Print Off, 1989: Shopland, 1995). The risk of lung cancer diminishes after smoking cessation, but not during the first 5 years,

and the relative risk never returns to that of a nonsmoker (Blot et al., 1996). Seet the normal lung and smoking lung as in below:



Inspite of the rising antitobacco sentiment in the United States and improvements in smoking cessation methods, approximately 25% of the U.S. adult population, about 47 million people, continues to smoke cigarettes (Anonymous, Cigarette smoking among adults-1995). An understanding of mechanisms of tobacco- induced lung cancer will lead to new strategies for decreasing lung cancer risk, for identifying highly susceptible individuals, and for developing innovative techniques for early detection. Even in the writings of distinguished scientists with great expertise in cancer causes and mechanisms, one can read statements such as: "The carcinogenic mechanisms of tobacco smoking are not well understood" (Ames et al., 1995).

Carcinogens form the link between nicotine addiction and lung cancer (Diagram-4). Nicotine addiction is the reason that people continue to smoke (Surgeon Genral, The health consequences of smoking; nicotine addiction. Washington (DC)

U.S. Govt. Print Off, 1988). While nicotine itself is not considered to be carcinogenic, each cigarette contains a mixture of carcinogens, including a small dose of polycyclic aromatic hydrocarbons (PAHs) and 4-(methylnitro- samino)-1-(3-pyridyl)-1-butanone (NNK) among other lung carcinogens, tumor promoters, and co-carcinogens (Hoffmann and Hoffmann, 1997; Hoffmann and Hecgt, 1990). Carcinogens such as NNK and PAHs require metabolic activation to exert their carcinogenic effects; there are competing

detoxification pathways, and the balance between metabolic activation and detoxification differs among individuals and will affect cancer risk. We know a great deal about mechanisms of carcinogen metabolic activation and detoxification (Hecht, 1988; Conney, 1982; Miller, 1994: Miller and Miller, 1981). The metabolic activation process leads to the formation of DNA adducts, which are carcinogen metabolites bound covalently to DNA, usually at guanine or adenine. There have been major advances in our understanding of DNA adduct structure and its consequences in the past two decades, and we now have a large amount of mechanistic information (Hemminki et al., 1994: Geacintov et al., 1997). If DNA adducts escape cellular repair mechanisms and persist, they may lead to miscoding, resulting in a permanent mutation. As a result of clever strategies that combine DNA adduct chemistry with the tools of molecular biology (Singer and Essigmann, 1991).

We know a great deal about the ways in which carcinogen DNA adducts cause mutations. Cells with damaged DNA may be removed by apoptosis, or programmed cell death (Wistuba II et al., 1997; Sekido et al., 1998). If a permanent mutation occurs in a critical region of an oncogene or tumor suppressor gene, it can lead to activation of the oncogene or deactivation of the tumor suppressor gene. Multiple events of this type lead to aberrant cells with loss of normal growth control and, ultimately, to lung cancer. While the sequence of events has not been as well defined as in colon cancer, there can be little doubt that these molecular changes are im- portant (Wistuba II et al., 1997; Sekido et al., 1998).

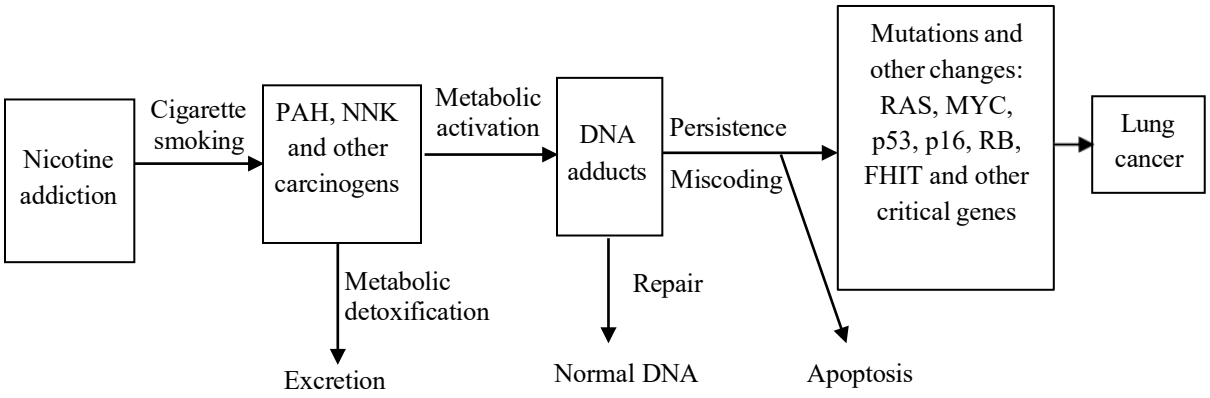


Diagram-4: Scheme linking nicotine addiction and lung cancer via tobacco smoke carcinogens and their induction of multiple mutations in critical genes. PAH4 polycyclic aromatic hydrocarbons, NNK 4 4-(methyl- nitrosamino)-1-93-pyridyl)-1-butanone.

Blocking any of the horizontal steps (Diagram-3) may lead to decreased lung cancer, even in people who continue to smoke. It will also consider other mechanisms of DNA damage via free radicals and reactive oxygen species. It will discuss mutations in oncogenes and tumor suppressor genes and their possible relationship to specific carcinogens and molecular epidemiologic investigations of carcinogen geneinteractions. On the basis of these data, it will evaluate the role of specific cigarette smoke carcinogens and other factors as causes of lungcancer. A detailed account of other aspects of the molecular pathogenesis of lung cancer has recently been published (Sekido et al., 1998).

Nicotine -Other diseases :

Heart disease : This is the biggest killer illness in the UK. About 120,000 people in the UK die each year from heart disease. About 1 in 6 of these is due to smoking.

Other cancers :of the mouth, nose, throat, larynx, gullet (oesophagus), pancreas, bladder, neck of the womb (cervix), blood (leukaemia) and kidney are all more common in smokers.

Circulation : The chemicals in tobacco can damage the lining of the blood vessels and affect the level of fats (lipids) in the bloodstream. This increases the risk of atheroma forming (sometimes called hardening of the arteries). Atheroma is the main cause of heart disease, strokes, poor circulation in the legs (peripheral vascular disease) and swollen arteries which can burst causing internal bleeding (aneurysms). All these atheroma-related diseases are more common in smokers.

Sexual problems : Smokers are more likely than non-smokers to have erection problems (impotence) or have difficulty in maintaining an erection in middle life. This is thought to be due to smoking- related damage of the blood vessels to the penis.

Rheumatoid

Arthritis : Smoking is known to be a risk factor for developing rheumatoid arthritis. One research study estimated that smoking is responsible for about 1 in 5 cases of rheumatoid arthritis.

Ageing : Smokers tend to develop more lines on their face at an earlier age than non-smokers. This often makes smokers look older than they really are.

Fertility : It is reduced in smokers (both male and female).

Menopause : On average, women who smoke have a menopause nearly two years earlier than nonsmokers.

Other conditions where smoking often causes worse symptoms.

These include:

- Asthma.
- Colds.
- Flu (influenza).
- Chest infections.
- Tuberculosis infection of the lungs.
- Long-term inflammation of the nose (chronic rhinitis).
- Eye damage due to diabetes (diabetic retinopathy).
- An overactive thyroid (hyperthyroidism).
- A disorder of the brain and spinal cord (multiple sclerosis).
- Inflammation of the optic nerve (optic neuritis).
- A condition causing inflammation of the gut (Crohn's disease).

Smoking increases the risk of developing various other conditions. These include:

- Dementia
- Optic neuropathy-this is a condition affecting the nerve supplying the eye.

- Cataracts.
- A breakdown of the tissue at the back of the eye (macular degeneration).
- Pulmonary fibrosis.
- A skin condition called psoriasis.
- Gum disease.
- Tooth loss.
- 'Thinning' of the bones (osteoporosis).
- Raynaud's phenomenon - in this condition, fingers turn white or blue when exposed to cold.

FREE RADICALS or REACTIVE OXYGEN SPECIES (ROS):

The term "free radicals" conjures up all kinds of images. Free radicals in the human body are involved in thousands of normal chemical reactions constantly occurring in the body, and are hydro-products of our "fire of life' oxidation reactions. Free radicals can be defined as chemical species possessing one or more unpaired electrons in their outer orbital. These free radicals formed by homolytic cleavage of a covalent bond of a molecule, by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule (Ray and Husain, 2002).

The mitochondria respiratory chain, NADPH cytochrome P_{450} in the endoplasmic reticulum, phagocytic cells, lypoxygenases and cyclo-oxygenases are also sources of basal ROS production (Halliwell and Gutteridge, 1999; Bestwick and Maffulli, 2004). Radicals are also produced blood processes other than normal metabolism-by ionizing radiation, smoking and other pollutants, herbicides and pesticides, and are even found in certain types of food (deep fat fried food) (Christophersen et al., 1991).

Reactive Oxygen Species (ROS) also referred as active oxygen species (AOS); reactive oxygen intermediates (ROM). ROS include a number of chemically reactive molecules derive from oxygen (Halliwell, 1996; Fridovich, 1999; Halliwell, 1999; Betteridge, 2000). Normal cellular metabolism involves the production of ROS such as superoxide ($O_2^{\cdot -}$), hydroxyl radical ($\cdot OH$), hydrogen peroxide (H_2O_2) and other reactive intermediates (McCord, 1993; Fattman et al., 2003). Some of those molecules are extremely reactive,

such as the hydroxyl radical, while some are less reactive (superoxide and hydrogen peroxide). Intracellular free radicals, i.e., free low molecular weight molecules with an unpaired electron, are often ROS and vise versa and the two terms are therefore commonly used as equivalents.

Free radicals and ROS can readily react with most bio-molecules, starting a chain reaction of free radical formation. It leads to cause oxidative stress and disrupt the balance between ROS production and antioxidant homeostasis. In order to stop this chain reaction, a newly formed radical must either react with another free radical, eliminating the unpaired electrons or react with a free radical scavenger- a chain- breaking or primary antioxidant (Nordberg and Arner, 2001). Low levels of ROS are vital for many cell signaling events and are essential for proper cell functions. For example, superoxide is necessary for proper immune function (Oury et al., 1996). Under physiological conditions a balance exists between the level of ROS produced during normal cellular metabolism and the level of endogenous antioxidants, which serve to protect tissue from oxidative damage. Disruption of this balance, either through increased production of ROS or decreased levels of antioxidants, produces a condition referred to as oxidative stress.

Free Radical Damage:

Oxygen derived free radicals or reactive oxygen species (ROS) have been implicated in the pathogenesis of a wide spectrum of disease as well as in the aging process. The over production of free radicals leads to variety of pathological conditions including cardiovascular diseases, neurological disorders, lung pathologies and accelerated aging (Delanty and Dichter, 1998; Bowjer and Crapo, 2002; Fukai et al., 2002). In addition to playing role in direct tissue damage, their generation may also amplify the body's general inflammatory response and promote further cell injury (Best et al., 1999). The free radicals and/or reactive oxygen species (ROS) may cause damage to various biological molecules including DNA, lipid and proteins in human tissues. The severity of oxidative damage can be gauged by accumulation of products generated from oxidative modification of macromolecules, such as lipids and proteins (Chevion et al., 2000).

Chemistry of Biological Oxidation:

When oxygen is partially reduced it becomes activated and reacts readily with a variety of bio-molecules. This partial reduction occurs in one-electron step, by addition of one, two and four electrons to O_2, which leads to successive formation of reactive oxygen metabolites (ROMs). These are five possible species: Superoxide anion ($O_2^{\cdot -}$), hydroperoxyl radical (HO_2^{+}), peroxide ion ($HO_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) (Chessman and Slater, 1993: Ray and Husain, 2002).

Superoxide ($O_2^{\cdot -}$):

The result of monovalent reduction of triplet oxygen is called superoxide, abbreviated as $O_2^{\cdot -}$. Superoxide is a radical; it is usually shown with a negative sign, indicating that it carries a negative charge of –1.

$^{\cdot}O$ □ $O^{\cdot}$ Triplet oxygen (Ground state)

↓ Monovalant reduction

$^{\cdot}O$ □ O: Superoxide

Superoxide anion is the first reduction product of oxygen (O_2). $O_2^{\cdot -}$ can be produced either by the univalent reduction of O_2 or by the univalent oxidation of H_2O_2. However, superoxide is not particularly reactive in biological system and does not by itself cause much oxidative damage. It is a precursor to other oxidizing agents, including singlet oxygen, peroxynitrite and other highly reactive molecules. Superoxide also acts as a signaling molecule needed to regulate cellular processes (Leeuwenburgh and Heinecke, 2001). Superoxide is also important in the production of the highly reactive hydroxyl radical ($^{\cdot}OH$). This is because superoxide donates one electron to reduce the metal ions that acts as the catalyst to convert hydrogen peroxide into hydroxyl radical ($OH^{\cdot -}$).

$$O_2^{\cdot -} + Fe^{3+} \longrightarrow {}^3O_2 + Fe^{2+}$$

The reduced metal (ferrous ion or Fe^{2+}) then catalyzes the breaking of the hydrogen-oxygen bond of hydrogen peroxide to produce a hydroxyl radical ($^{\cdot}OH$) and a hydroxyl ion (OH^{-})

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

Superoxide can react with the hydroxyl radical to form singlet oxygen ($^1O_2$·) which is not a radical but reactive nonetheless.

$$O_2^{\cdot -} + OH^{\cdot} \longrightarrow {}^1O_2^{\cdot} + OH^-$$

Hydrogen Peroxide (H_2O_2):

Hydrogen peroxide is the most stable reactive oxygen metabolite (ROMs). This is to say that it is the least reactive and the most readily detected. H_2O_2 may be generated directly by divalent reduction of O_2 or indirectly by univalent reduction of superoxide anion. H_2O_2 is the primary product of the reduction of O_2 by numerous oxidases such as xanthine oxidase (XO), uricase, localized in peroxisomes (Ray and Husain, 2002). Superoxide can undergo monovalant reduction to produce peroxide (O_2^{-2}) an activated form of oxygen that carries a negative charge of -2. Usually peroxide is termed "hydrogen peroxide" since in biological systems the negative charge of -2 is neutralized by two protons (two hydrogen atoms, each with a positive charge).

˙O □ O: Superoxide

↓ Monovalant reduction

H: O □ O: H hydrogen peroxide

H_2O_2 is not a free radical but is nonetheless highly important much because it can pass readily through the biological cell membranes and can't be excluded from the cell. H_2O_2 once produced by the above mentioned mechanisms is removed by at least three antioxidant enzyme systems namely; catalases, glutathione peroxidases and peroxyredoxins (Chae et al., 1999; Mates et al., 1999; Leeuwenburgh and Heinecke, 2001; Nordberg and Arner, 2001).

Hydroxyl Radical (˙OH):

Due to its strong reactivity with bio-molecules, ˙OH is probably capable of doing more damage to biological systems than any other ROS (Betteridge, 2000; Nordberg and Arner, 2001). Hydrogen peroxide in the presence of metal ions (Cu^+ / Fe^{2+}), is converted to a hydroxyl radical(˙OH)and hydroxide ion (OH^-).The metal ion is required for the breaking

of the oxygen-oxygen bond of peroxide. This reaction is called the **'Fenton Reaction'** and was discovered over a hundred years ago. It is important in biological systems because most cells have some level of iron, copper, of other metals which can catalyze this reaction.

H:O —- O:H hydrogen peroxide
Metal ion
HO· hydroxyl radical
and
HO: hydroxide ion

This reaction also be written

$$H_2O_2 + Cu^{+}/Fe^{2+} \longrightarrow {}^{\cdot}OH + OH^{-} + Cu^{2+}/Fe^{3+}$$

A hydroxyl radical can also react with superoxide to produce singlet oxygen and a hydroxide ion.

$$HO^{\cdot} + O_2^{\cdot -} \longrightarrow {}^1O_2^{\cdot} + OH^{-}$$

Like hydrogen peroxide the hydroxyl radical passes easily through membranes and cannot be kept out of cell. Hydroxyl radical is highly reactive. It can react with practically any molecule present in cells. For this reason it is short lived. This insufficient stability does not allow it to diffuse through the cells. The life span of ·OH at 37°C is 10^{-9} s. It does not survive for more than a few collisions after its formation. Due to such short lifetime, it is very difficult to investigate the ·OH by conventional methods (Pryor, 1986). This ·OH is produced following the reaction of $O_2^{\cdot -}$ and H_2O_2 in presence of metallic ions such as Cu^{+} / Fe^{2+} (Ray and Husain, 2002).

Singlet Oxygen ($^1O_2$):

Oxygen in the air we are breathing is in its "ground" (not energetically exited) state and is symbolize by the abbreviation $^3O_2$. It is a free radical in fact it is adradical, as it has two unpaired electrons. Molecules whose out most pair of electron has parallel spins (symbolized ↓↓) are in the "triplet" state: molecules whose outer most

pair of electrons has antiparellel spins (symbolized□□) are in the "single" state. Singlet oxygen is produced as a result of natural biological reactions and by photosensitization by the absorption of light energy. Like many other reactive species, this can be harmful at higher concentrations and at low levels may act as signaling molecules. Due to it's relatively long-life, $^1O_2$ can travel appreciable distance in the cellular environment and is capable of damaging various bio-molecules (Sies and Packer, 2000). In human plasma, which is rich in antioxidants, the lifetime of $^1O_2$ is calculated to be 1 μs. It can move freely across water-lipid interfaces. In can behave, like as a strong electrophile in solutions and reacts with bimolecular possessing regions of high electron density (for instance guanine in DNA). Oxidative damage in bio-molecules mediated by $^1O_2$ is rather frequent. Lipids, proteins and DNA are all at risk (Devasagayam and Kamat, 2002).

AGING:

Aging, an unwanted, unavoidable and universal biological phenomenon, is caused by time dependent progressive deleterious and irreversible changes occurring in cells, organs and in the total organism (Patel, 1981). Metabolic machinery of the body deteriorates at an increasing rate after the organism reaches its reproductive maturity (Shock, 1979). Aging may be described as a phenomenon which results from the accumulation of changes in informational biomolecules and is responsible for both the diminished bodily functions with advancing age and associated progressive increase in the chance of diseases and death (Harman, 1992; Masoro, 1993). Numerous definitions has been given by various scientists for aging.

Shock (1961, 1962) and Weiss, (1966) defined aging as, "the sum total of changes during an individual's life span which are common to all members of his species (or) strain".

Carl Leopold (1974) explained that, "aging is a process associated with accrual of maturity of time. The rat at which the developmental changes occurs slows with increasing age".

Masoro (1993) defined "aging as process accuring during the life time, which increasing the vulnerability of the organism to challenges, thereby increasing the likelihood of death"

Aging-Metabolic changes:

The aging process has been shown to result in an accelerated functional decline. The exact mechanisms that cause this functional decline are unclear. The free radical theory of aging, however, has gained strong support because it is able to explain some of the processes that occur with aging and the degenerative diseases of aging. This theory proposes that an increase in oxygen radical production with age by mitochondria produce an increase in cellular damage (Harman, 1996, 1998). Aerobic organisms are well-protected against oxidative challenges by sophisticated antioxidant defense systems. However, it appears that during the aging process an imbalance between oxidants and antioxidants balance may occur, referred to as oxidative stress. Oxidative stress induced by oxidant species occurs under conditions when antioxidant defenses are depleted or when the rate constants of the radical reactions are greater than the antioxidant defense mechanisms (Buettner, 1993). Oxidative damage to these biomolecules seems to depend on hydrogen peroxide and a reduced transition metal. Therefore, molecules that contain transition metals, such as aconitase (a Krebs cycle enzyme), are likely to undergo oxidative damage (Hausladen and Fridovich, 1994).

When animal models of aging have been used, a decrease of mitochondrial oxidative function as a cause of reduced aerobic capacity has been implicated by studies that show decreased oxidative enzyme activities in skeletal muscle homogenates (Stump et al., 1977; Hansford, 1983) as well as in isolated mitochondria (Sugiyama et al., 1993; Desai et al., 1996). Similarly, conflicting results were obtained in functional studies measuring the respiratory properties of isolated mitochondria, reporting either no changes (Farrar et al.,1981; Bayer et al.,1984) or a decrease in respiratory rates with some but not with other substrates (Hansford, 1983). These discrepant findings with respect to age on skeletal muscle aerobic capacity could in part be explained by the different strains and species used in the studies as well as the different muscles sampled (Holloszy et al.,1991).

Some evidences implicates oxidative damage of cellular constituents in aging, as well as in the pathogenesis of the degenerative diseases of later years (Baynes, 1991; Stadtman, 1992; Youngman et al., 1992; Ames et al., 1993; Viner et al., 1996). Reactive oxygen intermediates are potentially damaging to nucleic acids, lipids, and proteins (Stadtman, 1992;

Ames et al., 1993; Huggins et al., 1993; Orr and Sohal, 1994; Masoro, 1995; Esser and Martin, 1995). Several different metal-catalyzed oxidation systems convert certain amino acid residues to carbonyl derivatives (Stadtman, 1992). Protein-bound carbonyls are present at low levels in dermal fibroblasts isolated from young to middle-aged donors, but they are increased twofold in fibroblasts from people over the age of 60 years. Moreover, fibroblasts obtained from patients with diseases of accelerated aging have dramatically higher levels of protein carbonyls (Oliver et al., 1987). These results suggest that levels of oxidized proteins may increase with age, and that metal catalyzed oxidation reactions may be partly responsible for protein oxidation. Other factors, such as less efficient removal of oxidized proteins through proteolytic cleavage, may also promote the accumulation of protein carbonyls with aging (Ursine, 1990; Stadtman, 1992).

Aging - Oxidative stress:

The free radical theory of aging was first proposed by Denman Harman in the 1950s (Harman, 1956). There is now substantial evidence that supports that aging is associated with, if not the consequence of, free radical damage by various endogenous reactive oxygen species (Finkel and Holbrook, 2000; Harman, 2001). This role of reactive oxygen species in aging is thought to explain the observation that animals with higher metabolic rates have shorter lifespans, the so-called “rate of living” hypothesis (Finkel and Holbrook, 2000).

Aging is associated with evidence for deleterious changes to the molecular structure of DNA (deoxyguanosine derivatives), proteins (carbonyls), lipids (lipoperoxides, malondialdehydes), and prostaglandins (isoprostanes), all markers of oxidative stress (Harman, 1992, 1993) (Diagram-5).

