INTERACTION OF NICOTINE AND RED GRAPE EXTRACT ON ANTIOXIDANTS ENZYMES IN THE BRAIN OF MALE ALBINO RAT WITH REFERENCE TO AGING.

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ABSTRACT

Consumption of Red grape flavonoids has been shown to confer antioxidant protection, antioxidant has been assessed in nicotine administered rats to examine the effects of nicotine on the antioxidant defense systems in brain 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 50 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 animals were sacrificed after 24 hours after the last treatment by cervical dislocation and isolated the brain washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80^0 C for biochemical analysis and enzymatic assays. In the present study the Superoxide dismutase(SOD), Catalase(CAT), Glutathione (GSH) and Glutathione peroxidase (GSH-Px) were significantly decreased in nicotine treated rats in brain and enhance was observed in the combination treatment (Nt+RGEt), The study suggests red grape extract treatment may be beneficial to young and old age subjects due to nicotine intoxication.

Key words: Nicotine, Red Grape Extract, Superoxide dismutase (**SOD**), Catalase(**CAT**), Glutathione (**GSH**) and Glutathione peroxidase (**GSH-Px**), Brain and Male albino rats.

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INTRODUCTION

The Grape belongs to the berry family as it is found attached to the stem. Many berries make up a cluster or bunch of grapes. 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 *et al.*, 2002; Brouillard *et al.*, 2003). Most grapes come from cultivars of *Vitis vinifera.* 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.

Taxonomic position of Red grape

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

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). The antioxidant activity of grape seed

phenolic compounds is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders (Yilmaz, Y. and Toledo, R. T. 2004). Flavonoids, which are polyphenolic antioxidants, occur naturally in vegetables and fruits (Soong.Y.Y and Barlow. P.J. 2004). Flavonoids possess several physiological properties: antioxidant, antibacterial, antiviral, antiinflammatory, antimutagenic and antitumoral activity, as well as the activation or inactivation of certain enzymes (Rice-Evans, C. Packer, L.1998).

The fruit of the grape is one of the most palatably edible foods, having many established nutritional and medicinal properties for consumers. 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).

Water	(82%)
Carbohydrates	(12 – 18%)
Proteins	(0.5-0.6%)
Fat	(0.3-0.4%)
Potassium	(0.1-0.2%)
Vitamin C	(0.01-0.02%)
Vitamin A	(0.001-0.0015%)
Calcium	(0.01-0.02%)

Source: Mukesh yadav *et al*., 2009.

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). 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). In general, cigarette smokers switching from a higher to a lower-yield cigarette will compensate, i.e., will change the smoking pattern to gain more nicotine (USDHHS, 2001). Although the absorption through cell membranes is rapid for these more alkaline tobacco products, the rise in the brain nicotine level is slower than with

smoking. 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).

Nicotine binds to brain tissues with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers (Benwell *et al*., 1988; Breese *et al*., 1997; Perry *et al*., 1999). The increase in the binding is caused by a higher number of nicotinic cholinergic receptors in the brain of the smokers. The time course of nicotine in the brain and in other body organs and resultant pharmacologic effects are highly dependent on the route and rate of dosing. Smoking a cigarette delivers nicotine rapidly to the pulmonary venous circulation, from which it moves quickly to the left ventricle of the heart and to the systemic arterial circulation and to the brain. The lag time between a puff of a cigarette and nicotine reaching the brain is 10 to 20 s. Although the delivery of nicotine to the brain is rapid, there is nevertheless significant pulmonary uptake and some delayed release of nicotine as evidenced by pulmonary positron emission tomography data and the slow decrease in the arterial concentrations of nicotine between puffs (Lunell *et al*., 1996; Rose *et al*., 1999).

