CHANGES IN THE LUNG BY THE INTERACTION OF RED GRAPE EXTRACT AND NICOTINE IN THE MALE ALBINO RAT WITH REFERENCE TO AGING

M. Jayachandrudu and P.Sreenivasulu K. Chennaiah*

Department of Zoology, Sri Venkateswara University. Tirupati, Andhra Pradesh, India-517502.

ABSTRACT

Nicotine is a psychoactive drug, available in the tobacco leaves. Consumption of Red grape flavonoids has been shown to confer antioxidant protection. In the present study antioxidant has been assessed in nicotine administered rats to examine the effects of nicotine on the antioxidant defense systems in lung of male albino rat. Age matched rats were be divided into 4 groups of six in each group and treated as follows: Group I. Normal Control (NC) (Control rats received 0.9% saline). Group II. Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months). Group III. Red grape extract treated (RGEt). (Red grape extract at a doses of 25 mg/ kg body weight via orogastric tube for a period of 2 months). Group IV. Nicotine + Red grape extract treated (Nt+RGEt) (The forth group of rats were received the nicotine + red grape extract as followed by the second and third group). The enzymes such as Superoxide dismutase(SOD), Catalase(CAT), Glutathione (GSH) and Glutathione peroxidase (GSH-Px) were significantly decreased in nicotine treated rats in lung and increase was observed in the combination treatment (Nt+RGEt), This study suggests that red grape extract treatment may be beneficial for nicotine intoxications.

KEY WORDS: Nicotine, Red Grape Extract, Superoxide dismutase (SOD), Catalase(CAT), Glutathione (GSH) ,Glutathione peroxidase (GSH-Px), Lung and Male albino rats.

***Corresponding author:**
Dr.K.Chennaiah,
Asst. Professor,
Dept.of Zoology,
S.V.University,Tirupati
A.P.,India-517502.
Email::chennaiahk@gmail.com

INTRODUCTION:

Grape (*Vitis vinifera* L.) is one of the most commonly consumed fruit growing worldwide. The total amount about 80% is used in wine making (Maier *et al*., 2009) and the grape byproduct consists 20% of weight from winery process (Lafka *et al*., 2007). In Thailand, grape is usually processed into various products such as wine, juice and raisins. Black queen is one of the grape varieties that is normally processed into wine and juice and the large quantity of byproducts from both processes such as pomace (grape pulp, peels and seeds) were obtained and there has been several studies showing that these kind of by products could be a good source of antioxidants such as polyphenols and flavonoids. Wine is considered to be a high bioactive polyphenol content source. Many studies have revealed the key role played by phenolic compounds from grapes and wine on human health; cardiovascular diseases being the pathologies that have received much attention (Pozo-Bayón *et al*.,2012, Arranz *et al*.,2012).

Wine is a widely consumed beverage in the world, with thousands of years of tradition. The phenolic compounds in grape berries are responsible for some of the major organoleptic properties of wine, such as color, astringency, bitterness, and aroma (Minussi *et al.*, 2003; Pérez-Magariño and González-Sanjosé, 2006). During the red winemaking process, phenolic compounds from the skins of red grapes transfer to the must during the fermentation and any maceration steps (Salas *et al*., 2003). Based on their carbon skeleton, phenolic compounds are divided into two groups: flavonoid (anthocyanins, flavan-3-ols, flavonols) and non-flavonoid compounds (hydroxybenzoic and hydroxycinnamic acids, stilbenes). Different types of phenolic compounds endow grape varieties and wines with specific quality characteristics.

Nicotine is highly addictive (Grana *et al.*, 2014; Holbrook and Bradley, 2016). An average cigarette yields about 2 mg of absorbed nicotine, and in lesser doses of that order, the substance acts as a stimulant in mammals, while high amounts (50–100 mg) can be harmful (Mayer, 2014). This stimulant effect is a contributing factor to the addictive properties of tobacco smoking. Nicotine's addictive nature includes psychoactive effects, drug-reinforced behavior, compulsive use, relapse after abstinence, physical dependence and tolerance (Caponnetto *et al*., 2012). Nicotine is a natural ingredient acting as a botanical insecticide in tobacco leaves. It is the principal tobacco alkaloid, occurring to the extent of about 1.5% by weight in commercial

cigarette tobacco and comprising about 95% of the total alkaloid content. Oral snuff and pipe tobacco contain concentrations of nicotine similar to cigarette tobacco, whereas cigar and chewing tobacco have only about half the nicotine concentration of cigarette tobacco. An average tobacco rod contains 10–14 mg of nicotine (Kozlowski *et al*., 1998), and on average about 1–1.5mg of nicotine is absorbed systemically during smoking (Benowitz and Jacob 1984). Nicotine in tobacco is largely the levorotary *(S)*-isomer; only 0.1–0.6% of total nicotine content is *(R)*-nicotine (Armstrong *et al*., 1998). Chemical reagents and pharmaceutical formulations of *(S)*-nicotine have a similar content of *(R)*-nicotine (0.1–1.2%) as impurity since plant-derived nicotine is used for their manufacture.