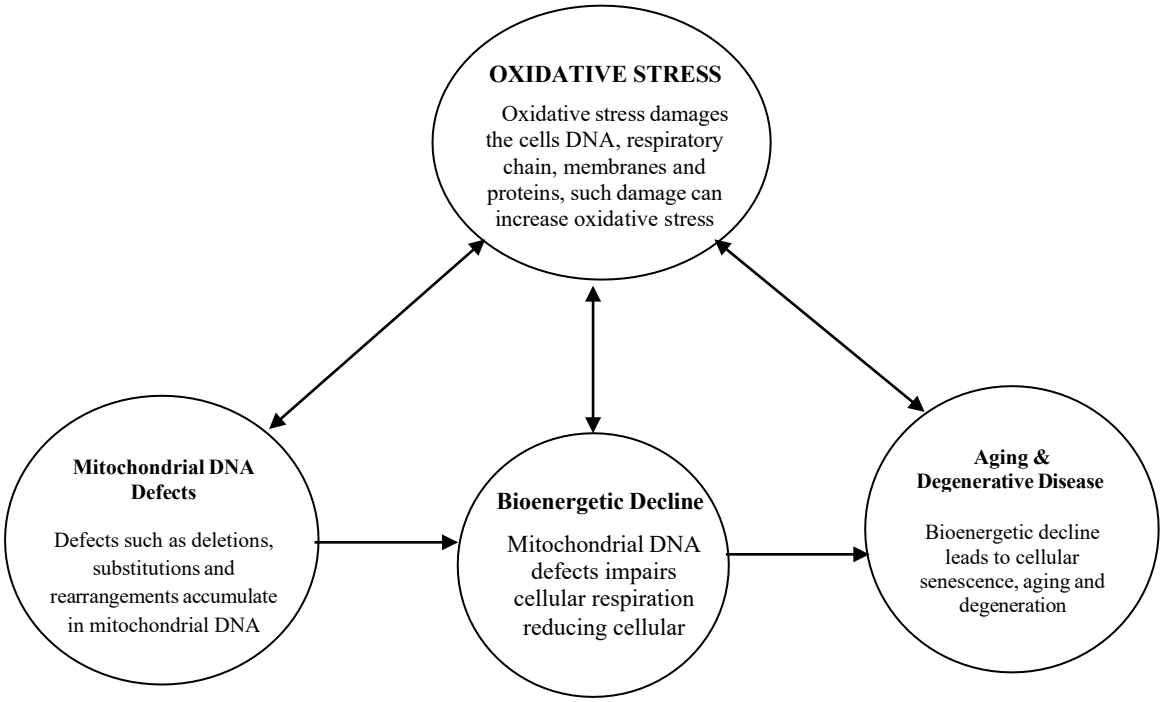


Diagram-5

The "error catastrophe" theory of aging proposes that the accumulation of these molecular changes, particularly in proteins, constitutes the basis of cell aging and leads to death. More recently, it has been recognized that reactive oxygen species also play a role in normal signaling processes and that their generation is essential to maintain homeostasis and cellular responsiveness (Droge, 2002).

Reactive oxygen species include superoxide and hydroxyl radicals and other activated forms of oxygen such as hydrogen peroxide and singlet oxygen. In 1972 it was suggested that the primary sites of production of reactive oxygen species were the mitochondria, as a byproduct of oxidative metabolism (Harman, 1972). Other major sources of reactive oxygen species include phagocytic processes, prostaglandin synthesis, cytochrome P_{450} enzymes, nonenzymatic reactions of oxygen, and ionizing radiation (Finkel and Holbrook, 2000; Harman, 2001). Enzymatic defenses that minimize oxidative injury include superoxide dismutase, catalase, glutathione peroxidase, glutathione transferases, peroxidases, and thiol-specific antioxidant enzymes. These, together with a host of low-molecular-weight compounds such as ascorbate, glutathione, carotene, tocopherol, uric acid, and bilirubin serve as free radical scavengers (Harman, 2001).

Aging–Lung :

Respiratory conditions are among the leading causes of morbidity and mortality worldwide. Although they are currently listed as the fifth leading cause of death in Canada, respiratory diseases are predicted to be the third leading cause of mortality by the year 2020, following ischemic heart disease and stroke **(**Murray and Lopez, 1997). Furthermore, since the prevalence of these conditions increases with age, the adverse impact of respiratory illnesses on the Canadian health care system will grow enormously over the next few decades as the overall population ages **(**Randall, 1993**)** and treatments for other common conditions, such as ischemic heart disease, stroke and diabetes, improve. Agood understanding of the aging process of the respiratory system is clearly needed to formulate better strategies to prevent, diagnose and manage respiratory conditions.

The lungs of elderly persons are subject to a lifetime of exposure to known and unknown harmful agents. Decades may pass before the physical manifestations of cigarette smoke, pollution and other noxious environmental agents become clinically apparent. Furthermore, the respiratory system of an elderly person may have "battle scars" from repeated bouts of upper and lower respiratory tract infections, aspiration pneumonia, hypersensitivity reactions and other acute insults. The accumulated "left- over" damages from these acute episodes may build up over the years to produce functional impairments. Elderly individuals also may have many more comorbid illnesses than their younger counterparts. Comorbidities that affect oxygen delivery such as congestive heart failure, anemia or cardiac ischemia frequently exacerbate dyspnea associated with underlying respiratory conditions, and make it more refractory to standard therapy. Finally, as we will discuss further, the pulmonary system ages physiologically, making elderly individuals susceptible to frequent respiratory illnesses.

Physiologic Age-related Changes of the Lung :

With the aging process, the elastin component of the lung matrix decreases while the amount of type III collagen increases, **(**Murray, 1986**)** making the pulmonary system more compliant. Because of this, during respiratory cycles alveolar units can become destabilized, leading to fluid/mucous retention and atelectasis.

Alveolar basement membrane also thickens with aging (Niewoehner and Kleinerman, 1974), which reduces diffusion capacity and may result in poor gas exchange (i.e., increased alveolar-arterial oxygen gradient). Indeed, the arterial oxygen tension (PaO_2) decreases with aging independent of any superimposed disease processes such as chronic obstructive pulmonary disease (COPD), pulmonary hypertension or heart failure. Even among elderly individuals without any associated lung disease, the alveolararterial oxygen gradient is larger than that observed in the young, healthy population, making it imperative that prediction equations for oxygen tension take into account age-related changes to partial pressure of oxygen. Carbon dioxide tension, on the other hand, is not materially influenced by age.

Airways :

Even in the absence of any material insult or injury to the airways, bronchiolar diameter diminishes significantly after age 40, largely in response to decreased elastin fiber deposition in the supporting connective tissues (Niewoehner and Kleinerman, 1974). Physiologically, this leads to a marked reduction in the "tethering" forces of the surrounding matrix to keep the adjacent airways patent. This can increase airway resistance and promote premature airway closure,and as such, elderly individuals can demonstrate mild airflow obstruction at low lung volumes even in the absence of any disease. Since airway inflammation is not a prominent feature of age-related changes in the airways, corticosteroids and bronchodilators are largely ineffective in reversing mild airflow obstruction associated with aging.

Chest Wall :

There are two important components of the chest wall that become adversely affected with the aging process: the bony structures and the respiratory muscles. With bone demineralization and osteoporosis, kyphoscoliotic changes may become apparent in some elderly individuals, which may diminish chest wall expansion and produce lung restriction. Moreover, some may develop significant calcification and fusion of rib-vertebrae joints, which can produce similar physiologic impairments. Although muscle fiber atrophy has not been consistently demonstrated with aging (Tolep and Kelsen, 1993), there is a progressive loss in muscle fibers after the fifth decade, which probably explains the reduced respiratory muscle strength associated with aging. Because the diaphragm is also involved, additional ventilator loading (during acute illnesses) can quickly

lead to respiratory muscle fatigue and respiratory failure in some elderly individuals (Polkey et al., 1997).

Ventilatory Responsiveness to Hypercapnia and Hypoxia :

Ventilatory responsiveness as well as neural output from central and peripheral chemoreceptors are diminished with aging (Mc Connell and Davies, 1992). The changes are most prominently observed with hypercapnia (Peterson et al., 1981). Compared to healthy young individuals, elderly persons have a blunted ventilator response to increases in carbon dioxide. They also have a reduced perception of dyspnea associated with hypercapnia, hypoxia and/or airflow obstruction, which can delay their presentations to health care providers for treatment of respiratory infections (Fanta, 1989). Such delays can markedly increase their risk for arrhythmias, respiratory failure and even sudden deaths.

Paradoxically, during exertion the elderly have a more vigorous ventilator response to exercise compared to younger individuals, which allows them to reach their ventilatory "ceiling" at lower workloads, thus limiting their overall exercise capacity (Brischetto et al., 1984). Peak oxygen consumption falls because of other agerelated physiologic changes, including loss of muscle mass, airflow obstruction and reduced stroke volume.

Although the effects of aging on the pulmonary vasculature are not well understood, pulmonary vessels become less distensible and more constricted with aging, principally due to vascular wall remodeling. This may limit the individual's ability to increase cardiac output during exertion (Priebe, 2000). However, age-related changes in the pulmonary circulation usually have minimal impact on cardiac output at rest (Priebe, 2000).

Age-related Changes in Pulmonary vasculature Function :

There are two directly opposing forces that govern lung expansion and contraction: elastic recoil pressure and expansive force of the chest wall. At end- expiration (or functional residual capacity [FRC]), the recoil pressure of the lung generated by elastic fibers is balanced perfectly by an equal but opposite (expansive) force generated by the chest wall. Because the lung recoil pressure decreases with age due to a reduction in elastic tissues, FRC increases.The residual volume (RV) is the principal component of FRC that is increased.

Vital capacity (VC), on the other hand, remains the same or decreases slightly with aging (Burr et al., 1985). If FRC exceeds the closing volume (i.e., the lung volume at which airways begin to close), premature airway closure can occur during normal expiration, leading to flowlimitation.

Owing to age-related physiologic changes to the airways, forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1 to FVC ratio also decrease with age. FEV1 is reduced by approximately 15–30cc/year among non- smoking men and 10–20 cc/year among non-smoking women (Knudson et al., 1983). The decline in FEV1 accelerates after age 65 and is further exacerbated by smoking. Inspiratory and expiratory pressures also decrease with age, reflecting the decline in respiratory muscle strength. The reduced surface area and increased thickness of alveolar walls cause a decline in diffusing capacity from midlife at a rate of 2.03mL/min/mmHg per decade in men and 1.47mL/min/mmHg per decade in women (Neas and Schwartz, 1996).

Impact of Age on Diagnosis of Respiratory Disorders :

Most of the deaths in asthma and COPD are among elderly patients. There is a general under-recognition and undertreatment of these disorders in the elderly, which may, in part, be related to decreased patient perception and reporting of symptoms and to the frequent occurrence of comorbidities that may confound the diagnosis and lead to an under-appreciation of disease severity (Chan and Welsh, 1998). Decreased perception of airflow obstruction, hypoxia and hypercapnia may also lead to a significant delay in elderly patients seeking medical care. Even when care is sought, the patients may trivialize the significance of their symptoms, leading to inappropriate management (Chan and Welsh, 1998). Whenever possible, clinicians should supplement patient's history with objective measurements of lung function through spirometric testing in order to establish disease severity.

Because pneumonia is a common problem among the elderly, vaccination should be considered. Although elderly individuals have diminished humoral immune responses to vaccines in general, influenza vaccination is still very effective in preventing clinically relevant and severe influenza infections. Accordingly, all individuals 65 years of age and older should receive yearly influenza vaccination, regardless of their clinical

status (Nichol, 2000). The clinical efficacy of pneumococcal vaccination is modest. However, it has been demonstrated to be effective in preventing pneumococcal sepsis and possibly reducing lung infections. Elderly patients, and especially those with significant comorbid illnesses, should receive pneumococcal vaccination.

Because of the physiologic effects of aging on the respiratory system and accumulated exposures to harmful infectious and non-infectious respiratory agents, obstructive airway disease and other respiratory conditions are common in the elderly population. In the presence of dyspnea and/or cough, spirometry should be used to confirm the diagnosis of obstructive airway disease and therapy should be instituted, when necessary. If medications are implemented, the lowest possible dose should be used and their effects need to be carefully followed and monitored.

OBJECTIVES OF THE PRESENT STUDY

The above reviews of literature reveal the effect of red grape, nicotine and aging on carbohydrate metabolism, oxidative and antioxidative enzymes in the lung tissue. Nicotine and aging are the principle sources for free radical production. These free radicals or reactive oxygen species create oxidative stress by disturbing the homeostasis in the tissue (cell). Both aging and nicotine consumption have been found to produce changes in antioxidant defense system. It is logical, therefore, that differences in the lung tissue response to nicotine consumption, when comparing young with old individuals may exist. Elderly people may be more susceptible to oxidative stress induced by nicotine consumption than youth. As the number of elder smokers in the population is quite large, who are also taking regular red grape juise, the effects of regular red grape on aging and nicotine induced oxidative injury deserve thorough investigation. So far, not much work has been focused on the interaction of red grape extract and nicotine combination on the lung system in two different age groups of male albino rats with reference to age.

Hence, in the present study an attempt has been made to investigate whether endurance red grape extract would improve the carbohydrate metabolism, oxidative and antioxidative enzymes in the lung tissue of nicotine induced and aged subjects.

The main objectives of the present investigation are:

- To examine the age related changes in the levels of carbohydrate metabolites, Oxidative and Antioxidant enzyme system in the lung tissue of two different age groups of rats i.e., young and old age groups, with reference to red grape extract and nicotine treatment.
- To examine whether two months period of endurance red grape extract has beneficial anyway by alter the carbohydrate metabolites, Oxidative and Antioxidants enzymes in the lung tissue of male albino rats.
- To evaluate the interactive effect of red grape extract and nicotine (combination treatment) with reference to lung tissue and aging in male albino rats.

PROGRAMME OF THE PRESENT STUDY:

The following aspects of carbohydrate metabolic profiles, oxidative and antioxidant enzymes in physiologically different age groups in the lung tissue of control, nicotine treatment, red grape extract treatment, combined effect of red grape and nicotine treatment in young (3 months) and old rats (18 months) have been studied.

- The levels of total carbohydrates, glycogen and total free amino acids were assayed in young and old age groups of rats.
- To elucidate the oxidative enzymes regulation due to the effect of red grape extract, nicotine and combined effect, such as LDH, ICDH, SDH, MDH and G-6-PDH were assayed.
- To assess the extent of changes in the antioxidant enzymes, such as SOD, CAT, GSH and GPX were assayed in the control as well as experimental age groups in the lung tissues.

The results obtained in the current investigation have been described and discussed in detail in the light of available supportive and critical reports to understand the response of carbohydrate metabolism, oxidative and antioxidant enzymes in physiologically different age groups in the lung tissue such as young and old of male albino rats during nicotine and age induced stress conditions, with reference to beneficial role of endurance red grape extract.

MATERIALS AND METHODS

CARE AND MAINTENANCE OF EXPERIMENTAL ANIMALS

Pathogen free, wistar strain male albino rats of two age groups (3 months and 18 months) 3 months age group considered as 'Young age' and 18 months age group considered as 'Old age' as per the life span of Wistar strain, (Jang et al.,2001) were used in the present study. The usage of animals was approved by the Institutional Animal Ethics Committee (No.10/(i)/a/CPCSEA/IAEC/SVU/ZOOL/KC/ Dt.08.07.2012). The rats were housed in clean polypropylene cages under hygienic conditions with photoperiod of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai) and water ad libitum.

Selection of age group:

In the book, entitled "International care and treatment of rabbits, mice, rats, guinea pigs and Hamsters" published by W.B. Saunders Co., Philadelphia, USA. Schuchman, (1989) given a detailed table regarding the age and life span of different strains of laboratory animals. As per this study the maximum life span of a rat is 3 years. Cao and Cutler, (1995) studied aging process from 6 months age through 12 and 24 months. Jang et al., (2001) studied age related changes in 2.5, 5, 10 and 23 months of Wistar strain rats. In their study 12 months age group rats were considered as the second highest age group. Thus, the literature pertaining to selection of age group in the field of "aging" is variable in various studies.

Maintenance of animals for three years long period to attain maximum aging in the laboratory is practically difficult. The puberty of the rat reaches in between 50 – 60 days (i.e., 2 months). So, any time after 2 months is considered as "matured age". The rat attained 12 months age considered as "middle age" and after that age is the "old age". However, between 12 months and 36 months the animal becomes older and with diminished physiological functions. On the basis of the physiology of the animal, in the present study "3 months age" group considered as "Young" and "18 months age" group was considered as "Old" for effective comparison of aging process in relation to red grape extract treatment and nicotine treatment.

SELECTION AND MODE OF NICOTINE TREATMENT

Nicotine was first distilled from tobacco sap in 1809. Nineteen years later, the main base of tobacco was isolated and separated in pure form from fermented as well as non-fermented tobacco by Posselt and Reimann (Pailer, 1964). They called it nicotine and characterized it as a water-clear liquid, boiling under atmospheric pressure at 246ºC, miscible with water, alcohol and ether. Historically nicotine had been recommended for treatment of numerous symptoms.

PHYSICAL AND CHEMICAL PROPERTIES OF NICOTINE

1)	Nicotine Scientific name	:	Nicotiana tobacco
2)	Nicotine Family	:	Solanaceae
3)	Chemical formula	:	$C_{10}H_{14}N_2$
4)	Molecular Weight	:	162.23
5)	IUPAC Name	:	3-[2-(N-methylpyrrolidinyl)]pyridine
6)	Appearance	:	Oily, colourless hygroscopic liquid,
7)	Characteristic odour	:	Turns brown on exposure to air
8)	Boiling point (decomposes)	:	246 °C
9)	Density	:	1.01 g cm-3
10)	Solubility in water	:	miscible

DOSAGE OF NICOTINE

The dose administration of nicotine was followed as per the protocol given by (Shoaib and Stolerman, 1999; Helen et al., 2003) 0.6 mg / kg body weight (0.5ml) was chosen as the dose, for this study.

GROUPING OF ANIMALS

Age matched (young and old) rats were divided into 4 groups of six in each groups. i) Control, ii) Nicotine treatment (Nt), iii) Red Grape extracts treatment (RGEt) and iv) Nicotine + Red Grape extract treatment (Nt+RGEt).

Group I – Control:

Six rats were treated with normal saline (0.9%) orally via orogastic tube for a period of 2 months.

Group II – Nicotine treatment (Nt):

Rats were received the nicotine at a dose of 0.6 mg/kg body weight (0.5ml) by subcutaneous injection for a period of 2 months.

Group III – Red Grape extract treatment (RGEt):

Rats were received red grape extract 25mg/kg body weight via orogastric tube for a period of 2 months.

Group IV – Nicotine + Red Grape extract treatment (Nt+RGEt):

Rats were received the nicotine at a dose of 0.6 mg/kg body weight (0.5ml) by subcutaneous injection and red grape extract 25mg/kg body weight via orogastric tube for a period of 2 months.

The animals were sacrificed after 24 hrs after the last treatment session by cervical dislocation and the lung tissue were isolated at -4^0, washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80^0 for biochemical analysis and enzymatic assays. Before assay, the tissues were thawed, sliced and homogenized under ice-cold conditions. Selected parameters were estimated by employing standard methods.

BIOCHEMICAL ANALYSIS

Total Carbohydrates:

The total carbohydrate content was estimated by the method of Carroll et al., (1956). The lung tissue was homogenized in 10% Trichloro acetic acid (TCA) to prepare 1% (W/V) homogenates. The proteins precipitated were removed by centrifuging the homogenates for 15 minutes at 3000g at 4^0C. The clear supernatant was taken for the estimation of total carbohydrates. To 0.5 ml of supernatant, 5 ml of anthrone reagent was added and kept in a boiling water bath for 15 minutes. Then,

the contents were cooled and read at 620 nm against the reagent blank. The total carbohydrate content was expressed as mg of glucose/gm wet weight of the tissue.

Glycogen:

The Glycogen was estimated by the method of Kemp and Van Hejnigen, (1954). The lung tissues were homogenized in 80% (W/V) methanol to prepare 5% (W/V) homogenates. The suspension was centrifuged at 3000g for 15 minutes at 4^0C the supernatant containing glucose was decanted. (The glycogen content present in the lung tissue homogenates was estimated after extraction of the glucose with 80% methanol). Now the lung tissue residue was suspended in 5 ml of deproteinizing solutions (5% TCA containing 0.1% silver sulphate) and the fluid level was marked on centrifuge tube and the tube was covered with a glass cap and placed in a boiling water bath for 15 minutes. Then the tube was cooled in running tap water and deproteinizing solution was added up to the mark to compensate the loss due to evaporation. The contents were centrifuged at 5000g for 15 minutes at 4^0C. 1ml of clear supernatant was added to 3ml of concentrated sulphuric acid in a wide mouthed test tube mixed by vigorous shaking. The mixture was heated in a boiling water bath for exactly 6.5 minutes and subsequently cooled under running tap water. The intensity of the pink colour developed was read against the blank at 520 nm in a spectrophotometer. The glycogen content was expressed in mg of glucose/gram wet weight of the tissue.

Total Free Amino Acids:

The total free amino acids were estimated by the method of Moore and Stein, (1954). 5% (W/V) homogenates of lung tissues were prepared in 10% (W/V) trichloro acetic acid (TCA) and centrifuged the contents at 2000g for 15 min at 4^0C. To 0.5 ml of supernatant, 2.0ml of Ninhydrin reagent was added and the contents were exactly boiled for 6½ minutes in a boiling water bath. The contents were cooled to laboratory temperature. The samples were made upto 10 ml with distilled water and the colour intensity was read at 570 nm in a spectrophotometer against the reagent blank. The total free amino acid content was expressed in mg of free amino acids per gram wet weight of the tissue.

Lactate Dehydrogenase (LDH) (L-lactate: NAD^+ Oxidoreductase– E.C:1.1.1.27):

Lactate Dehydrogenase activity was determined by the method described by Nachlas et al., (1960) as suggested by Prameelamma and Swami, (1975) with slight modifications. 10% homogenates of the lung tissue were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4^0C. The supernatant fraction was used for enzyme assay. The reaction mixture in a final volume of 2 ml contained 40 µ moles of sodium lactate, 100 µ moles of phosphate buffer (pH 7.4), 0.1 µ mole of NAD and 4 µ moles of INT. The reaction was initiated by the addition of
0.2 ml of homogenate containing 20 mg of tissue and incubated for 30 minutes at 37^oC and the reaction was stopped by the addition of 5 ml of glacial acetic acid. Zero time controls (ZTC) were maintained by addition of 5 ml of glacial acetic acid prior to the addition of the enzyme source to the incubation mixture. The formazan formed was extracted over night into 5 ml of toluene at 5^0C. The color developed was measured at 495 nm in a Spectrophotometer against the toluene blank. The enzyme activity was expressed in µ moles of formazan formed / mg protein / hour.

Isocitrate Dehydrogenase (ICDH) (Isocitrate: $NADP^+$ oxidoreductase- E.C:1.1.1.42):

Isocitrate dehydrogenase was assayed by the method of Korenberg and Pricer, (1951) as modified by Mastanaiah et al.,(1978).10% homogenates of lung tissues were prepared in 0.25M ice cold sucrose solution and centrifuged at 1000g for 15 minutes at 4^0C. The supernatant was used for the enzyme assay. The reaction mixture in a final volume of 2.0 ml contained 40µ moles of DL-isocitrate, 100 µ moles of magnesium chloride, 100 µ moles of sodium phosphate buffer (pH-7.4), 4 µ moles of INT (2-P-iodophenyl 3-P-nitrophenyl 5-phenyl tetrazolium chloride), 0.2 µ moles of ADP and 0.2 µ moles of NADP (for $NADP^+$-ICDH).

The reaction was initiated by the addition of 0.2 ml supernatant containing 20mg of the enzyme source and the contents were incubated at 37^0C for 30 minutes. After incubation, the reaction was stopped by adding 5.0 ml of glacial acetic acid and the formazan formed was extracted overnight at 5^0C into 5.0 ml of toluene. The colour was measured at 495nm in a spectrophotometer against toluene blank. The enzyme activity was expressed as µ moles of formazan formed/mg protein/hour.