Nicotine is metabolized primarily in the liver. In animals, nicotine metabolism has been shown to occur to a small extent in extrahepatic organs such as lung, kidney and brain (Gorrod and Jenner, 1975; Williams *et al*., 1990a; Vahakangas and Pelkonen, 1993; Jacob *et al*., 1997). The brain is deficient in oxidative defense mechanisms and hence is at great risk of damage mediated by reactive oxygen species (ROS) resulting in molecular and cellular dysfunction (Gupta YK *et al*., 2003). The central nervous system (CNS) is vulnerable to free radical damage because of brain's high oxygen consumption, its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues (Skaper *et al.*, 1999). Moreover, brain has a high ratio of membrane surface area of cytoplasmic ratio, extended axonal morphology prone to injury, and neuronal cells are non-replicating. ROS can increase the permeability of blood brain barrier, after tubule formation, and inhibit the mitochondrial respiration. If unchecked it can lead to a geometrically progressing lipid peroxidation (Gilman SC *et al*., 1993).

MATERIALS AND METHODS:

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 male albino rats (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*.

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).

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.

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 246^{o}C, 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

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% 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.

Treatment schedule:

Age matched rats divided into 4 groups of six in each group and treated as follows:

Ggroup: i) Control rats (Rats received 0.9% saline). Group:ii) Nicotine treatment(Nt) (Rats were received the nicotine at a dose of 0.6 mg/kg body weight by subcutaneous injection for a period of 2 months).Group:iii) Red Grape Extract treatment(RGEt) (Rats were received red grape extract 50mg/kg body weight via orogastric tube for a period of 2 months).Group:iv) Nicotine + Red grape extract(Nt+RGEt) (Rats were received the nicotine at a dose of 0.6 mg/kg body weight by subcutaneous injection and red grape extract 50mg/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 brain was isolated at -4^{0}C, washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80^{0}C, 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 Investigation:

In the present study Superoxide dismutase (SOD), Catalase(CAT), Glutathione (GSH) and Glutathione peroxidase (GSH-Px) were analyzed. Superoxide dismutase activity was determined according to the method of Misra and Fridovich (1972). The SOD Activity expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. Catalase activity was measured by a slightly modified version of Aebi (1984). One unit of activity is equal to the moles of H_2O_2 degraded / mg protein / min. Glutathione content was determined according to the method of Theodorus *et. al.,* (1981). The glutathione content was expressed in nano moles / gram wet weight of the tissue. Glutathione peroxidase (GSH-Px) was determined by a modified version of Flohe and Gunzler (1984). 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.

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-value

RESULTS:

Superoxide dismutase (SOD):

In the present study the Superoxide dismutase activity was decreased in both (young and old) nicotine treatment rats (young by -24.78 %; old by -11.29 %) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 5.05 %; old by 2.92 %). In the combination treatment

(Nt+RGEt) slightly increased was observed when compared to control rats of both age groups. (Table.1).

Catalase :

In the present study the Catalase activity was decreased in both (young and old) nicotine treatment rats (young by -28.36 %; old by -32.21%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 11.06 %; old by 2.42 %). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table.2).

Glutathione (GSH)

In the present study the Glutathione content was decreased in both (young and old) nicotine treatment rats (young by -10.45 %; old by -9.47 %) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 6.25 %; old by 7.49%). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table.3).

Glutathione Perox1dase:

In the present study the glutathione peroxidase activity was decreased in both (young and old) nicotine treatment rats (young by -31.62 %; old by -21.81%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 6.56 %; old by 11.48 %). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table.4).

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. 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). In the present investigation reduce was absorbed in SOD activity in the brain tissue of both age groups, due to nicotine treatment. 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 brain 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 brain 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.

Comparable 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.,* (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 treatment in the hepatic tissue. Somani and Husain, (1997b) reported significant reduce 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, Lieber, 2004). 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 current reading brain 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 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. 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 brain 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 brain tissue. These results were also agreed 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 (Carillo *et al.,* 1992). Miquel, (1980) and others postulated that mitochondrial decay is a significant factor in aging, caused, 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 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 brain tissue. Moderate red grape extract treatment produce a beneficial effect by decreasing the levels of oxidative stress markers in the mitochondria of brain and prevent the age associated decrease of antioxidant enzyme activities in the same organ. In the combination treatment observed (Nt+RGEt)

upregulation of antioxidant enzyme activity, decrease in oxidative stress and increased activity of mitochondrial electron transfer enzymes, are logically related.

