INTERACTION OF RED GRAPE EXTRACT AND NICOTINE ON AGING

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Abstract

Cigarette smoking (*Nicotiana Tabacum. L*) is also a risk factor for respiratory tract and other infections, osteoporosis, reproductive disorders, adverse postoperative events and delayed wound healing, duodenal and gastric ulcers and diabetes. 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 heart of male albino rat. Age matched rats were 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 heart tissue 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), Heart tissue and Male albino rats.

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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 are 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 (Kozłowski *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, nor nicotine 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, nicotine, nornicotine, nicotine *N*-oxide, 2, 3_-bipyridyl, and metanicoline. 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 (Siegmond *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 heart 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 (Regd.No. 438/01/a/CPCSEA/dt.17-2-2001) in its resolution number 9/IAEC/SVU/Zool/dt.4-3-2002. 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 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% (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 orogastric 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 heart tissue, were isolated at -4⁰, washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80⁰ 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 heart 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 40C in cold centrifuge. The supernatant

was separated and used for enzyme assay. 100 μ l of tissue extract was added to 880 μ l (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; and 20 μ l 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 heart 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 40C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 μ l of 100% EtOH was added to 100 μ l 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 μ l of Triton X-100 RS. In a cuvette containing 200 μ l of phosphate buffer and 50 μ l of tissue extract was added 250 μ l of 0.066 M H₂O₂ (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 H₂O₂ degraded / mg protein / min.

Glutathione (GSH) Content:

Glutathione content was determined according to the method of Theodorus *et.al.*, (1981). The heart 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 40C. 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% NaHCO₃), 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 370C 5% (W/V) of heart 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 40C in cold centrifuge. The resulting supernatant was used as enzyme source. The reaction mixture consisted of 500 μ l of phosphate buffer, 100 μ l of 0.01 M GSH (reduced form), 100 μ l of 1.5 mM NADPH and 100 μ l of GR (0.24 units). The 100 μ l of tissue extract was added to the reaction mixture and incubated at 370C for 10 min. Then 50 μ l of 12 mM t-butyl hydroperoxide was added to 450 μ l of tissue reaction mixture and measured at 340 nm for 180 s. The molar extinction coefficient of 6.22 X 10³ 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 -26.91 %; old by -12.58%) when compared to control rats. In red grape extract treated rats of both (young and old) an increased was observed when compared to the control rats (young by 14.33 %; old by 13.30 %). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups. (Table-1).

Table-1: Changes in **Superoxide dismutase (SOD)** activity in Heart 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
Heart	157.84 ±12.37	123.53** ±8.45 (-21.73)	183.82** ±9.09 (+16.45)	160.32@ ±10.90 (+1.57)	151.21 ±11.73	119.12** ±4.60 (-21.22)	154.52** ±8.78 (+2.18)	152.43@ ±11.73 (+0.80)

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

In the present study the Catalase activity was decreased in both (young and old) nicotine treated rats (young by -31.43 %; old by -22.94%) when compared to control rats. In red grape extract treated rats of both (young and old) an increased was observed when compared to the control rats (young by 12.82 %; old by 22.58 %). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table-2).

Table-2: Changes in **Catalase (CAT)** activity in Heart tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in μ moles of H₂O₂ cleaved/mg protein/min.

Name of the tissue	Young				Old			
	Control	Nt	RGEt	Nt+RGEt	Control	Nt	RGEt	Nt+RGEt
Heart	118.48 ±7.16	85.84** ±7.33 (-27.54)	127.23** ±5.70 (+7.38)	120.20@ ±6.03 (+2.03)	104.60 ±9.57	83.00** ±7.25 (-20.65)	113.91** ±5.70 (+8.90)	106.28@ ±7.26 (+1.60)

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

In the present study the Glutathione content was decreased in both (young and old) nicotine treated rats (young by -11.00 %; old by -9.84 %) when compared to control rats. In red grape extract treated rats of both (young and old) an increased was observed when compared to the control rats

(young by 6.87 %; old by 8.17 %). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table-3).

Table-3: Changes in **Glutathione (GSH)** activity in Heart 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
Heart	175.30 ±7.87	157.15* * ±5.95 (-10.35)	185.44** ±5.13 (+5.78)	177.10@ ±7.87 (+1.02)	165.54 ±3.46	154.98* * ±2.78 (-6.37)	175.74** ±3.27 (+6.16)	167.36@ ±2.93 (+1.09)

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

In the present study the glutathione peroxidase activity was decreased in both (young and old) nicotine treated rats (young by -34.95 %; old by -25.22%) when compared to control rats. In red grape extract treated rats of both (young and old) an increased was observed when compared to the control rats (young by 7.74 %; old by 11.64 %). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table-4).