Succinate Dehydrogenase (SDH) (Succinate acceptor oxidoreductase– E.C: 1.3.99.1):

The specific activity of SDH was assayed by the method of Nachlas et al., (1960) as suggested by Prameelamma and Swami, (1975) with slight modifications. 10% homogenates of the lung tissue were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4^0C. The supernatant fraction was used for enzyme assay. The reaction mixture in a final volume of 2 ml contained 40 µ moles of sodium succinate, and 100 µ moles of phosphate buffer (pH 7.0) and 4 µ moles of INT. The reaction was initiated by adding 0.2 ml of homogenate containing 20 mg of tissue. The incubation was carried out for 15 minutes at 37^0C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The subsequent steps were followed same as described for LDH. The activity was expressed in µ moles of formazan formed / mg protein / hour.

Malate Dehydrogenase (MDH) (L-Malate NAD^+ Oxidoreductase– E.C: 1.1.1.37):

The specific activity of MDH was measured by the method of Nachlas et al., (1960) as suggested by Prameelamma and Swami, (1975) with slight modifications. 10% homogenates of the lung tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4^0C. The supernatant fraction was used for enzyme assay. The total volume 2 ml of reaction mixture contained 100 µ moles of phosphate buffer (pH 7.0) 40 µ moles of sodium malate, 0.1 µ mole of NAD and 4 µ moles of INT. The reaction was initiated by the addition of 0.2 ml of homogenate containing 20 mg of tissue. The incubation was carried out at 37^0C for 30 minutes and the reaction was arrested by adding 5 ml of glacial acetic acid. The rest of the procedure was same as described earlier for LDH. The activity was expressed in µ moles of formazan formed / mg protein / hour.

Glucose-6-Phosphate Dehydrogenase (G-6-PDH – E.C: 1.1.1.49):

Glucose-6-posphate dehydrogenase activity was assayed by the method of Lohr and Waller (1965), as modified by Mastanaiah et al.,(1978). 10% lung tissues homogenates were prepared in 0.25 M ice cold sucrose solution and centrifuged at 1000 g for 15 min at 4^0C. The reaction mixture in a total volume of 2 ml contained 100 µ moles of sodium phosphate buffer (pH 7.4), 20 µ moles of glucose-6-phosphate, 2 µ moles of INT and

0.3 µ moles of NADP. The reaction was initiated by adding 0.5ml containing 50 mg of enzyme source. The reaction mixture was incubated at 37^0C for 30 min and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted with 5 ml of toluene at 5^0C. The optical density of the formazan was read at 495 nm against the toluene blank. The activity was expressed in µ moles of formazan formed/ mg protein / hour.

Superoxide Dismutase (SOD – EC: 1.15.1.6):

Superoxide dismutase activity was determined according to the method of Misra and Fridovich, (1972) at room temperature. The lung tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (W/V). The homogenates were centrifuged at 10,000 rpm for 10 min at 4^0C in cold centrifuge. The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min on a Hitachi U-2000 Spectrophotometer. Activity expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit.

Catalase (CAT – EC: 1.11.1.6):

Catalase activity was measured by a slightly modified version of Aebi, (1984) at room temperature. The lung tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (W/V). The homogenates were centrifuged at 10,000 rpm for 10 min at 4^0C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 µl of 100% EtOH was added to 100 µl of tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 10 µl of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer and 50 µl of tissue extract was added 250 µl of 0.066 M H_2O_2 (in phosphate buffer) and decreases in optical density measured at 240 nm for 60 s in a UV spectrophotometer. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded / mg protein / min.

Glutathione (GSH) Content:

Glutathione content was determined according to the method of Theodorus et.al., (1981). The lung tissue was homogenized in 0.1M ice cold phosphate buffer (pH 7.0) containing 0.001M EDTA and protein is precipitated with 1 ml of 5% sulfosalicylic acid (W/V) and the contents were centrifuged at 5000 g for 15 min at 4^0C. The resulting supernatant was used as the enzyme source. The reaction mixture in a total volume of 2.5 ml contained 2.0 ml of 0.1M potassium phosphate buffer, 0.05 ml of NADPH (4 mg / ml of 0.5% $NaHCO_3$), 0.02 ml of DTNB (1.5 mg / ml), 0.02 ml of glutathione reductase (6 units/ ml) and required amount of tissue source. The reaction was initiating by adding 0.41 ml of enzyme source and change in absorbance was recorded at 425 nm against the reagent blank. The glutathione content was expressed in nano moles/ gram wet weight of the tissue.

Glutathione Peroxidase (GSH-PX – EC: 1.11.1.9):

Glutathione peroxidase (GSH-Px) was determined by a modified version of Flohe and Gunzler (1984). At 37^0C 5% (W/V) of lung tissue homogenate was prepared in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 4^0C in cold centrifuge. The resulting supernatant was used as enzyme source. The reaction mixture consisted of 500 µl of phosphate buffer, 100 µl of 0.01 M GSH (reduced form), 100 µl of 1.5 mM NADPH and 100 µl of GR (0.24 units). The 100 µl of tissue extract was added to the reaction mixture and incubated at 37^0C for 10 min. Then 50 µl of 12 mM t- butyl hydroperoxide was added to 450 µl of tissue reaction mixture and measured at 340 nm for 180 s. The molar extinction coefficient of 6.22 X 10^3 M cm^{-1} was used to determine the activity. One unit of activity is equal to the mM of NADPH oxidized / mg protein/ min. The enzyme activity was expressed in µ moles of NADPH oxidized/ mg protein / min.

PROTEIN ASSAY:

Protein content where ever mentioned was estimated by the method of Lowry et al., (1951) using bovine serum albumin as standard.

PROCUREMENT OF CHEMICALS

All the chemicals used in the present study were Analytical grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

In the present investigation, Barnstead Thermoline water purification plant for nano pure water, Kubota KR 200000T centrifuge for centrifugation of the homogenates and Hitachi U-2000 Spectrophotometer for measuring the optical density values, were used for high-quality results.

RED GRAPE, COLLECTION AND EXTRACTION

Red Grapes, as large clusters with red berries, were brought from local surroundings in Bangalore and identified as Vitis vinifera L. (Family Vitaceae). The grape were crushed (whole fruit) for juice and dried in shade, powdered and extract by maceration with 70% (v/v) alcoholic for 72 hours in ambient temperature. The Red Grape extract was filtered and then solvent evaporated to dryness under reduced pressure in a rotary evaporator. The residual Red Grape extract was used for this study.

VALIDITY OF EXPERIMENTAL PROCEDURES

General:

For the entire enzyme studied in the present investigation, the assays were standardized in both experimental and normal control lung tissue by conducting preliminary tests to determine the optimal pH, temperature, enzyme and substrate concentrations and these optimal conditions were subsequently followed for each enzyme assay. Any changes in the carbohydrates metabolism, oxidative and antioxidative enzymes activities of experimental tissues were compared with their respective control ones.

Aliquots for assay:

Aliquots were selected such that initial rates were approximated as nearly as possible yet providing sufficient product to fall in a convenient range of spectrophotometric measurement.

Enzyme Units:

Enzyme activities were expressed in standard units i.e., µ moles of product formed or substrate cleaved / mg protein / hour.

Substrate Requirement:

All the enzyme assays were done under the conditions following zero order kinetics unless otherwise stated.

Lambert-Beer Law:

All most all the products of the reactions were measured by the spectrophotometric procedures in which the optical density (absorbance) of the resulting colored complexes was proportional to the concentrations of the reaction products.

Enzyme nomenclature:

The nomenclature of enzymes used in the present study is according to the report of the commission of the "International Union of Biochemistry" (IUB).

Assay of dehydrogenases using INT:

Tetrazolium salts are unique class of oxidation-reduction indicators in the study of dehydrogenases. The advantages of using Tetrazolium salts as electron acceptors are:

i). The tetrazolium salts give a stable color or reduction.

ii). They are highly soluble in aqueous solutions.

iii). They can be reduced both aerobically and anaerobically.

iv). They have high redox potential which makes the reduction easier.

v). They are freely permeable through membranes.

The first developed tetrazolium salt was Triphenyl Tetrazolium Chloride (TTC). Following the application of TTC, new tetrazolium salts were developed. Various tetrazolium salts receive electrons from various sites of electron transport

system (Oda et al., 1958; Nachlas et al., 1960), which is due to the inherent difference in the redox potentials of various tetrazolium salts. The phenyl ring was observed to increase its redox potential. Karmaket et al., (1959) reported that INT (2- paraidophenyl 3-paranitophenyl 5-phenyl tetrazolium chloride) was superior to the most of the tetrazolium salts as electron acceptor for the assay of various dehydrogenases.

STATISTICAL TREATMENT OF THE DATA

The mean and standard deviation (SD) were calculated by using the method of Pillai and Sinha (1968). The formulas used for calculating SD, percent deviation were as follows.

$$SD\ (\tilde{\sigma}) = \sqrt{\Sigma X^2 - (\Sigma x/n)^2 / n-1}$$

Where, X = individual observations
n = total number of observations

Percent deviation = CM – EM / CM x 100
Where, CM = mean of control value
EM = mean of experimental value

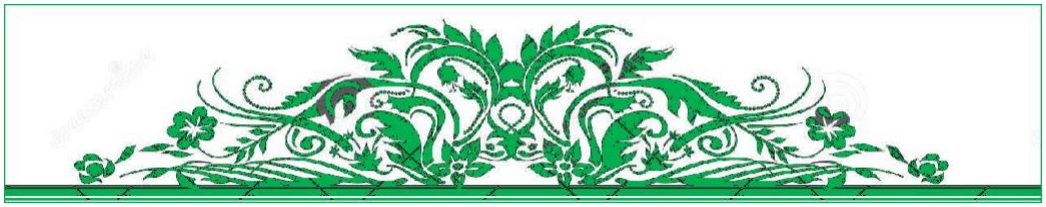
STATISTICAL TOOLS

Each experiment has 3 factors, namely1) Tissue (Lung), 2) Age (young and old) and 3) Treatment, age matched rats were divided into 4 groups of six in each group and treated as follows: i) Control, ii) Nicotine treatment, iii) Red Grape extract treatment and iv) Nicotine treated+ Red Grape Extract treatment.

Statistical analysis has been carried out using 1^3 factor design ANOVA with replication. In each combination of the factor levels six animals were examined, using SPSS 13.0 version. The mean, standard deviation, percent changes and t-test were analyzed in the Ms-Excel programme. The data has been analyzed for the significance of the main effects of tissue (lung), age (young and old) and treatment Control,

Nicotine treatment (Nt), Red Grape Extract treatment (RGEt), Nicotine + Red Grape Extract treatment (Nt+ RGEt**)** along with their interactions.
The results were presented with the F-value. In most of the cases F-value was found to be significant with p value less than 0.01**. This indicates that the effect of factors is significant. Those effects which are not significant have been indicated in the tables with @ mark.

RESULTS AND DISCUSSION



Chapter–1

CARBOHYDRATE METABOLISM

TOTAL CARBOHYDRATES

A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen, usually with a hydrogen oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula $C_m(H_2O)_n$. (Some exceptions exist; for example, deoxyribose, a component of DNA, has the empirical formula $C_5H_{10}O_4$). Carbohydrates are one of the three major food groups needed for proper nutrition (proteins : 20-25%, carbohydrates : 50-60%, Fat : 20-30%). Carbohydrates in food are important and immediate source of energy for the body. Starch refers to carbohydrates found in plants (grains), vegetables and fruits are a source of starch and are broken down to sugar or glucose.

Carbohydrates are present in at least small quantities in most food, but the chief sources are the sugars and the starches. Carbohydrates may be stored in the body as glycogen for future use. If they are eaten in excessive amounts, however, the body changes them into fats and stores them in that form. If carbohydrates are not properly broken down before they are absorbed, then adverse health consequences may occur. To some extent every cell depends on glucose. The cells of the nervous system and the brain almost exclusively use glucose for energy. Fibers are different than starches in that they cannot be broken down by the digestive system, and therefore they provide little or no energy for the body. Fiber has been shown to protect against heart disease and diabetes by lowering cholesterol and glucose levels.

The then U.S. Surgeon General C.Everett Koop recommends in his 1988 report increasing consumption of complex carbohydrates as the best alternative to eating fats and cholesterol. The report implies that Americans already eat enough protein and should not increase their intake of this nutrient. Building glycogen reserves may improve endurance in some activities, such as running marathons. However, Sharon Vitousek, M.D., a physician and an athlete adds that glycogen-loading diets for sports may be less effective than originally thought. Diabetics also may benefit from a diet high in complex carbohydrates and low in fat and sugar, according to the American Dietetic Association. Some researchers believe that dietary fiber improves the ability of diabetics to process blood sugar.

Results and Discussion:

In the present study the total carbohydrates content was decreased in both age groups (young and old) of nicotine treatment rats (young by –23.00%; old by –33.96%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase (young by 3.68%; old by 5.34%) was observed than the control rats. In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table.1, Fig.1).

In the present investigation it was observed that the age induced slight elevation in total carbohydrate content in the lung, which may be due to decreased metabolic utilization in the old animals. The impaired alterations in the activities of enzymes involved in the carbohydrate metabolism contribute to the reduction of carbohydrate catabolism and elevation in age-related accumulation of tissue carbohydrates. The age-related slowing down and impairment in carbohydrate metabolism appears to play a role in the expression of cellular senescence (Tollefsbol, 1987).

The decrease in total carbohydrate levels in the lung of old rats after nicotine treatment suggest possible utilization of carbohydrates to meet the energy demand during nicotine toxicity. Nicotine produces stress in the body both in vivo in vitro (Suleyman et al., 2002). Barry and Mizock, (1995) reported stress causes to the alteration in the carbohydrate metabolism. These alterations include enhanced peripheral glucose uptake and utilization, hyperlactatemia, increased glucose production, depressed glycogenesis, glucose intolerance, and insulin resistance. The hyper-metabolic state is induced by the area of infection or injury as well as by organs involved in the immunologic response to stress; it generates a glycemic milieu that is directed toward satisfying an obligatory requirement for glucose as an energy substrate.

The ability to metabolize carbohydrates is reduced with advancement of age. An age related decrease in respiratory activity and metabolic utilization of carbohydrates has been observed in kidney (Sailaja, 1997; Gurumurthy, 2001; Khalindar Basha et al., 2013), heart tissue slices (Bilwanath, 1996;Subhan et al., 2013), liver tissue (Sivasankar et al., 2014). Enzymes of Kreb's-citric acid cycle show diminished activities with age (Ermini, 1972). Cartee et al., (1993) reported decreased activity levels of glucose-6-phosphate dehydrogenase and glucose-6-

Table-1: Changes in **Total Carbohydrates content** due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in mg/gram wet weight of the tissue.

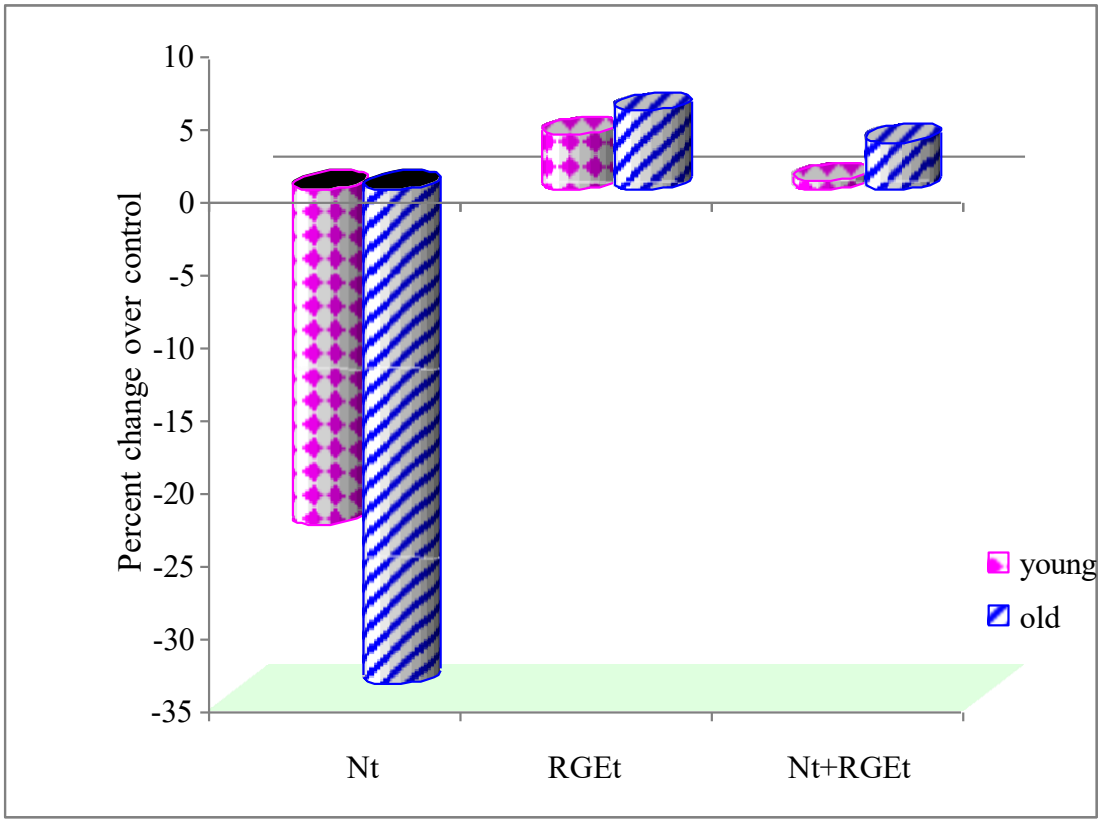
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	43.91 ±2.39	33.81** ±1.34 (-23.00)	45.53** ±1.49 (+3.68)	43.25@ ±0.77 (+0.50)	48.25 ±0.85	31.86** ±0.66 (-33.96)	50.83** ±1.02 (+5.34)	49.79@ ±0.88 (+3.19)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	8.68	1.74	0.77427*
Treatment (C, Nt, RGEt, Nt+RGEt)	3	1850.87	616.96	275.0646**
Age (Young, O ld)	1	133.57	133.57	59.5496**
Tissue & Treatment	15	15.52	1.03	0.4613@
Tissue & Age	5	9.31	1.86	0.8298@
Treatment & Age	3	114.81	38.27	17.0628**
Error	16	35.89	2.24	---
Total	48	2168.65	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.1: Per cent change over respective control in **Total carbohydrates** content in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



phosphofructokinase with advancement of age. Young rats can more readily maintain high levels of oxygen consumption accompanied by a more efficient use of fats, carbohydrates as an energy source compared to old ones (Somani et al., 1992). The decreased glycolytic and Kreb's-citric cycle enzymes which are necessary for the catabolic process of carbohydrates, may lead to increase the total carbohydrate content in the lung of old age rat.

In the present study total corbohydrate content was decreased in the Nt rats of both ages. The decrease in the total carbohydrates under nicotine treatment clearly suggests that the substrates derived from the total carbohydrates constitute the important functional role in the supply of energy. Similar sort of results were obtained under several stress conditions (Nihira, 1982; Kabeer Ahammad et al., (1978) and Linda and Charles, (1983). Several authors have been reported decreased total carbohydrate in afferent tissue with reference to different toxic treatments. Subramanyam, (1984) reported decreased total carbohydrate content in different tissues with acetaldehyde toxicity. It also explains a biochemical situation where in much of the metabolic functions of glycolsis carbohydrate interconversions would be high. This leads to the greater availability of transporting monosaccharides which are the immediate source, for energy supply.

We observed in the present study the induced effect of red grape extract treatment (RGEt) increase the total carbohydrates content in both age groups at the same time decrease was observed in the nicotine treatment (Nt) rats when compare to control rats. Interestingly, in the present investigation with combination treatment (Nt+RGEt) an increase in the levels of total carbohydrate content was found in the lung tissue of both age groups of rats. Thus, these results clearly suggest that, the red grape extract treatment (RGEt) was beneficial for nicotine subjects.

GLYCOGEN:-

Glycogen is a multi branched polysaccharide that serves as a form of energy storage in animals (Sadava et al., 2011). In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, and functions as the secondary long-term energy storage (with the primary energy stores being fats held in adipose tissue). The amount of glycogen present in tissues varied widely with diet and physiological status (Nelson and Cox, 2001). Glycogen is the analogue of starch, a glucose polymer in plants, and is

sometimes referred to as animal starch, having a similar structure to amyl pectin but more extensively branched and compact than starch. Glycogen is found in the form of granules in the cytosol/cytoplasm in many cell types, and plays an important role in the glucose cycle. Glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose, but one that is less compact than the energy reserves of triglycerides (lipids). Polysaccharide represents the main storage form of glucose in the body. Found in the liver and muscles, muscle glycogen is converted into glucose by muscle cells, and liver glycogen converts to glucose for use throughout the body including the Central Nervous System.

In the liver hepatocytes, glycogen can compose up to eight percent of the fresh weight (100–120 g in an adult) soon after a meal (Campbell et al., 2006). Only the glycogen stored in the liver can be made accessible to other organs. In the muscles, glycogen is found in a low concentration (one to two percent of the muscle mass). The amount of glycogen stored in the body especially within the muscles, liver and red blood cells (Moses et al., 1972; Ingermann and Virgin, 1987; Miwa and Suzuki, 2002). Mostly depends on physical training, basal metabolic rate, and eating habits such as intermittent fasting. Small amounts of glycogen are found in the kidney and even smaller amounts in certain glial cells in the brain and white blood cells. The uterus also stores glycogen during pregnancy to nourish the embryo (Campbell et al., 2006). Glycogen is a branched biopolymer consisting of linear chains of glucose residues with further chains branching off every ten glucoses or so. Glucoses are linked together linearly by α (1→4) glycosidic bonds from one glucose to the next. Branches are linked to the chains they are branching off from by α (1→6) glycosidic bonds between the first glucose of the new branch and a glucose on the stem chain (Berg et al., 2012). Due to the way that glycogen is synthesised, every glycogen granule has at its core a glycogenin protein (Berg et al., 2012).

Results and Discussion:

In the present study the glycogen content was decreased in both age groups (young and old) of nicotine treatment rats (young by -26.93%; old by -24.04%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 10.85%; old by 11.89%).

In the combination treatment (Nt+RGEt), slightly increased (young by 3.89%; old by 2.74%) was observed when compared to control rats of both age groups (Table.2; Fig 2).

From the present investigation it was observed that the lung tissue glycogen levels were decreased in the nicotine treatment (Nt) rats in both age groups. Several authors have been reported decreased glycogen content in afferent tissues with reference to different toxic conditions. Vijayakumar Reddy, (1990) reported decreased glycogen levels in the kidney, liver and muscle, under guanidine toxicity. Hariprasad, (1996) observed decreased Glycogen content in fish with ammonium toxicity. Decrement in tissue glycogen levels has been reported during ammonia stress (Santhi, 1991; Nadamuni Cherry, 1992 and Obula Reddy, 1994). The decreased glycogen content in the lung tissue with nicotine treatment rats observed in the present study indicates its greater metabolic utilization possibly to meet higher energy demands to mitigate nicotine toxicity (or) decreased rate of its synthesis. This could be accomplished either through glycolysis (or) the alternative pathway namely the Hexose Monophosphate Pathway (HMP).

In our present findings, it is observed that the glycogen content was increased in RGEt rats in the lung tissue of both age groups when compared to control rats. Red grape extract literature is not available regarding glycogen in this matter. How ever other evidence indicates that, Shibib et al., (1993) reported Momordica charantia (Bitter Milon,Family of Cucurbitaceae) fruit juice increase the hepatic glycogen synthesis and decreases the hepatic gluconeogenesis. So that in our studies glycogen content was increased may be due to upregulation of glycogen metabolism by the RGEt rats.