Catalase :

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 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). In the present study we found that the administration of nicotine the reduce was observed in CAT activity in the brain tissue. Similar studies have been reported by several authors. Avati *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) reported the decreased CAT activity in brain tissue of rat due to nicotine toxicity. The depletion of CAT activity should be due to dispose of the freeradical, produced by the nicotine toxicity. Similar changes in CAT activity was reported in should be 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, (1997b) 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 current 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 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. It was observed that decline in SOD activity with age may result in lower CAT activity in the brain tissue. Rao *et al.,* (1990) reported that the CAT activity was decreased in the tissues of brain, liver 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 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 $O_2^{\cdot-}$ it self 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 brain 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 brain, suggesting that RGEt may help to develop a resistance in the brain to cope with nicotine induced oxidative injury and maintains the antioxidant system.

Glutathione (GSH)

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). A deceased of this ratio indicates that the production of ROS exceeds the reducing capacity of GSH and other antioxidants. In the present study we found that the administration of nicotine showing the

decreased in GSH content in the brain 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), and induces oxidative tissue injury (Ashakumary and Vijayammal, 1991; Bhagwat *et al*., 1998).

A similar change in GSH activity was reported in various toxic conditions by should be various 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 content was increased in both age groups supplemented with RGEt in the brain 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 brain 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 ommited 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 brain 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 brain tissue.

Glutathione Perox1dase:

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. GSH-Px system is a

critically important enzymatic defense system against oxidative stress in the tissues (Ji *et al.*, 1998). In view of this GSH-Px activity was assayed in the brain 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, hepatic GPx activity was decreased in mice (Vijayan and Helen, 2007), Wistar rats (Avati *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 should be various 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. 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 aggrement 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 brain glutathione peroxidase activity in both age groups of rats when compared to their respective controls. GSH-Px activity increased in kidney 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 brain 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 brain 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. 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 brain 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 brain 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 brain tissue in young and as well as old age rats.

CONCLUSION:

This investigation draw a conclusion stating that this much of red grape juice extracts to the old age as well as young age male subjects may be beneficial, especially for the nicotine subjects to improve the health status and life span.

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Table–1: 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 Brain 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
Brain	145.93 ±13.22	109.76** ±10.35 (-24.78)	153.31** ±9.14 (+5.05)	148.40$^{@}$ ±12.09 (+1.69)	140.62 ±10.62	124.73** ±10.42 (-11.29)	144.73** ±12.92 (+2.92)	142.43$^{@}$ ±10.47 (+1.28)

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.

Table –2: 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 Brain 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
Brain	118.16 ±7.93	84.64** ±9.23 (-28.36)	131.23** ±11.95 (+11.06)	120.81$^{@}$ ±10.66 (+2.24)	102.43 ±81.63	81.63** ±9.02 (-32.21)	123.35** ±10.45 (+2.42)	124.38$^{@}$ ±8.84 (+3.27)

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.

Table–3: 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 Brain 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
Brain	172.92 ±7.95	154.84** ±9.18 (-10.45)	183.74** ±4.71 (+6.25)	175.09$^{@}$ ±9.65 (+1.25)	166.16 ±9.46	150.42** ±6.44 (-9.47)	178.61** ±11.41 (+7.49)	168.30$^{@}$ ±5.41 (+1.28)

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.

Table –4: 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 Brain 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
Brain	105.83 ±6.82	72.36** ±4.65 (-31.62)	112.78** ±7.49 (+6.56)	108.41$^{@}$ ±11.24 (+2.43)	90.20 ±12.94	70.52** ±3.02 (-21.81)	100.56** ±5.82 (+11.48)	93.42$^{@}$ ±5.06 (+3.56)

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.

Normal Control (NC) (Control rats received 0.9% saline).Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months).Red grape extract treated (RGEt).(Red grape extract at a doses of 50 mg/ kg body weight via orogastric tube for a period of 2 months).Nicotine + Red grape extract treated (Nt+RGEt).

Brain tissue (Young and Old age) Superoxide dismutase (**SOD**), Catalase(**CAT**), Glutathione (**GSH**) and Glutathione peroxidase (**GSH-Px**), Brain and Male albino rats.

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