Table-4: Changes in **Glutathione peroxidase (Gpx)** activity in heart 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
Heart	108.89 ±7.07	72.51** ±4.58 (-33.40)	118.23** ±9.36 (+1.23)	111.82@ ±7.07 (+2.69)	92.76 ±11.95	68.67** ±9.55 (-25.97)	103.20** ±7.84 (+11.25)	95.15@ ±8.83 (+2.57)

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

DISCUSSION:

Superoxide Dismutase (SOD)

In the present study an decrease was absorbed in SOD activity in the heart 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 heart 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 heart tissue 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.

The results of the present study showed a fall in SOD activity in the heart tissue, in the nicotine treated groups. SOD, dismutate $O_2^{\cdot -}$ and the same in turn is a potent inhibitor of CAT (Ashakumari and Vijayammal, 1996). The depletion in SOD activity was may be due to dispose off the free radicals, produced due to nicotine toxicity. Beside this, on nicotine administration, H_2O_2 produced by dismutation of superoxide anion, may have been efficiently converted to O_2 by CAT and the enzyme activities showed a marked reduction. The depletion of antioxidant enzyme activity was may be due to inactivation of the enzyme proteins by nicotine-induced ROS generation, depletion of the enzyme substrates, and/or down-regulation of transcription and translation processes.

In the present study the SOD activity was decreased due to nicotine treatment in the male albino rat in heart tissue. 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 observed 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.*, (2001) 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 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, Licher, 2004). Evidences are exist that ethanol intake increases the oxidative stress in the liver (Chen and Cohen, 1995) and its toxicity is associated with elevated s 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 heart 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 quinone methide radical intermediates which is excreted via bile (Rukkumani *et al.*, 2005). Dani *et al.*, (2007) reported the SOD activity was increased in rats when treated with organic grape juice. The activities of two major antioxidant enzymes, mitochondria

SOD and cytosolic glutathione peroxidase (GSH-Px) were significantly 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 heart 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 heart tissue. These results were also agree with previous findings, which reported the decreased SOD activity with advancement of age (Rao *et al.*, 1990). 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). Miquel, (1980) and others postulated that mitochondrial decay is a significant factor in aging, caused, in part, 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 steady 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 heart tissue. Moderate red grape extract treatment produce a beneficial effect by decreasing the levels of oxidative stress markers in the mitochondria of heart and prevent the age associated decrease of antioxidant enzyme activities in the same organ. In the combination treatment (Nt+RGEt) observed upregulation of antioxidant enzyme activity, decrease in oxidative stress and increased activity of mitochondrial electron transfer enzymes, are logically related.

CATALASE (CAT)

In the present we found that the administration of nicotine was observed the decrease in CAT activity in the heart tissue. Similar studies have been reported by several authors. Chennaiah *et al.*, (2006) reported due to nicotine treatment CAT activity was decreased in the muscle tissue. Helen *et al.*, (2000) reported the decreased CAT activity in brain tissue of rat due to nicotine toxicity. Avati *et al.*, (2005) reported chronic administration of nicotine the CAT activity was decreased in the rat kidney, liver and lung. The depletion of CAT activity 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 various 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 / 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 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 quinone methide radical intermediates which is excreted via bile (Rukkumani *et al.*, 2005). Similar studies have been reported by several authors. Dani *et al.*, (2007) 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. It was observed that decline in SOD activity with age may result in lower CAT activity in the kidney tissue. Rao *et al.*, (1990) reported that the CAT activity was decreased in the tissues of liver, brain and kidney 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 H₂O₂ generation were found to increase with age in mammals (Sohal *et 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.

GLUTATHIONE (GSH)

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

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, 2001; 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 from 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 hepatic tissue of ethanol fed rats and inhibition of GR activity are indicative of ethanol induced oxidative stress in the hepatic tissue. Depletion of hepatic 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 heart 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 heart 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. 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. 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 heart 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 heart tissue.

GLUTATHIONE PEROXIDASE (GSH-Px)

The present study reveals that the activity of glutathione peroxidase was decreased in nicotine treated 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 has been reported by varies authors in different toxic conditions. Kazeem *et al.*, (2011) reported the GSH-Px activity was decreased in the hepatic tissue. Recently 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 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 H₂O₂, 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 the heart tissue glutathione peroxidase activity in both age groups of rats when compared to their

respective controls. GSH-Px activity increased in heart tissue at a high level indicating an efficient elimination of organic peroxides (Husain and Somani, 1997a). 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 tissues. 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 heart tissue of old rats. The reason for higher GSH-Px activity in red grape extract treatment 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 GSH-Px activity was decreased in different animals and different tissues reported by various authors. The age related decrease in heart tissue GSH-Px activity in the current study was supported by earlier reports also. 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 kidney 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 hepatic 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 heart tissue in young and as well as old age rats.

In the present investigation the impact of red grape extract treatment on nicotine induced oxidative stress has been studied in the heart 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 in fact 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 heart tissue with reference to aging.

CONCLUSION:

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

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