From the present investigation it was observed that the lung glycogen levels significantly decreased due to aging (Table; 2; Fig.2). The decrease in the glycogen content with advancement of age may be due to augmented glycogen degradation, through glycolysis or due to decreased in the synthesis of glycogen during aging, Takahashi et al., (1970); Khalindar Basha et al., (2013); Sivasankar et al., (2014) reported reduction in glycogen levels with advancement of age. The decrease in glycogen, ATP (Ermini and Verzar, 1968, Frubel Osipova, 1969), ATP/ADP ratio (Ermini et al.,1971) and Creatine phosphate (Ermini, 1970) levels with advancement of age.

Table-2: Changes in **Glycogen content** due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in mg/gram wet weight of the tissue.

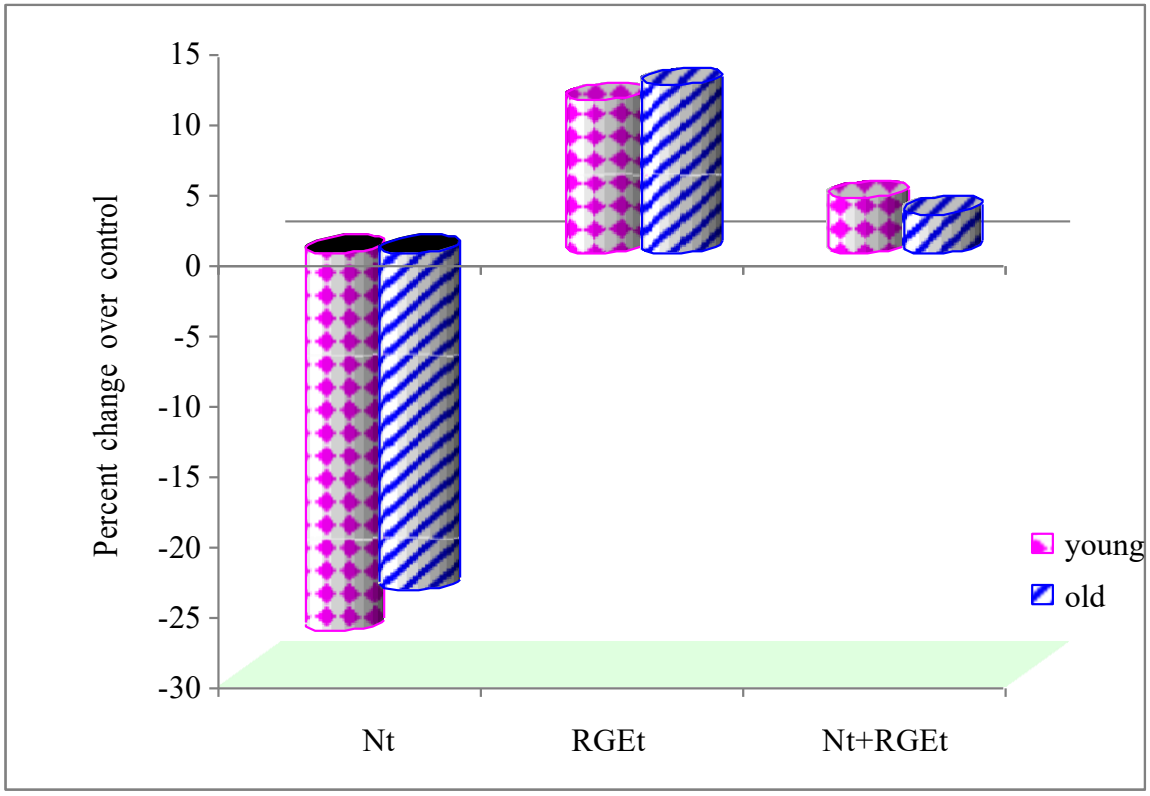
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	56.02 ±0.79	40.93* ±0.68 (-26.93)	62.10** ±0.78 (+10.85)	58.20@ ±0.68 (+3.89)	51.44 ±0.96	37.92** ±2.38 (-24.04)	57.56** ±0.69 (+11.89)	53.48@ ±1.95 (+2.74)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	9.24	1.85	2.397@
Treatment (C, Nt, RGEt, Nt+RGEt)	3	2646.40	882.13	1144.172**
Age	1	200.25	200.25	259.73**
Tissue & Treatment	15	22.66	1.51	1.9592@
Tissue & Age	5	8.16	1.63	2.1173@
Treatment & Age	3	21.01	7.00	9.083**
Error	16	12.34	0.77	---
Total	48	2920.06	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.2: Per cent change over respective control in **Glycogen** content in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



The decreased mitochondrial oxidation revealed by decreased activity of ICDH, SDH and MDH (Tables. 5, 6 and 7) clearly indicates the prevalence of hypoxic conditions in the tissues, which normally increases glycogen utilization. In the present investigation elevated glycogen content levels were observed in the lung of both age groups in the combination treatment (Nt+RGEt), suggesting RGEt may beneficial for the nicotine subject to improve the glycogen content under induced nicotine conditions.

TOTAL FREE AMINOACIDS:-

Amino acids are the building blocks of proteins, have a pivotal role to play in cellular metabolism. The diverse physical, chemical and biological properties of the proteins are dependent on the nature and arrangement of their constituent's namely free amino acids. A hydrolysis of dietary proteins as well as the breakdown of endogenous proteins result in the of a large amino acid pool in the body conversely amino acids are re-precursors for the synthesis of various cellular proteins. In addition, they also precursors for gluconeogenesis, glycogen synthesis as well as ketoacids and hence they are of greater significance. A pool of free-ammo acids which form the precursors for protein synthesis and gluconeogenesis will be present in every tissue (Nelson and Cox, 2001; Murray et al., 2000). All types of physiological processes relating to sports energy, recovery, muscle/strength gains and fat loss, as well as mood and brain function are intimately and critically linked to amino acids. It's no wonder aminoacids have become major players in athletes' supplementation, especially among bodybuilders. For all practical purposes, free amino acids are the currency through which protein metabolism operates.

There will be a constant flux of amino acids from plasma to tissue and vice versa. Because of the heavy traffic of this vital molecules which contribute to various metabolic pathways as well as to the structural machinery, of cells, sensitive control mechanisms are warranted to keep the amino acid pools in each tissue at an equilibrium. The physiological state of the cell also depend upon the free amino acid reserve (Adibi, 1980). The breakdown of proteins, the transport of amino acids across the cell membranes and the rate of incorporation of amino acids into cellular proteins and their oxidation towards other metabolic pathways are the factors that govern the size of amino acid pools. Free amino acids (FAA) also play an important role in the

maintenance of osmotic pressure in the cells. The quality and quantity of free amino acid pool can be considered as a best diagnostic tool to decide the physiological state of the cell.

In view of the extensive traffic passing through the free aminoacids (FAA) pool, one may expect sensitive control mechanisms to maintain the size of the pool constantly. Since the FAA represent intermediates protein metabolism, consequently the size of the amino acid pool is the resultant of a balance between input and removal. Any abnormality in the protein or amino acid metabolism of lung will have its own consequences not only in the host tissue but also in other tissues due to heavy traffic of these protein catabolic products. In view of this, the levels of total FAA in the lung of control and experimental rats were studied to gain an insight into the pattern of amino acid turnover.

Results and Discussion:

In the present study in total free amino acids content was decreased in both (young and old) nicotine treatment rats (young by –60.39%; old by –41.84%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 17.17%; old by 23.26%). In the combination treatment (Nt+RGEt) slightly increase (Young by 6.55%; old by 8.03%) was observed when compared to control rats of both age groups (Table. 3; Fig. 3).

In the present investigation more amount of free amino acids (FAA) were found in the lung tissue of young age group compared to old age group. Obled and Arnal, (1991) suggested that, with advancement of age protein synthesis was decreased and FAA concentration was increased. In general, we can conclude that the age by including tissue proteolysis, elevated free amino acids with a decline in protein synthesis in rats. In the present study total free amino acids content was decreased due to nicotine treatment in both age groups. This decrease may be due to the effect of nicotine products on the FAA content in the lung tissue. However, contradictory reports are also available regarding the influence of nicotine on total free aminoacid pool. Besides these, the enhanced level of FAA may be due to ammonia intoxication (Krishna Mohan Reddy, 1986).

Table-3: Changes in **Total free amino acids** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in mg/gram wet weight of the tissue.

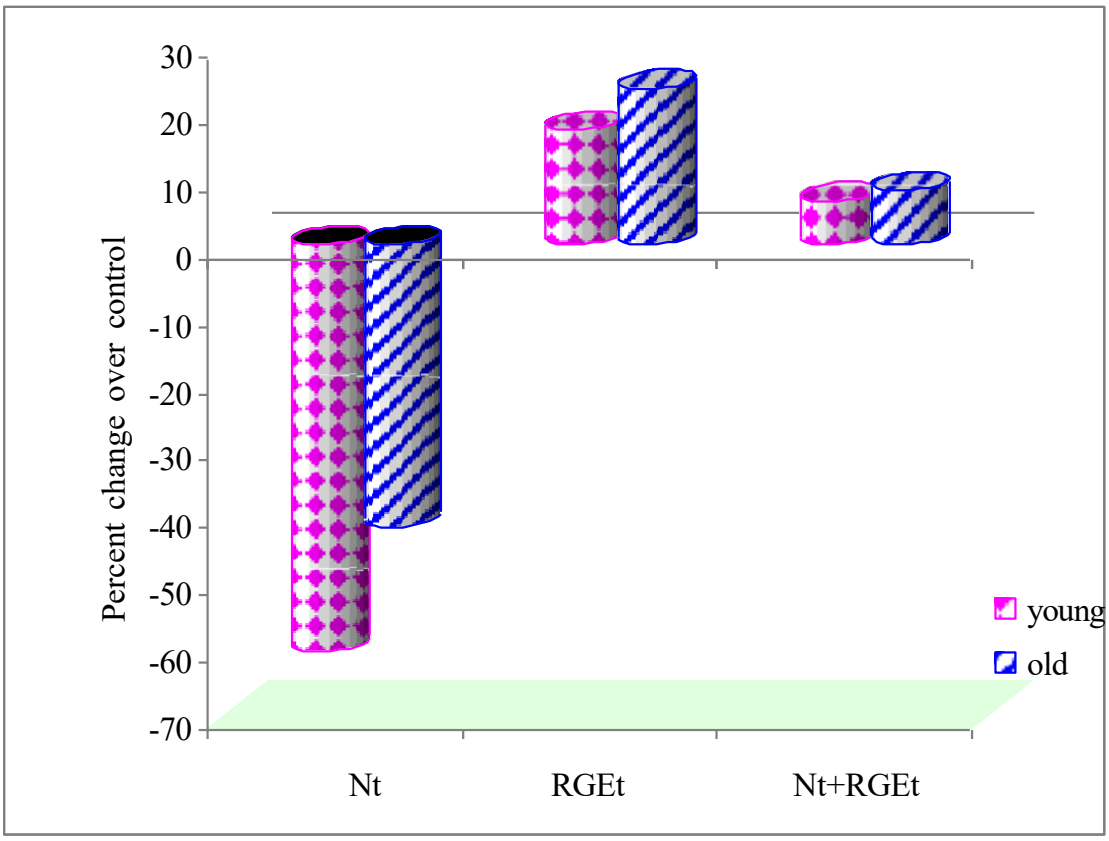
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	28.53 ±0.73	11.30* ±0.66 (-60.39)	33.43** ±0.79 (+17.17)	30.40@ ±1.24 (+6.55)	21.53 ±0.69	12.52** ±0.69 (-41.84)	26.54** ±0.84 (+23.26)	23.26@ ±0.77 (+8.03)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	2.92	0.58	1.0364@
Treatment (C, Nt, RGEt, Nt+RGEt)	3	2277.81	759.27	1346.541**
Age (Young, Old)	1	294.57	294.57	522.419**
Tissue &Treatment	15	11.84	0.79	1.3997@
Tissue & Age	5	3.34	0.67	1.1859@
Treatment & Age	3	152.26	50.75	90.012**
Error	16	9.02	0.56	---
Total	48	2751.77	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant

Fig.3: Per cent change over respective control in **Total free amino acid** content in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



The total FAA content was increased in the RGEt rats, in the both the age groups of lung tissue. Amino acids are added to the pool through the synthesis of non- essential amino acids and precursors within the tissue and through release of amino acids from the breakdown of dietary and cellular proteins in the tissue. The increased amino acid content in the RGEt lung tissue may be due to augmented activity of acidic, alkaline and neutral proteases. This elevation in amino acid level may also be attributed to the enhanced proteolysis as well as decreased amino acid utilization for protein synthesis (Bylund-Fellenius et al., 1984). The low levels of FAA in the lung tissue due to nicotine treatment may also be due to high utilization of these to carbohydrate sources via gluconeogenesis pathway to meet the energy demand under the influence of nicotine intoxication. In the present study we observed an elevation of FAA pool in the lung tissue due to combination (Nt+RGEt), suggests that RGEt enhances the supply of FAA content to counter the nicotine toxicity.

Chapter–2

OXIDATIVE METABOLISM

LACTATE DEHYDROGENASE (LDH)

Lactate dehydrogenase (L-lactate:NAD oxidoreductase) plays an important role in the regulation of anaerobic glycolsis through reoxidation of NADH (Javed et al., 1995). The structure of vertebrate LDH has been investigated by a number of investigators (Everse and Kaplan, 1973; Li et al., 1989) who have shown that it is a tetramer composed of two subunits, A (type-s) and B (type-l). The combinations of various forms of subunits produce five isozymes (Javed et al., 1995) these isozymes differ in various physicochemical, immunological and physiological properties (Javed and Waqar, 1993; Javed et al., 1997). Baba and Sharma, (1971) employed combined histochemical and electron microscopy techniques .and were apparently the first to find lactate dehydrogenase (LDH) localized in the mitochondria of rat heart and skeletal muscle. Subsequently, Kline et al., (1986) and Brandt and Murphy(1987) used cell fraction techniques to demonstrate the presence of LDH in rat liver, kidney and heart mitochondria. Brooks et al., (1999) reported that LDFI is located in the mitochondrial matrix + inner membrane compartment. The distribution of LDH was very similar to that of pyruvate kinase, except in the protease formation (Rasmussen et al., 2002).

Lactate dehydrogenase (LDH) is a key enzyme of anaerobic glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalyzed by LDH interlinks anaerobic and aerobic oxidation of glucose. In view of its role in glucose oxidation the NAD dependent LDH activities were assayed to assess the metabolic significance of this enzyme in compensatory mechanism operating in the tissues of rat during nicotine, aging and red grape extract and nicotine induced oxidative stress conditions.

Results and Discussion:

In the present study the lactate dehydrogenase activity was increased in both age groups (young and old) of nicotine treatment rats (young by 9.09%; old by 16.77%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase (young by 4.85%; old by 8.59%) was observed when compared to control rats. In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table. 4; Fig. 4).

Table-4: Changes in **Lactate Dehydrogenase (LDH)** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as µ moles of formazan formed/mg protein/hour.

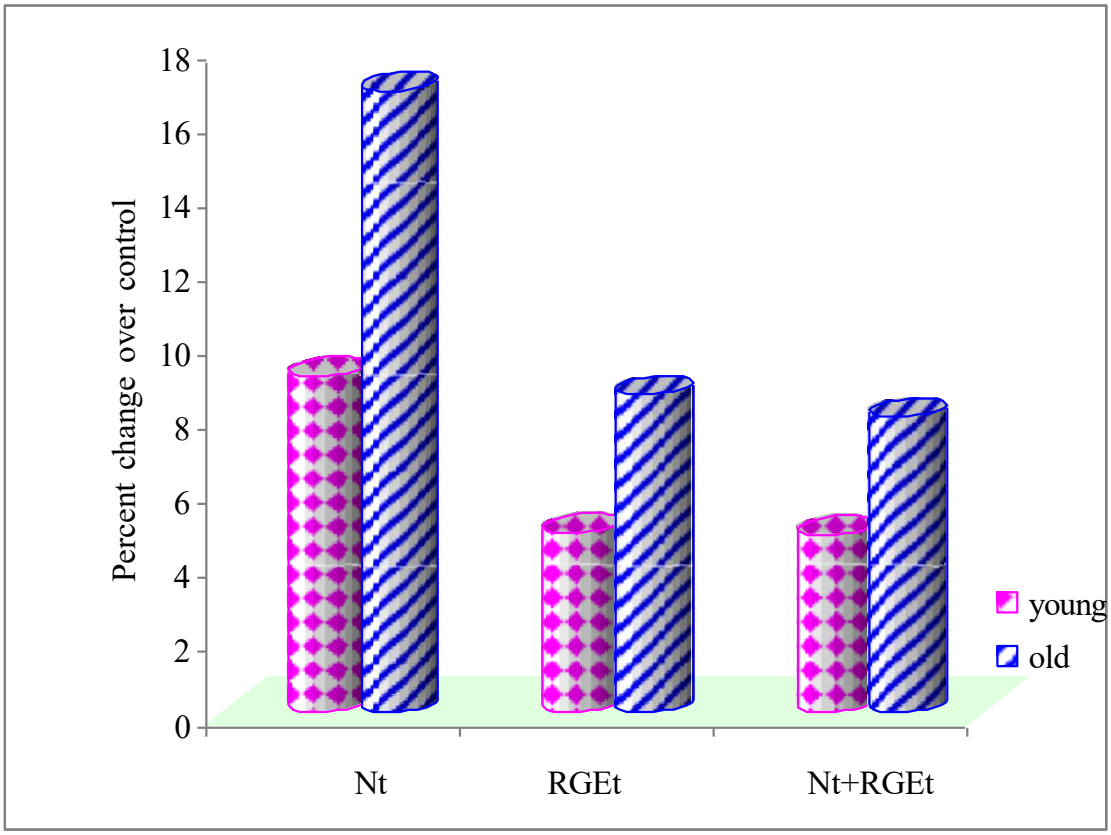
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	17.49 ±0.75	19.08** ±1.62 (+9.09)	18.34** ±0.67 (+4.85)	18.33@ ±0.75 (+4.80)	14.31 ±0.89	16.71** ±1.23 (+16.77)	15.54** ±0.69 (+8.59)	15.45@ ±1.24 (+7.96)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	9.46	1.89	2.2053@
Treatment (C, Nt, RGEt, Nt+ RGEt)	3	23.92	7.97	9.2925**
Age	1	94.61	94.61	110.2761**
Tissue & Treatment	15	17.51	1.17	1.3608@
Tissue & Age	5	2.35	0.47	0.5481@
Treatment & Age	3	1.05	0.35	0.406@
Error	16	13.73	0.86	---
Total	48	162.63	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.4: Per cent change over respective control in **Lactate Dehydrogenase (LDH)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



The rats witch, received nicotine showed a elevation of LDH activity in the lung tissue in both the age groups. Moreover, the high percent elevation of LDH was noticed in old (by 16.77%) group than the young group (9.09%) compared to their respective control rats. Several authors have reported increased LDH activity in afferent tissues with reference to different toxic conditions. LDH is a cytosolic enzyme, which allows the assessment of the process of anaerobic energy production by the cell. This enzyme is a marker of metabolic activity of renal glomeruli. A dose- dependent increase in LDH activity, evident after 24 weeks of cadmium (Cd) exposure, in the main tubules and glomeruli reflects intensification of anaerobic respiration (Malgorzata et al., 2004). Cunningham and Ivester, (1999) reported that significant ethanol-related increase in lactate dehydrogenase activity released from both periportal and perivenous cell that occur under toxic conditions or at low oxygen tensions. The release of LDH was greatest in perivenous-ethanol hepatocyte, but was significantly different from control hepatocytes in both cell types. Strubelt et al., (1999), Hanene Dhouib et al., (2014) reported the increased LDH activity levels with alcohol treatment in the hepatic tissue of rat. In clinical as well as experimental studies, hepatotoxicity may be analyzed by assaying liver cytosol-derived enzymes such as lactate dehydrogenase.

An increased leakage of these enzymes indicates damage of the cell membranes, which are attacked primarily by the toxic agents. McKarns et al., (1997) evaluated the release of LDH by rat liver epithelial cells in vitro after acute exposure to 11 short chain alcohols. Many studies have shown that ethanol exerts hepatotoxicity due to metabolic changes that are induced following its oxygenation by alcohol dehydrogenase (Lieber, 1994; Lieber, 2004; Venkatraman et al., 2004). Alcohol treatment increases the lactate/ pyruvate ratio within the tissue (Strubelt et al., 1999). According to Yildiz D et al., (1999) LDH activity was increased due to nicotine induced oxidative stress. In our present investigations, the increased levels of LDH activity in nicotine treatments. This is due to the increased generation of ROS by nicotine that leads to cell damage and also indicated the low capacity to combat against ROS.

The lung tissue LDH activity was increased with RGEt rats in both age groups when compared to control rats. Several authors reported in different tissues, the supplementation of RGEt the LDH activity was increased in heart tissue (Subhan et al., 2013), in the kidney tissue (Khalindar Basha et al., 2013), in brain tissue

(Ramaiah et al., 2015) and in skeletal muscle tissue (Chennaiah et al., 2015). This reports suggesting enhanced oxidative metabolism in RGEt rats to meet the increased energy demands of the animal. An increase in NAD dependent LDH activity in the lung tissue of rat subjected to RGEt, indicate the possible shift in the metabolic profile from the anaerobiosis to aerobiosis i.e., the NAD-LDH activity helps in the efficient conversion of lactate to pyruvate and its subsequent utilization in TCA cycle oxidative reactions. The lactate taken up by the tissue may be oxidized to carbon dioxide and water or used for glycogenesis. In both cases pyruvate is the first product (Rasmussen et al., 2002). Due to increased lactate levels in the lung tissue, the LDH activity may also increase to convert the high amount of lactate to pyruvate during red grape extract treatment (RGEt).

The lactate dehydrogenase activity was decreased with advancing of age in the lung tissue, young rats have high amount of activity. Generally during aging process oxidative metabolism decreases in the cell. Decreased formation of lactate / pyruvate leads to the decrease in LDH activity the tissues. The age related decrease in total LDH activity in the right ventricle of old age rats was reported by Anitha and Devi, (1996). The changes in the activity of enzymes related to the energy metabolism suggest that decreased glycolysis to lactate and simultaneously cause the low energy production in mitochondria. It is now generally accepted that the mitochondrial function decreases with advancing of age and decline with age associated respiratory function (Wei and Lee, 2002). Mitochondrial decay has been observed with aging in different tissues. This mitochondrial decay includes decreased mitochondrial content, decreased oxidative capacity and decreased enzyme activities (Rooyakers et al., 1996).

The lactate dehydrogenase activity was elevated with the combination treatment in both the age groups rats. According to Stuewe et al., (2000) increased glyceraldehyde dehydrogenase' may increase glycolysis and thereby elevated levels of lactic acid. The formation of pyruvate from lactate is catalyzed by lactate dehydrogenase, which is present in the sacroplasm at very high levels (Rasmussen et al.,2002).This has lead to the proposal of an intracellular lactate shuttle, which involves transport of lactate into mitochondrial matrix followed by oxidation to pyruvate, catalyzed by LDH (Brooks et al., 1999). However, the direction of LDH is determined by the lactate / pyruvate ratio multiplied by the NAD / NADH ratio.