In most tobacco strains, nor nicotine and anatabine are the most abundant of minor alkaloids, followed by anabasine. This order of abundance is the same in cigarette tobacco and oral snuff, chewing, pipe, and cigar tobacco (Jacob *et al*., 1999). However, nornicotine levels are highest in cigar tobacco, anatabine levels are lowest in chewing tobacco and oral snuff, and anabasine levels are lowest in chewing tobacco (Jacob *et al*., 1999). Small amounts of the *N*_-methyl derivatives of anabasine and anatabine are found in tobacco and tobacco smoke. Several of the minor alkaloids are thought to arise by bacterial action or oxidation during tobacco processing rather than by biosynthetic processes in the living plant (Leete, 1983). These include myosmine, *N*_-methylmyosmine, cotinine, nicotyrine, nornicotyrine, nicotine *N*_-oxide, 2, 3_-bipyridyl, and metanicotine. Myosmine is found not only in tobacco but also in a variety of foods including nuts, cereals, milk, and potatoes (Tyroller *et al*., 2002). Also, nicotine is found in low levels in vegetables such as potatoes, tomatoes, and eggplants (Siegmund *et al*., 1999).

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. Hence, this study was

designed to investigate the effects of red grape extract on nicotine induced oxidative stress in the lung tissue of male albino rat with reference to aging.

METERIALS 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: 2012/ 2013 / (i) a / CPCSEA/ IACE/ SVU/ KC/ dt. 01/07/2012). 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*.

CHEMICALS:

Nicotine and other fine chemical were obtained from Sigma chemical company, St. Louis, USA. All other chemicals and reagent used were of analytical grade.

PREPARATION OF RED GRRAPE EXTRACTION:

Red Grapes, as large clusters with red berries, were brought from a local supermarket in Banglore 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% (W/V) alcoholic for 72 h in ambient temperature. The red grape extract was filtered and then solvent evapored to dryness under reduced pressure in a rotary evaporator. The residual red grape extract was used for this study.

EXPERIMENTAL DESIGN:

Age matched rats were divided into 4 groups of six in each groups. i) Narmal Control (NC) (Six rats were put on a six-channel, the rats were treated with normal saline (0.9%) orally via orogastic tube for a period of 2 months.). 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). iii) Red Grape extracts treatment (RGEt) (Rats were received red grape extract

25mg/kg body weight via orogastric tube for a period of 2 months), and 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:

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.

STATISTICAL ANALYSIS

Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance; the results were presented with the P-values.

RESULTS:

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-1).

Table-1: Changes in **Superoxide dismutase (SOD)** activity 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)

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 not significant.

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-2).

Table–2: Changes in **Catalase (CAT)** activity 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)

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 not significant.

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-3).

Table–3: Changes in **Glutathione (GSH)** activity 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)

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 not significant.

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.-4).

Table-4: Changes in **Glutathione peroxidase (Gp_x)** activity 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)

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 not significant.

DISCUSSION:

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. In the present study decrease was absorved in SOD activity in the lung tissue of both age groups, due to nicotine treatment. Similar studies have been reported by several authors in different tissues. 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. 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 cells.

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. 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).

In the present study, decreased SOD activity with advancement of age (Rao *et al.,* 1990). Several authores reported in varies tissues regarding the age related SOD activity. 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). 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)

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. In the present we found that the administration of nicotine CAT activity was decreased in lung tissue. Similar studiy have been reported by Avti *et al.,* (2006) chronic administration of nicotine the CAT activity was decreased in the rat kidney. The depletion of CAT activity was may be due to dispose of the free radical, produced by the nicotine toxicity. The two antioxidant enzymes namely SOD and CAT decreased in the lung tissue of nicotine administered rats suggesting the increased damage to this tissue as a result of uncontrolled generation of partially reduced oxygen species.

In the present study, in the both age groups of RGEt rats the CAT activity was increased. Dani *et al*., (2008) reported the CAT activity was increased in rats when treated with organic grape juice 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 cell against deleterious effects of hydroperoxides.

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. 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). 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.

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).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. Saner *et al*., (2005) reported chronic administration of nicotine the GSH activity was decreased in the rat tissues. Sokkary *et al.,* (2007) reported chronic administration of nicotine the GSH activity was decreased in the rat kidney. 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).

In the present study the GSH activity was increased in both age groups supplemented with RGEt in the lung tissue of rat. 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. 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.

Glutathione Perox1dase (GSH-Px)

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. 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.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 in different tissues , hepatic GPx activity was decreased in mice (Vijayan and

Helen, 2007) and 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. 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. So our results also suggesting the same in the lung tissue of male albino rat.

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.

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. 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.

CONCLUSION:

In the present study all the anti oxidant enzymes (SOD, CAT, GSH and GSH-Px), the upregulation was found with the response of combination (Nt+RGEt) in both age groups of rats. This study suggesting that RGEt may help to develop a resistance in the lung to cope with nicotine induced oxidative injury and maintains the antioxidant system.

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