Lactate oxidation only occurs if this mass action ratio is larger than the equilibrium constant (Rasmussen et al., 2002). These evidences support the age related decreased in LDH activity in old age rat of lung tissue, however in the combination treatment (Nt+RGEt) the LDH activity was increased in both age groups.

ISOCITRATE DEHYDROGENASE (ICDH-E: C-1.1.1.42)

ICDH is an important enzyme in Kreb's cycle which catalyses the reversible oxidation of isocitrate to oxalosuccinate followed by decarboxylation leading to the formation of □-ketoglutarate. During oxidation-reduction it utilizes NAD/NADP to accept electrons. NAD-ICDH localized in the mitochondria is an allosteric enzyme which requires ADP, Mg^{2+} or Mn^{2+} for its activity. In the presence of the positive modulator (ADP) the monomeric form aggregates to form the dimer. Such ADP- induced aggregation is prevented by NADH, a negative modulator. NADP-ICDH catalyzes, 1) the formation of oxalosuccinate from isocitrate and concurrent reduction of NADP to NADPH and 2) the decarboxylation of oxalosuccinate to □-ketoglutarate. The first step requires NADP but not divalent metal ions. The second step requires divalent metal ion but not NADP. The rate of dehydrogenation observed in the absence of divalent ions is lower than the rate of the overall reaction. Approximately 75-90% of NADP-specific enzyme is located in non-particulate fraction of the cytoplasm and the remainder in the mitochondria (Plaut, 1963). The intra- and extra- mitochondrial enzymes differ from each other in electrophoretic mobility and in immunological properties (Lowenstein, 1967).

Results and Discussion:

In the present study in isocitrate dehydrogenase content was decreased in both (young and old) nicotine treatment rats (young by –43.32%; old by –25.95%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 8.99%; old by 5.09%). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table.5; Fig.5).

In the present study a decrease of ICDH activity was observed in lung tissue of both the age groups of nicotine treatment rats. Various authors reported similar changes in the different tissues, in the liver tissue (Sivasankar et al., 2015), in brain

Table–5: Changes in **Isocitrate dehydrogenase (ICDH)** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the Control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as µ moles of formazan formed/mg protein/hour.

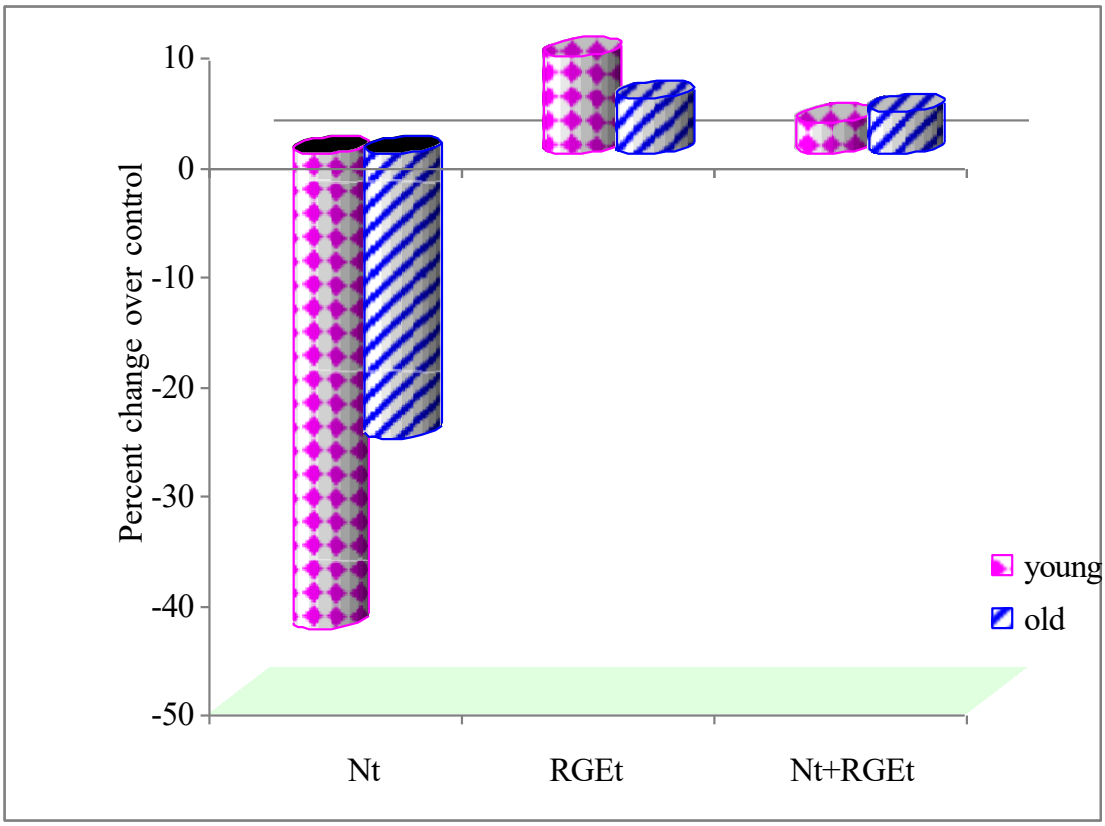
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	48.49 ±0.60	27.48* ±0.78 (-43.32)	52.85** ±1.43 (+8.99)	49.91@ ±2.85 (+2.92)	38.29 ±1.21	28.35** ±0.92 (-25.95)	40.24** ±2.59 (+5.09)	39.77@ ±2.30 (+3.86)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	11.58	2.32	0.9066@
Treatment (C, Nt, RGEt, Nt+RGEt)	3	2663.26	887.75	347.3657**
Age (Young, Old)	1	771.44	771.44	301.854**
Tissue & Treatment	15	56.05	3.74	1.4619@
Tissue & Age	5	19.31	3.86	1.5111@
Treatment & Age	3	328.34	109.45	42.8246**
Error	16	40.89	2.56	---
Total	48	3890.88	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.5: Per cent change over respective control in **Isocitrate dehydrogenase (ICDH)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



tissue (Ramaiah et al., 2015). The decrease in specific activity of NAD/NADP-ICDH as a consequence of induced nicotine toxicity suggests reduced conversion of isocitrate to □-Ketoglutarate. Similar changes in ICDH activity was reported in different animals treated with various toxic compounds (Joseph and Rao, 1990; Reddy and Rao, 1991). The changes in NADP-ICDH could be attributed to the mitochondrial damage caused by nicotine treatment. Interaction of enzyme with NADPH may result in an unfavorable conformation of the enzyme molecule (Plaut, 1963). Inhibition of ICDH by acetaldehyde results in alteration of activities of TCA cycle enzymes (Umadevi, 1992). During acetaldehyde metabolism the oxygen consumption diminishes (Cederbaum et al., 1977) resulting in the decreased mitochondrial oxido- reductase activity. Decreased NADP-ICDH activity as a consequence of acetaldehyde toxicity results in the reduced production of NADPH which plays a crucial role in the detoxification processes (Reed, 1986).

The lung NADP-ICDH was decreased, suggesting reduced mitochondrial oxidation of isocitrate in lung with advancement of age. This could be attributed to diminished supply of keto acids into citric acid cycle (Thalwar et al., 1989). From the data it was observed, the activity levels of ICDH in lung of RGEt rats were increased in young and old rats. The possible reason for an increase in NADP-ICDH activity during red grape extract treatment (RGEt) is to stepup NADPH production needed for the detoxification of ammonia formed. NADP-ICDH activity increases during periods of increased energy demands and lowered ATP/ADP ratio (Stein et al., 1967).

The NADP-ICDH activity levels decreased in the lung tissue of rats as a function of age. The decrease in NADP reduction through NADP-ICDH indicates lesser involvement of TCA cycle in the oxidative reactions during aging. The decrease in ICDH with advancement of age can be attributed to admonished supply of ketoacids into TCA cycle (Thalwar et al., 1989). Sanadhi, (1967) reported that the transfer of substrate by the mitochondrial membrane is altered in old cells because of rupture of the membrane. Age-dependent damage in individual process has been reported for several enzymes including mitochondrial oxidoreductases (Thalwar et al., 1989). The variation in the NADP-ICDH was found to be marginal during aging in the present investigation. The decrease in the specific activity of ICDH reflects reduced conversion of isocitrate to □-Ketoglutarate. In the present investigation we

observed a slight/marginal increase in ICDH activity when the nicotine treatment rats were supplemented by the (combination treatment (Nt+RGET). This restoration of ICDH activity reveals the normal operation of TCA cycle for high energy production to withstand the toxic conditions of nicotine metabolic profiles. This observation which is a beneficial to the organism to streamline the deranged metabolic machinery either due to aging or nicotine toxicity.

SUCCINATE DEHYDROGENASE (SDH - EC: l.3.99.1)

Succinate dehydrogenase (SDH) is a marker enzyme of mitochondria in the tissues. It is tightly bound to the inner surface of the inner mitochondrial membrane and has a molecular weight of 175 KD. This enzyme is a flavoprotein with four iron atoms and four inorganic sulfides in addition to the flavin moiety. The activity of SDH in mitochondria is usually far greater than the other enzymes in both the developing and adult animals (Sivarama Krishnan et al., l983). Since the specific activity of SDH indicates the state of oxidative metabolism in mitochondria, any alterations in its activity under age and ethanol induced oxidative stress reflects the extent of derangement in mitochondrial metabolism. SDH serves as a link between electron transport system and oxidative phosphorylation and the activity of the enzyme is depend on the integrity of intracellular structure (Sailaja, 1997). The oxidation of succinate and malate and their interplay in metabolic processes have been widely established in biological oxidants (Murray et al., 2000; Nelson and Cox, 2001). Succinic acids oxidized to form fumaric acids in the presence of SDH. In this redox reaction two hydrogen atoms are released and they are accepted by FAD and FAD becomes $FADH_2$. The reaction does not required NAD like other TCA cycle enzymes.

Results and Discussion:

In the present study the succinate dehydrogenase activity was decreased in both (young and old) nicotine treatment rats (young by -47.26%; old by -28.79%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 14.06%; old by 21.11%). In the combination treatment (Nt+RGEt) slightly increase was observed when compared to control rats of both age groups (Table.6; Fig. 6).

Table-6: Changes in **Succinate dehydrogenase (SDH)** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as µ moles of formazan formed/mg protein/hour

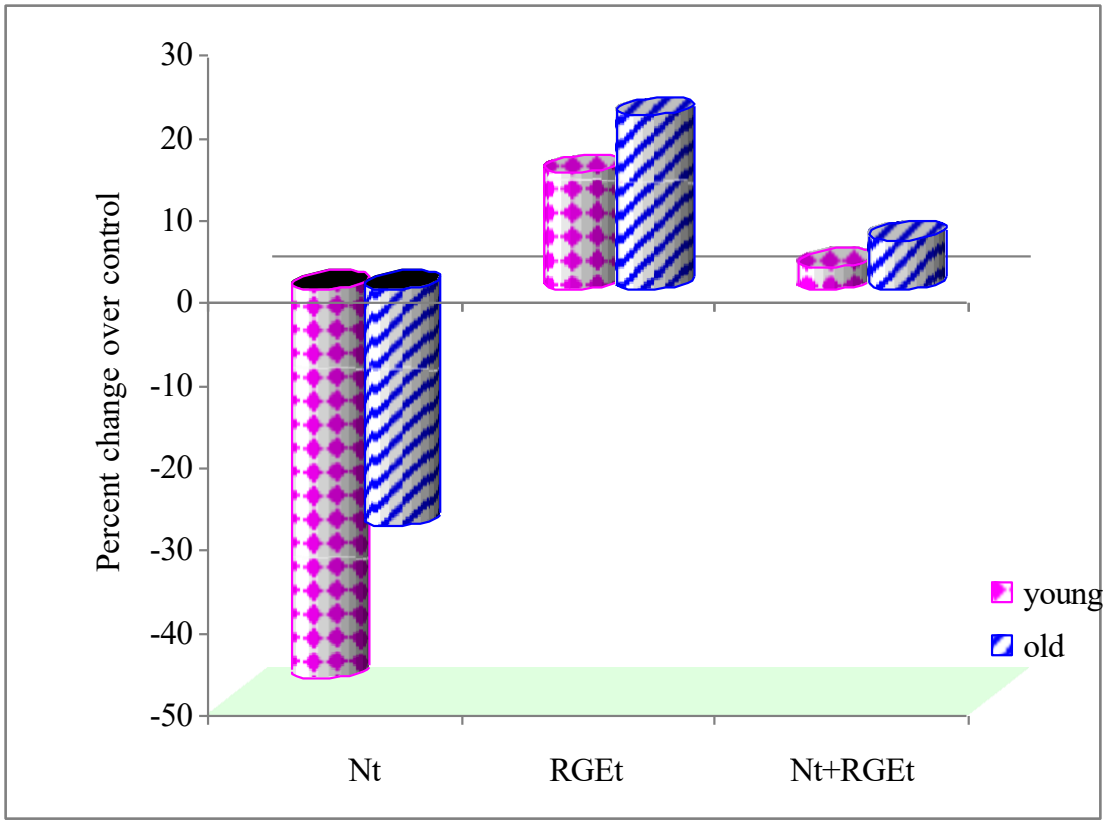
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	42.44 ±3.73	22.38** ±2.09 (-47.26)	48.41** ±1.77 (+14.06)	43.59@ ±2.26 (+2.70)	34.38 ±2.90	24.48** ±2.28 (-28.79)	41.64** ±2.44 (+21.11)	36.44@ ±2.29 (+5.99)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	56.08	11.22	2.3343@
Treatment (C, Nt, RGEt, Nt+RGEt)	3	3111.89	1037.30	215.872**
Age (Young, Old)	1	296.41	296.41	61.686**
Tissue & Treatment	15	88.03	5.87	1.221@
Tissue & Age	5	36.69	7.34	1.527@
Treatment & Age	3	202.63	67.54	14.056**
Error	16	76.88	4.81	---
Total	48	3868.63	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.6: Per cent change over respective control in **Succinate dehydrogenase (SDH)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



The decrease in SDH activity due to the nicotine stress condition indicates reduction in the conversion of succinate to fumarate resulting in decreased oxidative metabolism. Several authors reported in different tissues, the supplementation of RGEt the SDH activity was increased in the heart tissue (Subhan et al., 2013), in the kidney tissue (Khalindar Basha et al., 2013), in brain tissue (Ramaiah et al., 2015) and in skeletal muscle (Chennaiah et al., 2015). During stress conditions diversion of phosphoenolpyruvate leads to increased formation of fumarate resulting in product inhibition of SDH (Moorthy, 1983). Similar inhibition of SDH activity was reported in animals under induced different toxic conditions (Hamilton and Gould, 1987; Veerababu, 1988; Gupta et al., 1991; Reddy and Rao, 1991). Chennaiah et al., (2011) reported the decreased SDH activity was observed in all skeletal muscle fibres of rats treated with nicotine, indicating depressed oxidative metabolism in mitochondria. Since the activity of SDH is reduced, it is evident that this might affect the conversion of malate to oxaloacetate by MDH because of low succinate oxidation. A decrease in oxygen consumption in stress condition also leads to inhibition of mitochondrial oxido-reductases (Segal and Mason, 1979; Moorthy et al., 1985).

The reduced availability of oxidized form of flavoproteins needed for succinate oxidation results in decreased activity of SDH (Swami et al., 1983). Holownia et al., (1989) reported that an increase in ammonia levels and diminished levels of intermediates of citric acid cycle during acetaldehyde treatment result in mitochondrial swelling. Some studies also have reported conflicting results on SDH activity under the influence of alcohol. SDH, an enzyme of the tricarboxylic acid cycle, is a marker of the electron transport system in the inner membrane of the mitochondrion.

The changes observed in its activity as a result of chronic exposure to cadmium (Cd), indicate disorders in the respiratory mechanisms in cells and damage to the mitochondrial membranes. These were located in the main tubules (i.e. proximal convoluted tubules and straight tubules) and distal convoluted tubules. The focal decrease in kidney tissue in SDH activity was noted after exposure to 50 mg Cd, might result from poor glucose supply, on one hand, and from the inactivating effect of Cd, on the other. Strong reaction for SDH in the cytoplasm accompanied by an increase in its diffuse character, indicate substantial damage to the mitochondrial membranes and leakage of the enzyme to the cytoplasm or an increased availability of substrate (Malgorzata et al., 2004). Butler et al., (1985) reported a decrease in SDH activity levels in the heart of rat on chronic treatment with ethanol. Whereas, Cederbaum et al., (1976) reported a decrease in SDH activity following the

administration of acetaldehyde to rats. The results of the present study further confirm these earlier reports.

AcRell et al., (1974) reported a decrease in SDH activity due to the feedback- inhibition by oxaloacetate accumulated during stress condition. The possible accumulation of oxaloacetate may also lead to an increased oxidation of citrate and isocitrate to generate NADPH and divert the metabolites into other pathways resulting in diminution of citric acid cycle oxidations.

The molecular basis of toxicity has been suggested to be irreversible inactivation of bound flavin in SDH leading to eventual impairment of cellular respiration and oxidative phosphorylation capacity (Cederbaum et al., 1974; Wahid et al., 1980). Thus, the decreased SDH activity observed in the lung tissue of both age groups of rats treated with nicotine indicates depressed oxidative metabolism in mitochondria. Since the activity level of SDH is reduced, it is evident that this might affect the conversion of malate to oxaloacetate by MDH because of low succinate oxidation. The SDH activity was increased in the lung tissue of both the age groups supplemented with RGEt when compared to the control rats. The increase in maximal and specific activity of SDH by RGEt suggests the increased mitochondrial oxidative potential and energy synthesis utilizing carbohydrates and fats as substrates.

In the present study an increase was observed in the RGEt rats of both the age groups. The increase in specific activity of SDH in old age rats with response to RGEt suggests the increased mitochondrial oxidative potential and energy synthesis utilizing carbohydrates and fats as substrates function of mitochondria is energy production, isolated mitochondria generate reactive oxygen species during oxidative phosphorylation. Release of such intermediates accounts for an estimated 1 to 5% of the oxygen consumed during respiration, depending on the substrate and respiration state. However, most studies used isolated mitochondria and the flux of oxidants was often estimated indirectly.

In the present study illustrate that the specific activity of SDH was dramatically decreased with advancement of age. Similar age related decrease in SDH activity was reported by Sailaja (1997), Jhansi Lakshmi (1998) and Chennaiah (2006). The decrease in the SDH activity may also be due to diversion of TCA cycle intermediates such as α-ketoglutarate, for the conversion into glutamate and glutamine to counter the toxic effects of

ammonia produced during aging there by depleting TCA cycle intermediates. During physiological stress conditions diversion of phosphoenol pyruvate leads to increased formation of fumarate resulting in product inhibition of SDH. Aging may be considered as a type of stress, which leads to hypoxic condition in the tissues and consequent reduction on mitochondrial oxidoreductase activities (Moorthy et al., 1985). There is surprisingly little direct evidence for the generation of reactive species by mitochondria in intact cells of tissue (Leeuwenburgh and Heinecke, 2001). In the combination treatment with (Nt+RGEt) upregulation was observed in the lung tissue of both age groups. Thus differential response of SDH activity was observed in the lung tissue of both age groups in the present study.

MALATE DEHYDROGENASE- (MDH-EC- 1.1.1.37)

Malate Dehydrogenase (MDH) is an enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD^+ to NADH. This reaction is part of many metabolic pathways, including the citric acid cycle. Other malate dehydrogenases, which have other EC numbers and catalyze other reactions oxidizing malate, have qualified names like malate dehydrogenase ($NADP^+$). Malate dehydrogenase is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules. Pyruvate in the mitochondria is acted upon by pyruvate carboxylase to form oxaloacetate, a citric acid cycle intermediate. In order to get the oxaloacetate out of the mitochondria, malate dehydrogenase reduces it to malate, and it then traverses the inner mitochondrial membrane. Once in the cytosol, the malate is oxidized back to oxaloacetate by cytosolic malate dehydrogenase. Finally, phosphoenolpyruvate carboxykinase (PEPCK) converts oxaloacetate to phosphoenolpyruvate (PEP).

Several isozymes of malate dehydrogenase exist. There are two main isoforms in eukaryotic cells (Minarik et al., 2002). One is found in the mitochondrial matrix, participating as a key enzyme in the citric acid cycle that catalyzes the oxidation of malate. The other is found in the cytoplasm, assisting the malate-aspartate shuttle with exchanging reducing equivalents so that malate can pass through the mitochondrial membrane to be transformed into oxaloacetate for further cellular processes (Musrati et al., 1998). The activity levels of MDH indicate the status of prevailing oxidative metabolism.

Table-7: Changes in **Malate dehydrogenase (MDH)** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as µ moles of farmazan formed/mg protein/hour.

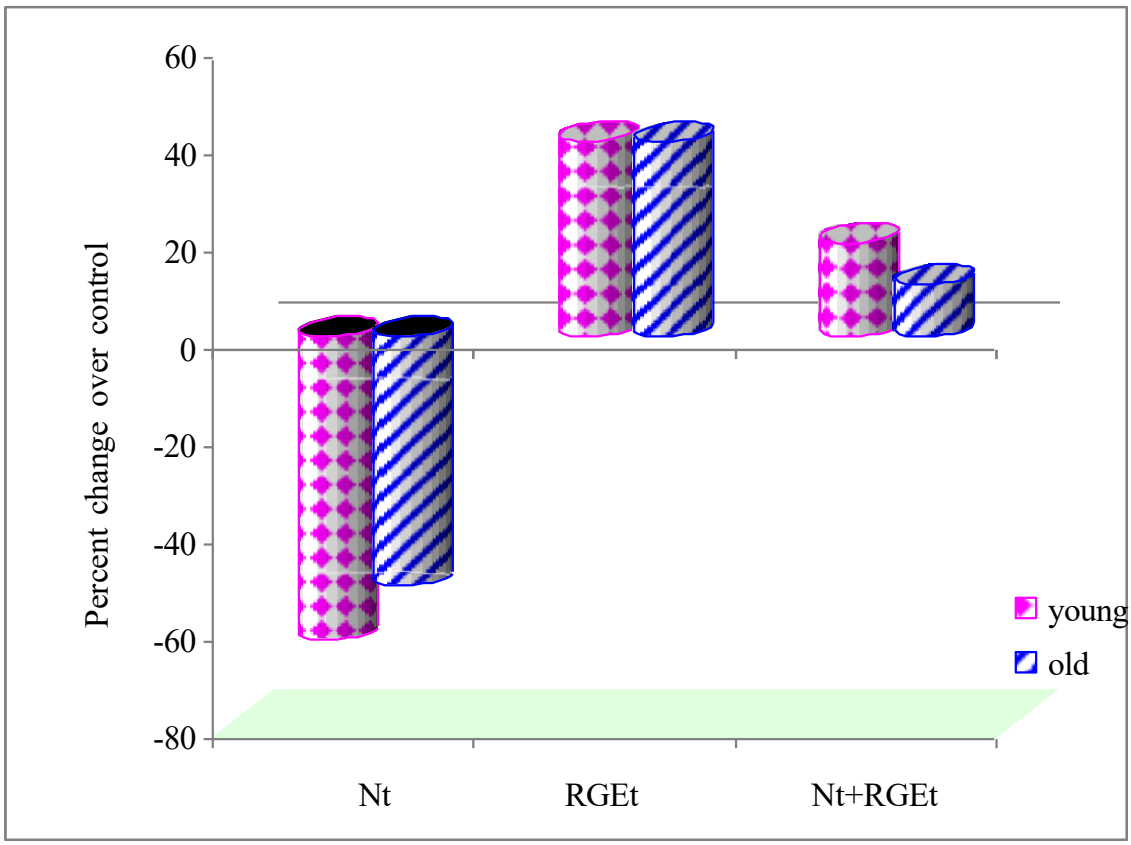
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	16.65 ±1.25	6.21** ±0.70 (-62.70)	23.32** ±0.88 (+40.06)	19.84@ ±1.21 (+19.15)	12.66 ±0.94	6.14** ±0.62 (-51.50)	17.72** ±0.88 (+39.96)	14.01@ ±1.42 (+10.66)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	4.49	0.90	0.9198@
Treatment (C, Nt, RGEt, Nt+ RGEt)	3	1336.39	445.46	455.886**
Age (Young, Old)	1	179.65	179.65	183.8485**
Tissue & Treatment	15	19.70	1.31	1.344@
Tissue & Age	5	2.34	0.47	0.4786@
Treatment & Age	3	63.88	21.29	21.7909**
Error	16	15.63	0.98	---
Total	48	1622.08	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.7: Per cent change over respective control in **Malate dehydrogenase (MDH)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



Hence, an attempt was made to examine the specific activity of MDH in the selected tissue of young and old age rats as a consequence of nicotine treatment(Nt), redgrape extract treatment (RGEt) and (Nt+RGEt).

Results and Discussion:

In the present study the malate dehydrogenase activity was decreased in both (young and old) nicotine treatment rats (young by –62.70%; old by –51.50%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 40.06%; old by 39.96%). In the combination treatment (Nt+RGEt) slightly increase (young by 19.15%; old by 10.66%) was observed when compared to control rats of both age groups (Table.7; Fig.7).

The decrease in specific activity of MDH in lung tissue of both age groups of rats as a consequence nicotine treatment suggests decreased utilization of malate. The reduced levels of TCA cycle intermediates may also be due to the decrease in MDH activity during nicotine-treatment. Concisely, the decreased MDH activity could be attributed to 1) low availability of substrate, 2) lesser conversion of succinate- fumarate-malate, and 3) the changes in the structural integrity of mitochondria. A significant decrease in the specific activity of NADP-ICDH (Table.5 .Fig.5) and as a consequence of nicotine-treatment observed in the present study indicates reduced formation of malate. The decrease in activity levels of dehydrogenases is consistent with the decreased CO_2 formation (Cederbaum et al., 1976).

An increase in proteolytic activity during nicotine intoxication may also be responsible for the decreased MDH activity. A similar study was reported by various authors in different tissues viz., in skeletal muscle fibers (Chennaiah et al., 2011), in the heart tissue (Subhan et al., 2013), in the liver tissue (Sivasankar et al., 2015) and in the brain tissue (Ramaiah et al., 2015), a decrease in specific activity of MDH was observed rats treated with nicotine, suggesting decreased utilization of malate. The decreased MDH activity could be attributed to low availability of substrate, lesser conversion of succinate-fumarate-malate, and the changes in the structural integrity of mitochondria. Similar inhibition of MDH activity was reported in animals under different toxic conditions (Veerababu, 1988; Tripathi and Shukla, 1990; Reddy and Yellamma, 1991). Inhibition of MDH activity was observed in the present study suggests the

prevalence of hypoxic condition in tissues and reduction in mitochondrial oxidative metabolism in tissues of rat administered with nicotine. Umadevi, (1992) reported decreased MDH activity with acetaldehyde treatment in hepatic tissue of rats. The activity of MDH depends on the rats of formation of oxaloacetate and phosphoenolpyruvate from malate the reduced oxidation of malate evidenced by decreased MDH activity indicates the possible diversion of malate through Wood- workman reaction undergoing decarboxylation leading to the formation of phosphoenolpyruvate. Alterations in the activities of TCA cycle enzymes cause mitochondrial dysfunction and integrity ultimately leading to energy crisis during induced nicotine toxicity.

Preliminary studies have shown that NAD concentration is a key factor in the activation of mitochondrial malate dehydrogenase (Stuewe et al., 2000). From the data it was observed, the activity levels of MDH were increased in the both age groups of lung tissue in the RGEt rats. The increased MDH activity both in response to age and red gape extract treatment suggests that higher utilization of malate. The elevation in MDH activity reflects the upturn of oxidative metabolism and the turnover of carbohydrates and energy output that is required during development (Murray et al., 2000).

Mitochondrial malate dehydrogenase enzyme activity was remarkably decreased in old rats compared to the younger rats. Previously several scientists reported the decrease in MDH activity with increase of age. Sailaja, (1997) reported a decrease in MDH activity in the skeletal muscle tissue of old albino rats. Jill and Thomas, (1991) observed a significant decrease in MDH activity in tissues of senile rats whereas training increased the enzyme activity levels. Thus, these reports attest the present findings. The decrease in MDH activity in senile rat tissues suggests the lower utilization of malate in the Kreb's cycle. The drop in the MDH activity denotes fluctuations of oxidative metabolism and also reflects the turnover of carbohydrates and energy output (Watanb and Aviado, 1974; Murray et al., 2000).

Mitochondrial dysfunction and accumulation of protein damage have been proposed to contribute to aging process (Bakala et al., 2003). It has been recently demonstrated that impairment in mitochondrial respiration and oxidative phosphorylation elicits an increase in oxidative stress (Yan and Sohal, 1998).

Moreover, oxidative damage and large-scale depletion and duplication of mitochondrial DNA have been found to increase with age in various tissues of human beings. In recent years, much data has been accumulated to suggest that mitochondria act like a timer that ticks all the way through the aging process (Wei and Lee, 2002). From the present study we report that the combination treatment (Nt+RGEt) exhibits a beneficial recovery of MDH activity in both the age groups of lung tissue. This suggests that red grape extract treatment is very much useful for the nicotine subjects to upregulate the decreased oxidative metabolism.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PDH: EC-1.1.1.49)

Glucose-6-phosphate dehydrogenase (G-6-PDH or G6PD) is the enzyme of the pentose phosphate pathway that is responsible for the generation of NADPH, which is required in many detoxifying oxygen derived free radicals (Tian et al., 1998; Salvemini et al., 1999). G-6-PDH, the first and rate limiting enzyme of the pentose phosphate pathway, has long been regarded as important in the biosynthesis of sugar moiety of nucleic acids (Luzzatro and Metha, 1995) and determines the amount of NADPH by controlling the metabolism of glucose via pentose phosphate pathway. It has been traditionally thought that G-6-PDH was a typical "housekeeping" enzyme that was regulated solely by the ratio of NADPH and NADP (Kletzien et al., 1994; Tian et al., 1999). The production of NADPH required for the regeneration of glutathione in the mitochondria is critical for scavenging the mitochondrial ROS through glutathione reeducates and glutathione peroxidase systems (Jo et al., 2001).

G-6-PDH may have directly reduced the basic ROS formation and as a consequence, increased the cellular concentration of glutathione (Salvemini et al., 1999). G-6-PDH plays a critical role in cell growth by providing NADPH for redox regulation (Tian et al., 1998). A major role of NADPH in erythrocytes is regeneration of reduced glutathione, which prevents the hemoglobin denaturation, preserves the integrity of red blood cell membrane sulfhydril groups and detoxify hydrogen peroxide and oxygen radicals in and on the red blood cells (Weksler, 1990). Glucose- 6-phosphate dehydrogenase enzyme is extra mitochondrial in location and highly specific for NADP as an electron acceptor. G-6-PDH is known to occur in two distinct forms, one located in cytosol, which is specific for NADP (Meizer et al., l977) and the other located in microsomes utilizes either NADP of NAD (Ashida et al., 1987). The overall equilibrium favors the formation of NADP,

which acts as electron donor in reductive biosynthesis.

G-6-PDH is an important enzyme in HMP shunt pathway, which occurs in the cytosol of the cell and is an alternate pathway of glucose oxidation. This pathway provides a major portion of the cell NADPH, which functions a biochemical reductant. It is particularly important in liver, mammary glands which are active in the biosynthesis of fatty acids. The activity of G-6-PDH is an index for determining the efficiency of HMP shunt. G-6-PDH is inhibited by NADPH and the inhibition is relieved by NADP (Candy, 1980). Since, HMP shunt is an alternate pathway for the supply of energy and reduced co-enzymes (NADPH), which function in detoxification process. Due to its importance an attempt has been made to examine the impact of G-6-PDH role in aging lung tissue with reference to nicotine metabolism and red grape extract treatment.

Results and Discussion:

In the present study the glucose-6-phosphate dehydrogenase activity was decreased in both (young and old) nicotine treatment rats (young by –49.08%; old by –28.55%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 23.53 %; old by 27.93%). In the combination treatment (Nt+RGEt) slightly increase was observed when compared to control rats of both age groups (Table.8; Fig.8).

Under the nicotine induced oxidative stress conditions the activity of G-6-PDH was decreased in the lung tissues of rats in both age groups. Nicotine induced stress condition is linked to the metabolism of nicotine in the tissues. In the similar studies, Gumustekin et al., (2005) reported that administration of nicotine inhibited the G-6-PDH in rat tissues. Similar decrease in G-6-PDH activity in rat during various induced toxic stress conditions in different tissues was also reported (Cartana et al., 1989; Cleary, 1991; Pugazhenthi et al., 1991). Chennaiah et al., (2011) reported the G-6-PDH activity was decreased in all the skeletal muscle fibres with the administration of nicotine. Khalindar Basha et al., (2013) reported in the kidney tissue decreased the G-6-PDH in the nicotine treated rats.

Table-8: Changes in **Glucose-6-phosphate dehydrogenase (G-6-PDH)** activity due to Nicotine treatment (Nt),Red Grape Extract treatment (RGEt), and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as μ moles of formazan formed/mg protein/hour.

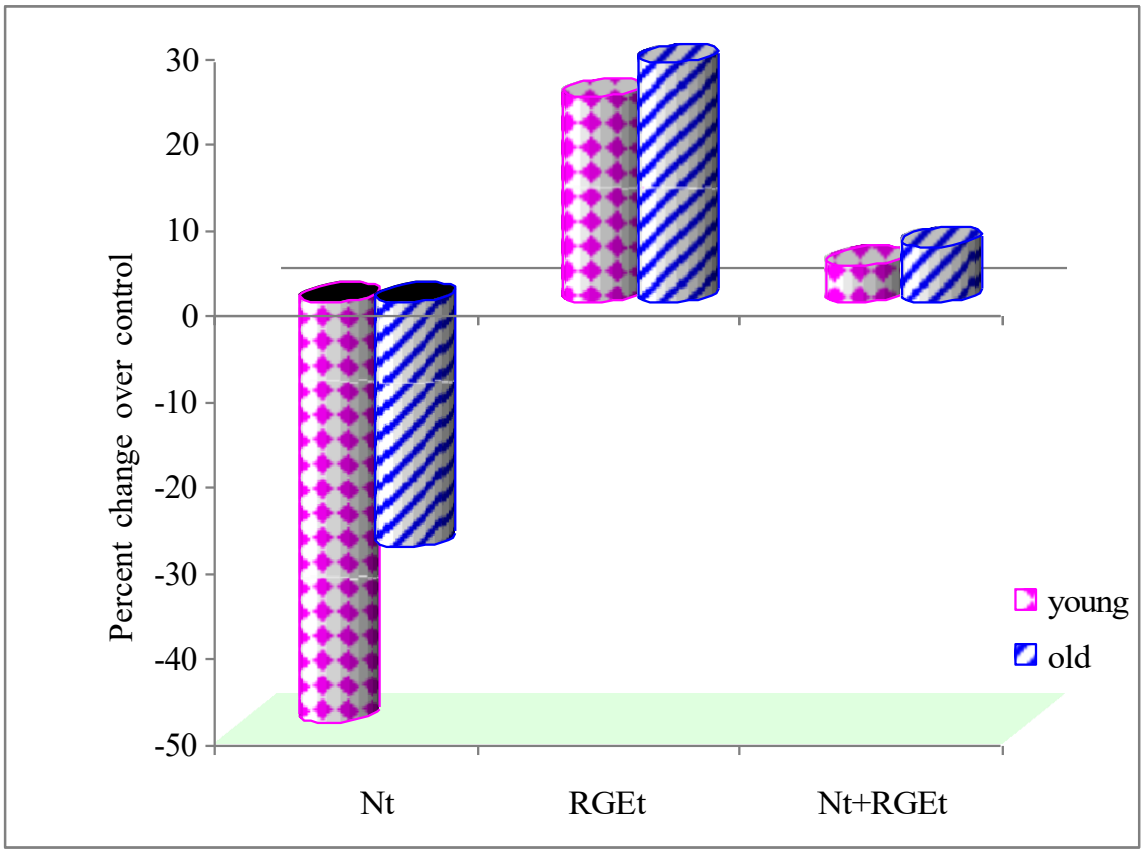
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	36.67 ±1.67	18.67** ±1.37 (-49.08)	45.30** ±1.33 (+23.53)	38.23@ ±1.44 (+4.25)	27.67 ±2.11	19.77** ±1.45 (-28.55)	35.40** ±1.52 (+27.93)	29.46@ ±1.62 (+6.46)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	13.32	2.66	1.632@
Treatment (C, Nt, RGEt, Nt+RGEt)	3	2820.23	940.08	576.3701**
Age (Young, Old)	1	529.54	529.54	324.666**
Tissue & Treatment	15	42.82	2.85	1.7503@
Tissue & Age	5	18.53	3.71	2.272@
Treatment & Age	3	241.84	80.61	49.4255**
Error	16	26.10	1.63	---
Total	48	3692.38	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
** Values are significant at $P < 0.01$
@ Values are non significant.

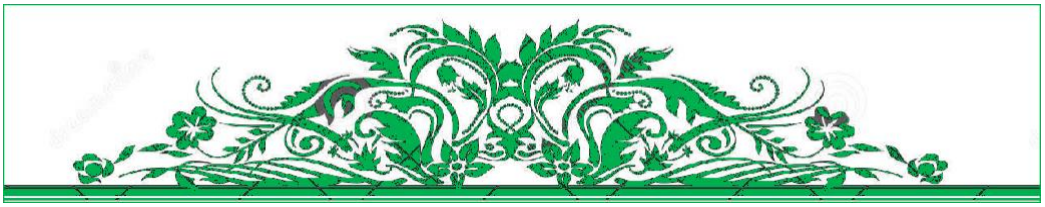
Fig.8: Per cent change over respective control in **Glucose-6-phosphate dehydrogenase (G-6-PDH)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



HMP shunt contributes the pentoses for the synthesis of ribonucleic acids. A decrease in G-6-PDH activity affects RNA synthesis and the nucleic acid metabolism which is of vital importance. Recently Das and Vasudevan, (2005) in their dose dependant studies on ethanol toxicity reported depleted activities of G-6-PDH at a significant level in the hepatic tissue of rat. Oh et al., (1997) reported significant decrease in G-6-PDH activity in the ethanol treated groups. Markku et al., (1997) reported a decrease in G-6-PDH activity followed by the administration of ethanol to the rats. Umadevi, (1992) also reported decreased G-6-PDH activity in the hepatic tissue of rat due to acetaldehyde induced stress conditions. The decreased G-6-PDH activity may be due to the decreased conversion of glucose-6-phosphate to 6- phosphogluconate leading to reduced formation of NADPH in HMP shunt. This cycle acts as an alternate source of energy during impairment of glycolytic and Kreb's cycle pathways.

Inhibition of oxidoreductases in the mitochondria results in the decreased energy supply for normal metabolic functions. The decrease in G-6-PDH activity by ethanol treatment indicates reduced oxidation of glucose in HMP shunt. Inhibition of G-6-PDH by acetaldehyde a product of ethanols could also be attributed to the reduced availability of NADP. The activity of G-6-PDH depends on SH groups in its active site (Jocelyn, 1972). It is likely that acetaldehyde may bind to these groups resulting in inhibition of G-6-PDH. The changes in SH groups observed in the tissues of acetaldehyde treated rats further support the view that SH-dependent enzymes including G-6-PDH are possibly affected (Umadevi, 1992). In the present study the G-6-PDH activity was increased in both the ages of lung tissue due to Red grape extract treatment, indicated the active participation of HMP shunt to overcome energy demand.

Aging is associated with the accumulation of inactive, or less active, more heat liable forms numerous enzymes. Comparatively old animals are more susceptible than young animals to protein damage during oxidative stress conditions (Berlett and Stadtman, 1997). Oxidative stress may cause the decrease of protein thiol which may consequently impair many enzymes. In such a situation G-6-PDH inhibition is closely associated with decreased proteins thiols. A decrease in protein thiol content is a consequence of an increase in intracellular oxidants. The inhibition of G-6-PDH may decrease cellular reducing equivalents,thus limiting anti-oxidative defense mechanism (Tian et al., 1999). In the combination treatment (Nt+RGEt) the G-6-PDH activity was increased in both age groups. This upregulation of G-6-PDH by RGEt, beneficial for nicotine subjects.



Chapter–3

ANTIOXIDANT ENZYMES

SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutase (SOD) is the key and primary antioxidant enzyme in the cell. Cellular defense against superoxide radicals is provided by the enzyme superoxide dismutase. Among other antioxidant enzymes, SOD considered as front line of defense against the potentially cytotoxic free radical cause oxidative stress. The superoxide dismutase catalyzes the dismutation of two superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen.

These enzymes obey first order reaction kinetics and the forward rate constants are almost diffusion limited. This results in steady state concentration of superoxide radicals in tissues that may be vary directly with the rate of superoxide generation and inversely with the tissue concentration of scavenging enzymes (Enghild et al., 1999; Fattman et al., 2003).It is well known that SOD is involved in destroying the superoxide radical and exists in several isoforms different in both cellular location and the metal co-factor bound to its active site. The Cu-Zn-SOD isoforms is located primarily in cytosol, the Mn-SOD isoforms is located in mitochondria and EC-SOD isoforms is found in extra cellular fluid,it is highly homologous to Cu-Zn-SOD (Powers and Lennon 1999; Enghild et al.,1999; Kao et al., 2002 ; Fattman et al., 2003).

The enzyme substrate interaction of the mitochondrial from does not appear to involve auto inactivation mechanism (Karuzina and Archakov, 1994) indicating that mitochondrial SOD levels are maintained for longer period of time compared to the cytosolic form increased levels of SOD are generally taken as indirect evidence an increased oxidant milieu. Since this is a sulfhydryl containing enzyme, decrease of its tissue level can also reflect oxidative denaturation. As with GSH biphasic fluxes are common and a change in either direction may relate to the presence of excess of ROS (Bondy, 1994; Kodavanti, 1999). Due to its important role in detoxifying the superoxide ion radical, the study was designed to find out to what extent SOD dismutase the superoxide radical under RGEt, nicotine and age induced oxidative stress conditions in the lung tissue.

Results and Discussion:

In the present study the Superoxide dismutase activity was decreased in both (young and old) nicotine treatment rats (young by –26.15 %; old by –11.30%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was

observed when compared to the control rats (young by 6.06%; old by 3.10%). In the combination treatment (Nt+RGEt) slightly increase was observed when compared to control rats of both age groups (Table.9; Fig. 9).

In the present study decrease was absorbed in SOD activity in the lung tissue of both age groups, due to nicotine treatment. The present results in the current investigation are in consistence with the previous findings. Among the generated free radicals due to nicotine metabolism, superoxide anion is the first derived free radical from nicotine. Thus, increased generation of superoxide radicals caused oxidative stress and damages the lung cells. In fact SOD scavenges the superoxide radicals in the tissues. In addition, the over production of superoxide radicals due to nicotine intoxication implies the over utilization of SOD, this may indicate its low activity under nicotine induced oxidative stress condition. The decrease in SOD activity due to nicotine consumption may impairs the other antioxidant enzyme activities like catalase and glutathione peroxidase. Because the superoxide radicals that are produced in the lung during nicotine metabolism are quickly scavenged to H_2O_2 by the enzyme superoxide dismutase. Under these circumstances, if SOD is not detoxifying the superoxide radical to hydrogen peroxide, there would be deficiency of substrate i.e., H_2O_2 for catalase and glutathione peroxidase enzyme activities. Thus, this kind of situation leads to impair the other antioxidant enzymes in the tissue metabolism.

Similar studies have been reported by several authors. Kazim Husain et al., (2001) reported a significant depression of renal SOD activity was observed in nicotine treated rats. The decrease in renal SOD activity may be a consequence of decreased de novo synthesis of enzyme proteins or oxidative inactivation of enzyme protein. Chennaiah et al., (2006) reported due to nicotine treatment SOD activity was decrease in the muscle tissue. The depletion of SOD activity was may be due to dispose of the free radical, produced by the nicotine toxicity. Helen et al., (2000) reported the decreased SOD activity in brain tissue of rat due to nicotine toxicity. Sokkary et al., (2007) reported chronic administration of nicotine the SOD activity was decreased in the rat liver and lung. Chattopadhyay and Chattopadhyay, (2008) reported due to nicotine treatment the SOD activity was decreased in ovary tissue. Similar changes in SOD activity was reported in various toxic conditions. Mahendran and Syamala Devi, (2001) reported decrease in SOD activity with 18% ethanol

Table–9: Changes in **Superoxide dismutase (SOD)** activity due to Nicotine treatment (Nt) ,Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in units of Superoxide anion reduced/ mg proteins.

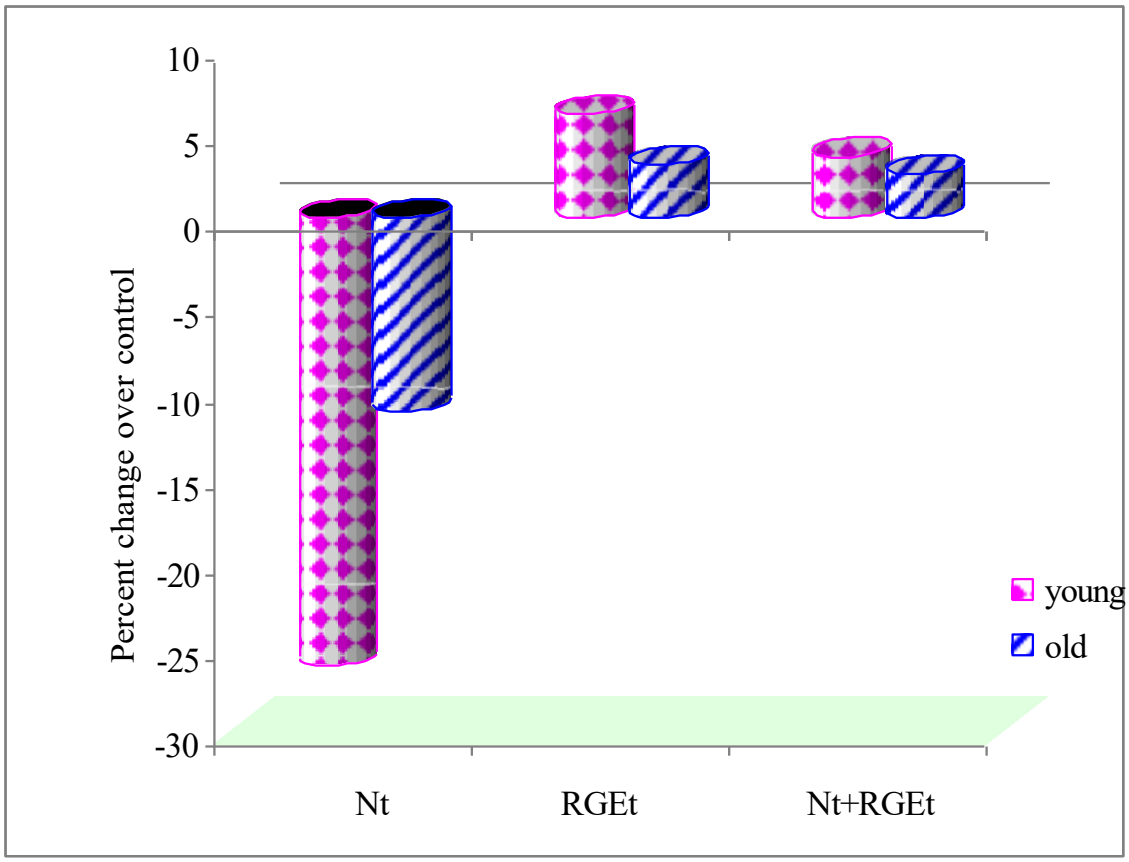
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	67.47 ±1.47	49.82** ±1.82 (-26.15)	71.56** ±1.99 (+6.06)	69.86@ ±1.27 (+3.54)	65.29 ±1.47	57.91** ±2.27 (-11.30)	67.32** ±2.46 (+3.10)	66.95@ ±2.22 (+2.54)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	14.72	2.94	1.3266@
Treatment (C, Nt, RGEt, Nt+RGEt)	3	1874.41	624.80	281.609**
Age (Young, Old)	1	1.17	1.17	0.5267@
Tissue & Treatment	15	78.76	5.25	2.3665*
Tissue & Age	5	18.60	3.72	1.6766@
Treatment & Age	3	288.91	96.30	43.405**
Error	16	35.50	2.22	---
Total	48	2312.07	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.9: Per cent change over respective control in **Superoxide dismutase (SOD)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



treatment in the hepatic tissue. Somani and Husain, (1997b) reported significant decrease in plasma and hepatic SOD activity with 20% of chronic ethanol treatment. When alcohol is metabolized in the liver by the MEOS pathway, a potentially dangerous by products such as, acetaldehyde and cytotoxic free radicals are generated (Temel et al., 2002; Licber, 2004). Chennaiah et al., (2012) reported in different toxic conditions SOD was decreased. Evidences are exist that ethanol intake increases the oxidative stress in the liver (Nordmann, 1994; Chen and Cohen, 1995) and its toxicity is associated with elevated generation of reactive oxygen species (Reinke et al., 1994). Among the generated free radicals due to ethanol metabolism, superoxide anion is the first derived free radical from ethanol. Thus, increased generation of superoxide radicals caused oxidative stress and damages the liver cells. Nordmann, (1994) showed that an acute ethanol load significantly enhanced superoxide generation in rat liver sub-mitochondrial particles.

In the present study lung SOD activity was increased with red grape extract treatment in both age groups of rats. This elevation was more pronounced in young age rats than old age rats. In vitro studies showed that grape juice has significant antioxidant activity and can inhibit oxidation of low density lipoprotein (LDL) (Castilla et al., 2006; O'Byrne et al., 2002). In addition to their antioxidant activity, polyphenols also possess many different biological properties. Normally phenolic compounds act by scavenging free radicals and quenching the lipid peroxidative side chain. It has been proposed that hydroxyl and hydroperoxy radicals initiate hydrogen abstraction from a free phenolic substrate to form phenoxy radicals that can rearrange to quinine methide radical intermediates which is excreted via bile (Rukkumani et al., 2005).

Dani et al., (2008) reported the SOD activity was increased in rats when treated with organic grape juice. Various authors reported in different tissues supplementation of red grape extract the SOD activity was increased (Sivasankar et al., 2013; Ramaiah et al., 2015). The activities of two major antioxidant enzymes, mitochondria SOD and cytosolic glutathione peroxidase (GSH-Px) were higher in red grape extract treated animals than the control animals. The increased generation of free radicals i.e., superoxide anion radicals would have triggered the induction of SOD enzyme and hence SOD activity was elevated during red grape extract treatment. Among the various antioxidant enzymes SOD provides the first line of defense against superoxide radicals, elevated SOD activity may reduce the exposure

of the hepatic tissue to superoxide radicals and perhaps hydroxyl radicals formed via the Haber-Weiss reaction (Halliwell and Gutteridge, 1989). In the present investigation increased lung SOD activity during red grape extract treatment helps in preventing accumulation of superoxide anion radicals in the tissue of old age rats by converting them to H_2O_2 which is considered to be an adaptational change by red grape extract treatment to mitigate superoxide toxicity.

This study supported a long standing hypothesis that generation of oxygen derived free radicals and other reactive oxidants may be increased in aged lung tissue. These results were also agree with previous findings, which reported the decreased SOD activity with advancement of age (Rao et al., 1990). Ramaiah et al., 2015;Vohra et al., (2001) reported the decrease in SOD activity in brain regions of 36 months old age guinea pigs. The reported decrease in SOD activity with age may further accelerated the aging process (Carilo et al., 1992). Some authors said that mitochondrial decay is a significant factor in aging, in rat, by the release of reactive oxygen species (ROS) as byproducts of mitochondrial electron transport.

Several authors quoted that during aging, inner mitochondrial membrane being a major intracellular site for the generation of superoxide anion radicals, which are toxic to the body (Yan and Sohal, 1998; Bejma and Ji, 1999). Mitochondria are the targets of oxidant byproducts. The study state and the percentage of oxygen converted to superoxide anion radical increased with age (Sohal et al., 1995; Perez et al., 1998; Sastre et al., 2000). SOD activity may also reduce in aging rats due to over utilization of SOD to counter the age induced free radicals in the lung tissue. Moderate red grape extract treatment produce a beneficial effect by decreasing the levels of oxidative stress markers in the mitochondria of Lung and prevent the age associated decrease of antioxidant enzyme activities in the same organ. In the combination treatment observed (Nt+RGEt) upregulation of SOD activity, decrease in oxidative stress and increased activity of mitochondrial electron transfer enzymes, are logically related.

CATALASE (CAT-EC: 1.11.1.9)

Catalase is one of the most important antioxidant enzyme, which can function either in the catabolism of hydrogen peroxide (H_2O_2) or in the peroxidative oxidation of small substances such as ethanol or methanol. Catalase has four subunits and each subunit contains a heme group. Heme consists of a protoporphyrin ring and a central

iron (Fe) atom. The iron can either be in the ferrous (Fe^{2+}) or the ferric (Fe^{3+}) oxidative state. This heme group is responsible for carrying out catalase activity. To maintain catalytic activity CAT requires Fe^{2+} as a co-factor (Powers and Lennon, 1999; Temel et a1., 2002). Catalase is widely distributed in the body comportments, tissues and cell. In many cases the enzyme is located in subcellular organelles such as, peroxisomes and cytosol of liver (Atalay and Laaksonen, 2002; Lesiuk et al., 2003). Mitochondria contain little amount of catalase. Catalase is a tetrameric peroxidative enzyme which converts the hydrogen peroxide to water and molecular oxygen and whose gene expression is regulated by H_2O_2. Catalase plays an important role in ROS metabolism and in adaptation to 'oxidant stress (Mates et al., 1999; Vaziri et al., 2003). Catalase catalysis the destruction of hydrogen peroxide into water and oxygen.

Hydrogen peroxide is produced in the cells by a number of enzymatic reactions including those catalyzed by SOD, which converts superoxide anion radical to hydrogen peroxide and water (Fridovich 1995; Nordberg and Arner, 2001).
Hydrogen peroxide is more toxic than $O_2^{\cdot-}$ because it can enter the cell easily than superoxide radical. This H_2O_2 by reacting with $O_2^{\cdot-}$ seems to responsible for the formation of highly reactive hydroxyl radical (^{-}OH) through metal catalyzed Haber- Weiss / Fentane reactions. The well-known fentane reaction is maintained when Fe comes in contact with H_2O_2. Hydroxyl radical is highly potent free radical initiating the sequence of events leading to the peroxidation of membranes (Halliwell and Gutteridge, 1999; Ray and Husain, 2002; Giordano, (2005). Peroxisomes are potent sources of cellular H_2O_2, because of high concentration of oxidases. The main site of $O_2^{\cdot-}$ and H_2O_2 production in eukaryotic cell is the electron transport chain in mitochondria. Due to its indispensable role in the antioxidant defense system, catalase activity was estimated to know its efficiency in red grape extract treatment and nicotine induced oxidative stress conditions in two different age groups of male albino rats.

Results and Discussion:

In the present study the Catalase activity was decreased in both (young and old) nicotine treatment rats (young by -31.59%; old by -23.18%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 10.91%; old by 21.59%).

In the combination treatment (Nt+RGEt) slightly increase was observed when compared to control rats of both age groups (Table.10; Fig. 10).

In the present we found that the administration of nicotine CAT activity was decreased in lung tissue. Similar studies have been reported by several authors. Avti et al., (2006) reported chronic administration of nicotine the CAT activity was decreased in the rat kidney. Chennaiah et al., (2006) reported due to nicotine treatment CAT activity was decreased in the muscle tissue. Helen et al., (2000), Ramaiah et al., (2015) reported the decreased CAT activity in brain tissue of rat due to nicotine toxicity. The depletion of CAT activity was may be due to dispose of the free radical, produced by the nicotine toxicity. Similar changes in CAT activity was reported in various toxic conditions by varies authors. Bindu et al., (2002) reported the decrease in CAT activity with 4g / kg body weight alcohol treatment for a period of 50 days in Sprague Dawley albino rats. Recently Das and Vasudevan, (2005b) reported a significant decrease in CAT activity with 2g by / kg body weight ethanol treatment for a period of 4 weeks in hepatic tissue of Wistar strain male albino rats.

This ethanol induced decrease in CAT activity may be due to enzyme protein oxidation as a result of accumulation of $H_2O_2$and other cytotoxic radicals (Somani et al., 1996). The decreased CAT activity with ethanol treatment indicates inefficient scavenging of hydrogen peroxide due to oxidative inactivation of enzyme. Husain and Somani, (1997a) reported a significant decrease in plasma CAT activity in alcohol treated rats. The lower levels of plasma CAT activity may be explained due to mobilization of iron, which can generate ROS and these species can release low molecular weight iron (Nordmann, et al., 1987). The two antioxidant enzymes namely SOD and CAT decreased significantly in the hepatic tissue of alcohol administered rats suggesting the increased damage to this tissue as a result of uncontrolled generation of partially reduced oxygen species (Mahendran and Shyamala Devi, 2001).

In the present study, in the both age groups of RGEt rats the CAT activity was increased. The increased catalase activity indicates its active involvement in the decomposition of hydrogen peroxide during red grape extract treatment. A change in the binding characteristics of enzyme to membrane or their release from peroxisomes has been proposed as a possible mechanism for the increased activity levels of CAT (Somani and Rayback, 1996). CAT and SOD are considered to be indispensable for the survival of the

Table–10: Changes in **Catalase (CAT)** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in μ moles of H_2O_2 cleaved/mg protein/min.

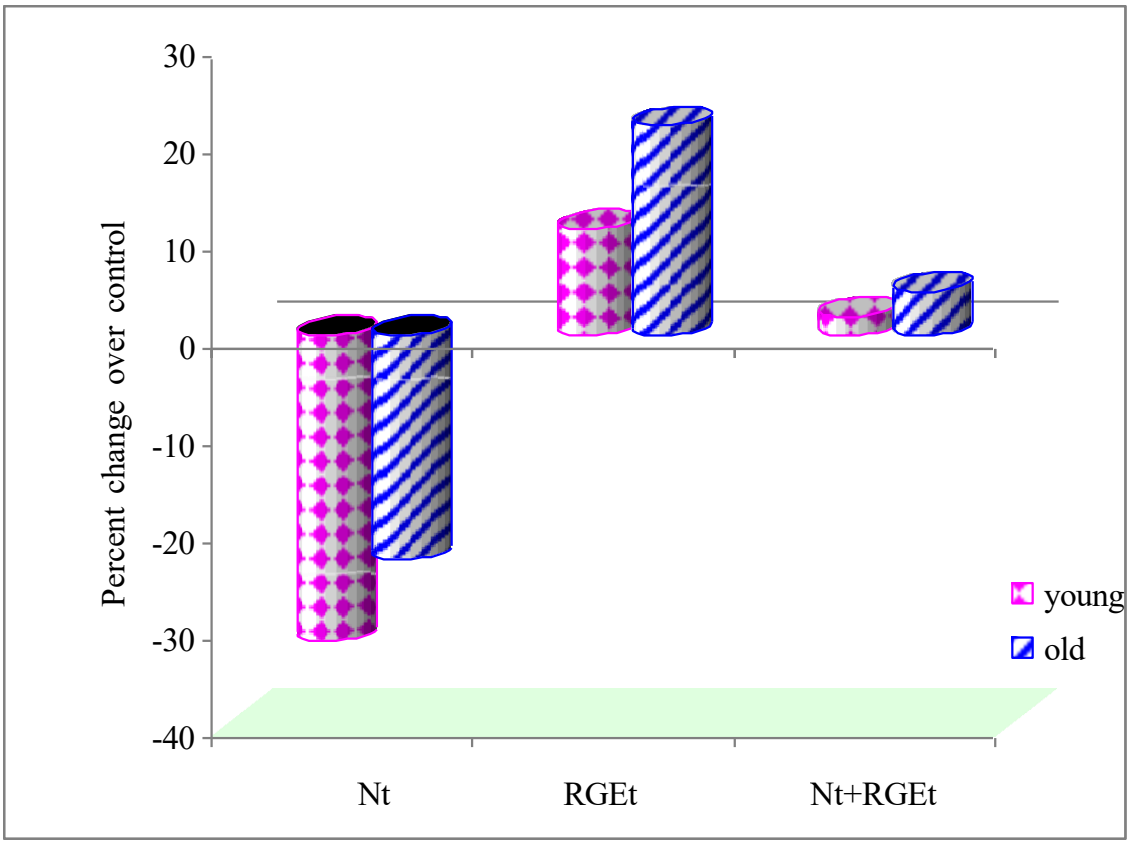
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	54.32 ±3.01	37.16** ±2.63 (-31.59)	60.25** ±2.30 (+10.91)	55.34@ ±3.53 (+1.87)	46.27 ±3.31	35.54** ±2.47 (-23.18)	56.26** ±2.81 (+21.59)	48.34@ ±3.47 (+4.47)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	135.58	27.12	3.6784*
Treatment (C, Nt, RGEt, Nt+RGEt)	3	3063.24	1021.08	138.51**
Age (Young, Old)	1	320.13	320.13	43.4253**
Tissue & Treatment	15	77.69	5.18	0.70261@
Tissue & Age	5	24.07	4.81	0.65306@
Treatment & Age	3	77.09	25.70	3.4857*
Error	16	117.95	7.37	---
Total	48	3815.75	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.10: Per cent change over respective control in **Catalase (CAT)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



cell against deleterious effects of hydroperoxides. The combination of SOD and CAT provide an efficient mechanism for removal of free radicals from the cell (Husain et al., 1996; Bhaskar Reddy, 2002). In vitro studies showed that grape juice has significant antioxidant activity and can inhibit oxidation of low density lipoprotein (LDL) (Castilla et al., 2006; O'Byrne et al., 2002). In addition to their antioxidant activity, polyphenols also possess many different biological properties. Normally phenolic compounds act by scavenging free radicals and quenching the lipid peroxidative side chain. It has been proposed that hydroxyl and hydroperoxy radicals initiate hydrogen abstraction from a free phenolic substrate to form phenoxy radicals that can rearrange to quinine methide radical intermediates which is excreted via bile (Rukkumani et al., 2004). Similar studies have been reported by several authors. Dani et al., (2008) reported the CAT activity was increased in rats when treated with organic grape juice.

In the current investigation, catalase activity was decreased with advancement of age. The decreased CAT activity in old age animals may be due to increase in the oxidative stress with age. Demaree et al., (1999) reported the decreased aortic CAT activity in old age rats than in young rats. Sivasankar et al., (2015) reported with aging CAT activity was decreased. It was observed that decline in SOD activity with age may result in lower CAT activity in the liver tissue. Rao et al., (1990) reported that the CAT activity was decreased in the tissues of kidney and liver, brain with aging. They also reported mRNA levels in the tissues of aged rats, which may result in the decreased activity of the enzyme in aged rats. Malsuo et al., (1992) also reported decreased CAT activity in the liver tissue between, 8, 14 and 32 months aged rats. The rates of mitochondrial superoxide and H_2O_2 generation were found to increase with age in mammals (Sohal el al., 1990; Jhansi Lakshmi, 1998). Age related increase in the hydrogen peroxide concentration in the tissues leads to decrease in CAT activity and cause oxidative stress in the tissues.

If may be because of high reactive oxygen metabolites production especially $O_2^{\cdot -}$ and H_2O_2 during aging process. Evidences suggest that $O_2^{\cdot -}$ itself affect directly the CAT activity (Kono and Fridovich, 1982). It is also been reported that CAT is inactivated by hydroxyl radical (Piegeolet and Corbisier, 1990). The increased rate of reactive oxygen metabolites production frequently elicits, as a response, an increase in the level of antioxidants. Under high rate of free radicals input, the enzyme inactivation

prevails and the enzymatic activities are reduced leading to autocatalysis of oxidative damage process (Escobar et al., 1996; Ray and Husain, 2002). Furthermore, iron is an essential co-factor in the catalase enzyme. An iron deficiency would not only impair oxygen transport in the body, but also compromise the body's antioxidant capacity by lowering catalase activity in cell (Halliwell and Gutteridge, 1999; Powers et al., 2004). If the animals take regularly the red grape the activity of CAT would increase. Red grape may capture the age induced hydrogen peroxides before escaping it from the cell and breakdown them to water and oxygen. In this way RGEt can maintain the ample catalase activity in the lung tissue under age induced oxidative stress condition. The upregulation in CAT activity was found with response of combination (Nt+RGEt) in both age groups of rats. The combination treatment augmented CAT activity in the lung, suggesting that RGEt may help to develop a resistance in the lung to cope with nicotine induced oxidative injury and maintains the antioxidant system.

GLUTATHIONE (GSH):

Glutathione (GSH) is the most abundant intracellular thiol based antioxidant present in milli molar concentrations in all living aerobic cells, but there is a wide variability in glutathione content across organs depending on their basal levels of free radical production (Nordberg and Amer, 2000; Powers et al., 2004). Glutathione serves as a sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of the cell system (Das and Vasudevan, 2005a). GSH is involved in several reactions in the body and is one of the most prominent non- enzymatic antioxidant (Meister and Anderson, 1991) that detoxifies reactive oxygen species (Mari et al., 2002). It functions as a substrate for glutathione peroxides and scavenges free radicals, oxy-radicals and singlet oxygen produced during stress conditions. These radicals also convert GSH to toxic GSSG, thereby increasing the levels of GSSG (Husain and Somani, 1997).

The reduced glutathione, a thiol containing tripeptide, plays a vital role in maintaining the cells in the reduced state and in protecting tissues from oxidative stress. GSH is likely involved in reducing radicals derived from a variety of antioxidants, such as α-tocopherol and ascorbic acid to the native structure (Yu, 1994). By donating a pair of hydrogen atoms glutathione (GSH) is oxidized to

glutathione disulphide (GSSG). As a result, the ratio of GSH to GSSG can be used as a hallmark indicator of intracellular glutathione redox status. A decrease of this ratio indicates that the production of ROS exceeds the reducing capacity of GSH and other antioxidants.

Glutathione is synthesized individually the constructive action of two enzymes, 1-GluCys synthetase uses glutamate and cysteine as a substrate forming the dipeptide. γ-GluCys which is combined with glycine in a reaction catalyzed by glutathione synthetase to generate glutathione. ATP is a Co-substrate for both enzymes. The intracellular levels of GSH are regulated by a feedback inhibition of γ- GlyCys synthetase by the end product of GSH (Mishra and Griffith, 1998). Therefore, cellular synthesis and consumption of glutathione balanced (Dringen, 2000). During detoxification of ROS, GSH is involved in two types of reactions. First, GSH reacts non-enzymatically with free radicals such as, the superoxide radical anion or hydroxyl radical (Singh et al., 1996) and second GSH is the electron donor for the reduction of peroxides in the GSH-Px reaction (Chance, 1979; Powers et a1., 2004). The final product of the detoxification of GSH is glutathione disulfide (GSSG).

Results and Discussion:

In the present study the Glutathione content was decreased in both (young and old) nicotine treatment rats (young by –10.49%; old by –9.09%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 5.99%; old by 9.34%). In the combination treatment (Nt+RGEt) slightly increase was observed when compared to control rats of both age groups (Table.11; Fig. 11).

In the present study we found that the administration of nicotine showing the decreased in GSH activity in the lung tissue. Similar studies have been reported by several authors. Sokkary et al., (2007) reported chronic administration of nicotine the GSH activity was decreased in the rat kidney. Saner et al., (2005) reported chronic administration of nicotine the GSH activity was decreased in the rat tissues. Chennaiah et al., (2006) reported due to nicotine treatment, GSH activity was decrease in the muscle tissue. Nicotine is oxidized primarily into its metabolite cotinine in the liver (Sastry et al., 1995), generates free radicals/ROS in tissues (Pryor and Stone, 1993; Wetscher et al., 1995),

Table–11: Changes in **Glutathione (GSH)** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in n moles of glutathione/ gm/ wet wt of tissue.

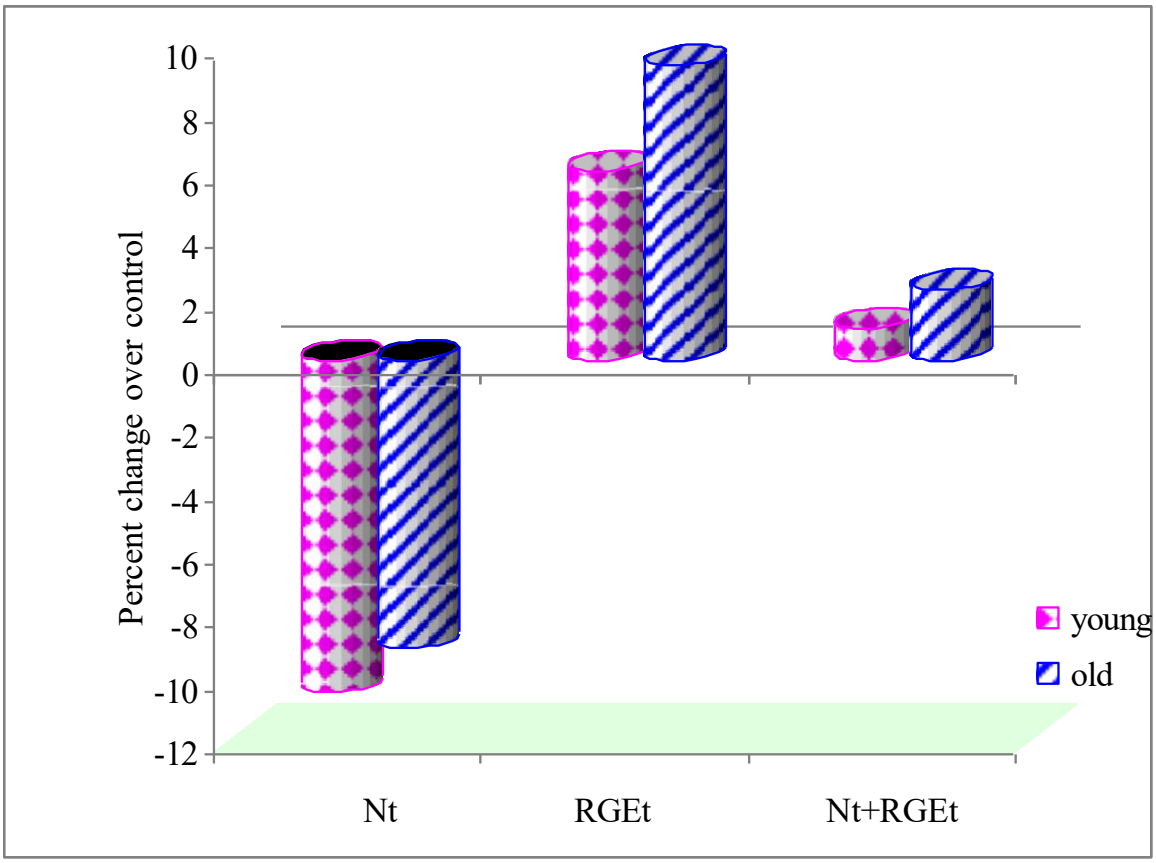
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	81.20 ±1.89	72.68** ±1.97 (-10.49)	86.07** ±2.75 (+5.99)	82.04@ ±1.66 (+1.03)	77.51 ±2.00	70.46** ±1.48 (-9.09)	84.75** ±1.61 (+9.34)	79.26@ ±1.42 (+2.25)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	8.00	1.60	0.269@
Treatment (C, Nt, RGEt, Nt+RGEt)	3	1187.16	395.72	66.7024**
Age (Young, Old)	1	75.50	75.50	12.7264**
Tissue & Treatment	15	34.12	2.27	0.3834@
Tissue & Age	5	6.28	1.26	0.2117@
Treatment & Age	3	8.94	2.98	0.50248@
Error	16	94.92	5.93	---
Total	48	1414.93	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.11: Per cent change over respective control in **Glutathione (GSH)** content in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



and induces oxidative tissue injury (Ashakumary and Vijayammal, 1991; Bhagwat et al., 1998; Park et al., 1998).

A similar change in GSH activity was reported in various toxic conditions by varies authors in varies tissues. Chronic ethanol consumption significantly depleted the GSH concentration in the hepatic tissue of different mammals like, rats (Mahendran and Shyamala Devi, 200l; Kim et al., 2003) mice (Zhou et al., 2002) and man (Kannan et al., 2004; Das and Vasudevan, 2005a). One important antioxidant that is affected by alcohol is glutathione. Liver cells contain an abundance of glutathione, especially with in structures called mitochondria, where most of each cell's energy is generated. The key enzymes in mitochondria are certain cytochromes that are integral components of inner mitochondrial membrane. Glutathione is not synthesized in mitochondria; adequate concentrations of glutathione are maintained there by active transport form the cytoplasm through the mitochondrial membrane.

Alcohol interferes with the transport of GSH through membranes, leading to its depletion from mitochondria. The resulting GSH deficiency may permit mitochondrial damage and cell death by means of unimpeded lipid peroxidation (Maher, 1997; Zhou et al., 2002). The decrease in GSH concentration in mitochondria would thus be highly responsible for ROS generation and the structural and functional damage in this organelle (Kannan et al., 2004). The decrease in GSH / CSSG ratio in the liver tissue of' ethanol fed rats and inhibition of GR activity are indicative of ethanol induced oxidative stress in the liver tissue. Depletion of liver GSH by chronic ethanol ingestion induced oxidative stress is well reputed.

In the present study the GSH activity was increased in both age groups supplemented with RGEt in the lung tissue of rat. Moreover, the percent elevation of GSH was more pronounced in old age group of rats compared to the young group of rats. Increased GSH content with RGEt may also due to the increase in the synthesis of precursors for GSH formation and increase the γ-Glutamyl-Cystineglycine enzyme, which is very essential for the GSH. The synthesis and degradation of GSH is referred as the γ-Glutamyl cycle. This cycle small responsible for the enhanced GSH concentration in the lung tissue with red grape extracts treatment.

Age related an alteration in the levels of reduced glutathione seems to be very complicated. Decreased tissue concentrations of GSH have been reported in several diseased states

and are associated with an increase risk to oxidative stress (Bray and Taylor, 1993). GSH decrease may be due to increased oxidation of GSH or decreased in the synthesis of GSH and low decreased availability of precursors for GSH formation. Low glutathione reductase activity may also contribute to the lower levels of GSH in the tissues (Jhansi Lakshmi, 1998). Vohra et al., (2001) reported the decreased glutathione peroxides and glutathione, reductase activities in old age animals, indicate inadequate concentrations of GSH for their action in the tissues.

The decreased antioxidants with nicotine treatment were recovered with combination treatment in both ages. The combination treatment (Nt+RGEt) has the beneficial effect by enhancing the decreased antioxidants in the lung tissue. These results clearly indicate that the combination treatment for a period of 2 month would provide the favorable, condition to the cells by decrease the nicotine and improving their antioxidant aging caused oxidative stress capacity and / or decreased the nicotine and aging caused oxidative stress conditions in the lung tissue.

GLUTATHIONE PEROX1DASE (GSH-Px - EC: 1.11.1.9)

Glutathione peroxidase (GSH-Px) is a well-known first line defense of the cell against oxidative challenge, which inturn requires glutathione as a co-substrate. The enzyme contains selenium as a co-factor, most probably at the active site, but no other prosthetic group such as heme, flavine or other metal constituents. Selenium is involved in the protection of biological system, especially membrane lipids against peroxidation (Umadevi, 1992). Based on the selenium, GSH-Px can be divided into two forms; Se-dependent and Se-independent GSH-Px. Glutathione peroxidase is located in both mitochondria and cytosol (Powers et al., 2004). GSH-Px system is a critically important enzymatic defense system against oxidative stress in the tissues (Ji et al., 1998). GSH-Px catalyzes the reduction of H_2O_2 or organic peroxides using reduced-glutathione as the electron donor (Lawler and Powers, 1998) to yield water or an alcohol along with oxidized glutathione (GSSH) (Halliwell and Gutteridge, 1999). GSH-Px can also terminate the chain reaction of lipid peroxidation by removing lipid hydro peroxides from the cell membrane (Sing and Pathak, 1990; Jung and Henke, 1996; Vaziri et al., 2003).

While, there is an overlap between the function of glutathione peroxidase and catalase at cellular level, the two enzymes differ in their affinities for H_2O_2 as a substrate. Mammalian GSH-Px has a much affinity for H_2O_2 at low concentrations compared with CAT. Thus, when cellular levels of H_2O_2 are low GSH-Px is more active than CAT in converting H_2O_2 from the cell (Powers and Lennon, 1999). This enzyme has been postulated to protect the tissues from damage by H_2O_2 and will reduce lipid hydro peroxides led to the hypothesis that this enzyme may be protect tissue against oxidative damage due to lipid peroxidation. Glutathione peroxidase appears to be primary site of selenium action. The liver is a major site of detoxification and the first target of ingested oxidants and a very important tissue in the study of the role of GSH-Px in protection from lipid peroxidation. In view of this GSH-Px activity was assayed in the lung tissue of young and old rats with reference to aging and nicotine treatments.

Results and Discussion:

In the present study the glutathione peroxidase activity was decreased in both (young and old) nicotine treatment rats (young by –33.70%; old by –23.77%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase was observed when compared to the control rats (young by 7.53%; old by 11.36%). In the combination treatment (Nt+RGEt) slightly increase was observed when compared to control rats of both age groups (Table.12; Fig.12).

The present study reveals that the activity of glutathione peroxidase was decreased in nicotine treatment rats in both age groups. Similar studies have been reported by several authors due to nicotine, hepatic GPx activity was decreased in mice (Vijayan and Helen, 2007), Wistar rats (Avti et al., 2006). The decreased GSH- Px activity in the current investigation may disturb the glutathione (GSH) homeostasis in the liver cell and ultimately it leads to the damage of hepatocytes. Several studies have been reported by varies authors in different toxic conditions. Kazeem et al., (2011) reported the GSH-Px activity was decreased in the hepatic tissue. Ostrowska et al., (2004) reported the decreased GSH-Px activity at a significant level in rat brain tissue for a period of 4 weeks ethanol intoxication. Ramaiah et al., (2015) reported decreased the GSH-Px in rat brain. Das and Vasudevan (2005a) reported the decreased GSH-Px activity in the liver homogenate with a series of ethanol treatments like, 0.8g, 1.2g, 1.6g and 2.0g / kg body

Table-12: Changes in **Glutathione peroxidase (GP_x)** activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in μ moles of thioether formed/ mg protein/min.

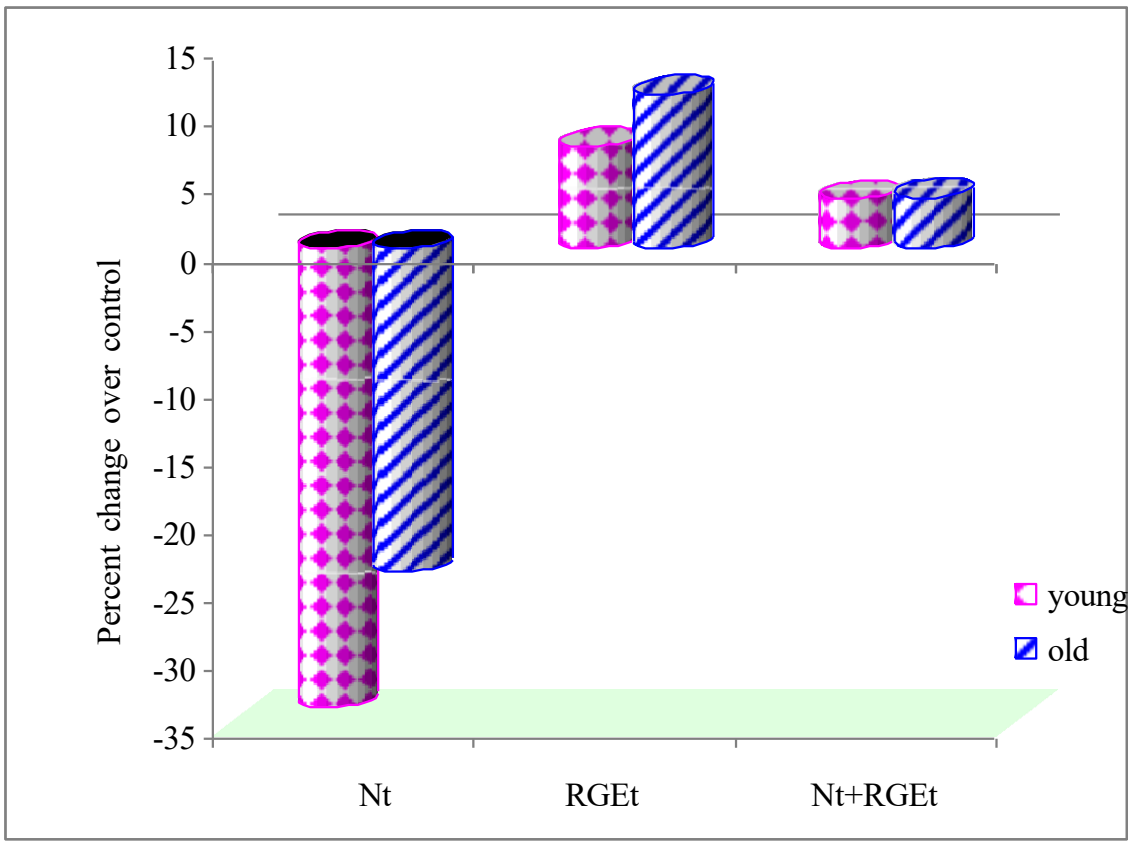
Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Lung	47.77 ±1.93	31.67** ±2.28 (-33.70)	51.37** ±1.66 (+7.53)	49.52@ ±1.60 (+3.66)	40.46 ±2.09	30.84** ±1.70 (-23.77)	45.06** ±1.93 (+11.36)	41.98@ ±1.94 (+3.75)

Three way ANOVA for six observation per cell

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Tissue (Lung)	5	9.74	1.95	0.6017@
Treatment (C, Nt, RGEt, Nt+RGEt,)	3	2065.51	688.50	212.6503**
Age (Young, Old)	1	363.17	363.17	112.1665**
Tissue & Treatment	15	48.00	3.20	0.98828@
Tissue & Age	5	36.09	7.22	2.2293@
Treatment & Age	3	89.57	29.86	9.22169**
Error	16	51.80	3.24	---
Total	48	2663.88	---	---

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
** Values are significant at $P < 0.01$
@ Values are non significant.

Fig.12: Per cent change over respective control in **Glutathione peroxidase (GPx)** activity in lung tissue, of young and old male albino rats in response to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt)



weight for a period of 4 weeks, our results also agreement with this. Decrease in GSH-Px activity may be due to either free radical dependant inactivation of enzyme or depletion of its co-substrate i.e., GSH and NADPH in the nicotine treatments. Similar studies, Santanu Kar Mahapatra et al., (2008) reported smoking decreases the Glutathione peroxidase in the serum of mans. GP_X works nonspecifically to scavenge and decompose excess hydro peroxides including H_2O_2, which may prevalent under oxidative stress (Somani et al., 1996)**.** In this study, decreased GP_X activity seems to indicate the smoking induced oxidative stress. The decreased level of GSH and activity of GSH- dependent enzymes i.e. GP_X, GR.

The results obtained from the present study reveals that red grape extract treatment enhanced in the lung glutathione peroxidase activity in both age groups of rats when compared to their respective controls. GSH-Px activity increased in lung tissue at a high level indicating an efficient elimination of organic peroxides (Husain and Somani, 1997). By accepting an electron from the peroxide (or donating a hydrogen ion), GSH is oxidized to half of disulphide (GSSH). This reaction is catalyzed by Se-containing GSH-Px enzyme. The elevation of glutathione peroxidase activity due to red grape extract treatment (RGEt) suggests an increased capacity to handle hydroperoxides in the lung tissue. It appears that red grape extract treatment provide the required substrate for a high increase in the GSH-Px activity.

Similar to the results obtained for SOD in this studies, the red grape extract treatment induced upregulation of GSH-Px activity, appears that SOD and GSH-Px are actively involving in decomposing the oxygen derived free radicals in the lung tissue of old rats. The reason for higher GSH-Px activity in red grape extract treatment (RGEt) rats may be due to higher production of ROS and increased activity of SOD in the rats. However, the available reports suggest the fact that higher SOD activity may be responsible in part, for higher GSH-Px activity (Ray and Husain, 2002).

The specific activity of GSH-Px was remarkably decreased in old rats compared to the young rats. With the aging, the GPx activity was decreased in different animals and a different tissue reported by various authors. The age related decrease in hepatic GSH-Px activity in the current study was supported by earlier reports also (Cand and Verdetti, 1989; Matsuo et al., 1992). Vohra et al., (2001)

reported both cytosolic and mitochondrial GSH-Px activities were decreased in different brain' regions of 32 months old guinea pigs. There appears to be an inter relationship between the activity of SOD and GSH-Px. The deficiency of SOD has been shown to be associated with decrease in the activity of GSH-Px vice-versa (Michiels et al., 1994). Both Se-dependent and Se-independent GSH-Px were decreased in old rats compared with the young rats. The production of free radicals and other reactive oxygen species are believed to increase with age in most tissues (Lawler and Powers, 1998).

These increased free radicals especially hydrogen peroxide (H_2O_2) may be responsible for the low activity of lung glutathione peroxidase in older rats. The decreased SOD activity in old rats which was also reported in the present study may also be responsible for the lower GSH-Px activity, because of their interrelation in detoxifying the toxic radicals. This age related decrease in lung GSH-Px activity was augmented with red grape extract compared to control rats. Thus, red grape extract play a prominent role in preventing nicotine induced oxidative stress by promoting the GSH-Px activity in the lung tissue in young and as well as old age rats.

SUMMARY AND CONCLUSIONS

In the present investigation the impact of red grape extract treatment on nicotine induced oxidative stress has been studied in the lung tissue with reference to aging on carbohydrate metabolic profiles, oxidative and antioxidant enzymes system by taking male albino rat as an experimental model.

Aging is the sum total of changes during an individual's life span which are common to all members of the species. If carbohydrates are infact the causative factors responsible for senescence, the maintenance of carbohydrate metabolism would minimize damage to physiological system and consequently the process of aging could be delayed. Red grape extract treatment enhances the ability to release energy by effective utilization of various metabolic fuels including stored ones, due to improved oxidative capacity. The survey of literature revealed that the reports on the effect of red grape extract, nicotine and aging is limited. Hence, the present investigation was programmed to elucidate the adaptive changes if any, induced by red grape extract treatment (RGEt), Nicotine treatment (Nt) and both Nt+RGEt on lung tissue with reference to aging.

Wistar strain male albino rats of two age groups (young and old) were maintained in polypropylene cages under laboratory conditions (Temperature 30□2^{0}C, Light: Dark =12:12 hours, Humidity=75%) and fed standard rat feed (Hindustan Lever Limited, Mumbai) and water ad libitum. The age matched rats were divided into four groups of 6 each. One group of rats were served as a control (C), the second group of rats were treated with nicotine (Nt), by subcutaneous injection (0.6mg/kg body weight for a period of 2 months). The third batch of rats were received red grape extract (25 mg/kg body weight, orally, via orogastric tube for a period of 2 months) and the fourth group treated with nicotine and red grape extract (Nt+RGEt) followed by the above groups II and III. All the groups of rats were sacrificed by cervical dislocation after 24 hrs the completion of experimental protocols for 2 months period. The lung tissue of both age groups were excised at -4^0, weighed immediately and homogenized in required media. The selected metabolites such as carbohydrate metabolic profiles and enzymes such as oxidative and antioxidative enzyme system were analysed.

1. The decrease in total carbohydrate levels in selected lung tissue after nicotine treatment in both age groups indicates utilization of carbohydrates to meet the energy demand during toxic stress conditions.

2. The lung glycogen concentration was increased after 2 months red grape extract treatment in both age groups due to mobilization of stored reserves including carbohydrates and fats. Whereas, in nicotine treated rats the glycogen content was decreased. However, with the combination treatment (Nt+RGEt) the glycogen was increased, which may be due to the induction of red grape extract treatment.

3. The total free amino acid pool was decreased in the lung tissue with the aging, when compared to control rats. The FAA content was decreased in nicotine treated rats in both the age groups when compare to control rats. However, in the combination treatment (Nt+RGEt) elevated the free amino acids (FAA) pool in old age rats to improve the amino acid pool reserves that were decreased due to nicotine treatment.

4. Decrease in NAD dependent LDH activity as a consequence of aging indicates poor clearance of lactate or an increased conversion of pyruvate to lactate as the LDH activity was estimated in the direction of pyruvate formation. The decreased LDH activity indicates the prevalence of hypoxic or anoxic conditions which normally alter the glycolytic rate. In the nicotine treatment rats, LDH activity was increased in both age groups, at the same time an increase was observed in the red grape extract treatment rats of both age groups. In the combination treatment (Nt+RGEt) upregulated the NAD dependent LDH activity suggesting increased conversion of lactate to pyruvate.

5. The specific activities of ICDH, SDH, MDH were assayed as markers of mitochondrial oxidative capacity. The decrease in NADP dependent ICDH activity indicates lesser involvement of TCA cycle in the oxidative reactions during aging. The possible reason for an increase in NADP-ICDH activity during red grape extract treatment is to step up energy production to meet the energy demands of lung tissue.

6. In the nicotine rats SDH activity was decreased in the lung tissue of both age groups. The increase in the specific activity of lung tissue SDH of red grape extract treatment rats suggests increased mitochondrial oxidative potential and energy synthesis utilizing fats as substrates by RGEt. The SDH activity was decreased with aging in both age groups, indicating depressed oxidative metabolism at the level of mitochondria leading to an overall deceleration of citric acid cycle operation thereby reducing the energy synthesis. A differential response of SDH activity in the lung tissue of young and old rats has been observed due to combination treatment (Nt+RGEt).

7. During aging malate oxidation was reduced in lung tissue of rats. The decreased activity of MDH may be due to restricted supply of substrates in view of lowered oxidative metabolism with advancement of age. In the nicotine treated rats also MDH was decreased in both age groups. The elevated activity levels of oxidoreductases (ICDH, SDH, MDH) in the RGEt rats suggest that the metabolic efficiency of biological transducer get adapted to increased energy requirement. In the combination treatment (Nt+RGEt) the MDH activity was upregulated due to mitochondrial activity by red grape extract treatment (RGEt).

8. In the present study the G-6-PDH activity was increased due to red grape extract treatment, indicate the active participation of HMP shunt to overcome energy demand and to generate reduced NADP for detoxification of free radicals generated due to red grape extract treatment in aged rat lung tissue. In the combination treatment the upregulation was observed by red grape extract treatment.

9. In the present study an elevation in SOD activity was observed with red grape extract treatment in both age groups, this was observed more in young age rats. However, with nicotine treatment the SOD activity was decreased. The combination treatment (Nt+RGEt) could able to elevate the SOD activity in the experimental rats suggesting that 2 months red grape extract treatment is beneficial to the nicotine animals. The elevation of SOD might be aimed at removal of superoxide radical anions generated either due to aging or nicotine toxicity.

10. Catalase an important antioxidant enzyme that consequence the hydrogen peroxide was found to decrease with aging. The upregulation of CAT activity was found with response to 2 months red grape extract treatment. The combined action of SOD and CAT worked as an efficient mechanism for the removal of ROS and also enhancement AOS system efficiently.

11. GSH is involved in a number of cell functions such as antioxidant defense. The GSH levels were decreased in the nicotine treated rats of both age groups. When GSH was measured, a decrease in its concentration observed in old age rats. This confirms increased susceptibility to oxidative stress during aging process. In the combination treatment (Nt+RGEt) GSH activity was increased due to red grape extract treatment. Elevated levels of GSH with red grape extract treatment in both age groups are a beneficial phenomenon to counter oxidative stress.

12. An age dependant decrease in GPx activity were observed in the present study. Nicotine treatment decreases the GPx activities. However, the combination treatment upregulated the GPx activities in both age groups in a good manner. This indicates an active participation of the enzyme in scavenging of the hydroperoxides that are generated due to toxicity nicotine.

To conclude, the present findings suggest that 2 months red grape extract treatment with the selected mg (25 mg/kg body weight) that was adapted may be beneficial in countering the age associated and nicotine induced alterations in carbohydrate metabolic profiles, oxidative and antioxidant enzyme activities in wistar strain male albino rats. The LDH, ICDH, SDH, MDH, G-6-PDH and antioxidant enzymes such as SOD, CAT, GSH, GPx activities are upregulated due to combination treatment (Nt+RGEt) in the lung tissue of both age groups. The augmentation of these enzyme systems due to 2 months red grape extract treatment will provide a significant advantage to overcome various pathological, physiological processes that occur in old age. This investigation draw a conclusion stating that (25 mg/kg body weight) red grape extract treatment to the old age as well as young age male subjects may be beneficial, especially for the nicotine subjects to improve the metabolic efficiency and thereby to improve the health status and survival capacity.

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