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ROLE OF RED GRAPE EXTRACT AGAINST NICOTINE INDUCED CHANGES IN THE LUNG TISSUE OF MALE ALBINO RAT WITH REFERENCE TO AGING

M. Jayachandrudu[1], P. Muni Lakshmi[2], S. Kishore[3], K. Khalindar Basha[4] and K. Chennaiah[5]*

[1,3,4,5]Department of Zoology, Sri Venkateswara University. Tirupati, Andhra Pradesh, India.
[2]Department of Zoology, Sri Venkateswara Arts College (TTD), Tirupati, Andhra Pradesh, India.

***Corresponding Author: Dr. K. Chennaiah**
Department of Zoology, Sri Venkateswara University. Tirupati, Andhra Pradesh, India.

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ABSTRACT
A grape (*Vitis vinifera*) is commercial juice product have been applied in medical research studies, showing potential benefits against the diseases. Nicotine, a major toxic component of cigarette smoking, has long been recognized to result in oxidative stress by inducing the generation of reactive oxygen species in the lung tissue. Wistar strain male albino rats were used in the present study, rats were divided into 4 groups of six in each group i) Normal Control (NC) (Control rats received 0.9% saline); ii) Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months); iii) Red Grape extract treated (RGEt) (red grape extract 25mg/kg body weight (after the standardization) via orogastric tube for a period of 2 months.); IV) Nicotine + Red Grape extract treatment (Nt+RGEt) as a mentioned in above Group II and Group III. The animals were sacrificed after 24 hrs after the last treatment by cervical dislocation and isolated the lung tissue. The levels of Succinate dehydrogenase (SDH), Isocitrate dehydrogenase (ICDH) and Malate dehydrogenase (MDH) were decreased in nicotine treated rats in the lung tissue and increases was observed in the combination treatment (Ni+RGEt) rats in both age groups. But at 25 mg/kg body weight were found more effective in both age groups. The Lactic dehydrogenase (LDH) activity was increased in all treated rats in the lung tissue then the control in both age groups, in nicotine treated rats in both age groups it is found more. This results stating that red grape extract treated rats are beneficial, especially for the nicotine subjects to improve the oxidative enzymes and thereby to improve the health status and life span.

KEYWORDS: Nicotine, Red Grape extract, SDH, MDH, ICDH, LDH, Lung tissue and Male albino rat.

INTRODUCTION
Tobacco is the most widely used drug in the word and the greatest causes of illness and premature death in developed and developing countries. Epidemiological studies have shown a relationship between smoking and increased risks of cardiovascular disorders, lung cancer and pulmonary diseases (Baggio *et al.*, 1998; Fagerstrom, 2002). Moreover, it has been shown that cigarette smoking may accelerate the progression of renal, pulmonary, and cardiac fibrosis (Goette *et al.*, 2007; Sekhon *et al.*, 2004; Zhang *et al.*, 2009). The detrimental effects of smoking have been extensively investigated by studies of direct administration of nicotine, a major pharmacologically active component of tobacco smoke (Schievelbein and Balfour, 1984), in animal and in a variety of cell systems. The predominant effects of nicotine in the whole intact animals or human consist of an increase in heart rate (10 to 20 beats/min), blood pressure (5 to 10 mmHg), release of catecholamines and free fatty acids and mobilization of blood sugar (Dani and Heinemann, 1996; Shivij *et al.*, 2006).

Many teenagers and adults think that there are no effects of smoking on their bodies until they reach middle age (AAP, 1998). Smoking caused lung cancer, other cancers, heart disease, and stroke typically don't occur until years after a person's first cigarette. However, there are many serious harms from smoking that occur much sooner. In fact, smoking has numerous immediate health effects on the brain and on the respiratory, cardiovascular, gastrointestinal, immune and metabolic systems. While these immediate effects do not all produce noticeable symptoms, most begin to damage the body with the first cigarette sometimes irreversibly and rapidly produce serious medical conditions and health consequences.

Vitis vinifera belongs to the Vitaceae family. *Vitis vinifera* L., grape vine, is a perennial, defoliating climber with a wooden often twisted stem which can reach a

length of 30 meters, whereas in culture it is usually cut back to one to three meters. The shrub develops climbing branches forking to twigs from where the long-stemmed, alternating arranged leaves protrude. The vine leaf is heart-shaped, thin, shows five to seven dendate lobes, divided by more or less deep and open sinuses and can reach a diameter of over 20 cm. At the lower tendrils the flower panicles with numerous yellow-greenish flowers are formed. The fruits, arranged in large and long clusters are soft and pulpy berries with yellow-green, reddish or purplish dark-blue skin (Pharmacopee Francaise, 1996).

Grapes *(Vitis vinfera* L.) are considered the world's most prevalent fruit crop. Their large amounts of phenolic compounds have made them the focus of extensive studies (Broussaud *et al.*,1999; Bozan *et al*., 2008). In grape berries, the phenolic compounds reside mainly in the skins and seeds (Rodriguez *et al*., 2006; Poudel *et al*., 2008). Flavonols are the most abundant phenolic compounds in grape skins, while grape seeds are rich in monomeric phenolic compounds, such as (+)-catechins, (-)-epicatechin and (-)-epicatechin-3-0-gallate, and dimeric, trimeric and tetrameric procyanidins. These compounds act as antimutagenic and antiviral agents (Kammerer *et al*., 2004; Rodriguez *et al.*, 2006), and inhibit the oxidation of human low-density lipoproteins (LDL) in vitro (Teissedre *et al*., 1996). They undergo partial extraction during the winemaking process. Phenolics play an important role in the quality of grapes and wines. They can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavonols) (Rodriguez *et al*., 2006). Anthocyanins are a family of phenolics that are directly responsible for colour in grapes and young wines. Anthocyanins may react with flavanols to produce more stable pigments, either directly or by means of different aldehydes (e.g. acetaldehyde, propionaldehyde)(Pisarra *et al*., 2003). Flavan-3-ols (monomeric catechins and proanthocyanidins) are another large family of phenolic compounds that are mainly responsible for the astringency, bitterness and structure of wines. They are also responsible for the browning reactions in grapes and wine (Macheix *et al*., 1991) and undergo different reactions with anthocyanins that lead to the stabilisation of colour in red wines. Finally, phenolics, particularly certain phenolic acids, participate in the phenomenon of co-pigmentation. The last group of flavonoids are flavonols (quercetin, myricetin, kaempferol, isorhamnetin and their glycosides), which are potent antioxidants. Phenolic compounds in grapes and wine have attracted much interest due to their antioxidant properties (Kanner *et al*., 1994; Llobera and Canellas, 2007) and their potentially beneficial effects on human health (Teissedre *et al*., 1996; Vitseva *et al*., 2005). Recognition of the health benefits of catechins and procyanidins has led to the use of grape seed extract as a dietary antioxidant supplement (Santos Buelga and Scalbert, 2000; Guendez *et al.*, 2005; Bozan *et al*., 2008; Maier *et al*., 2009). The main phenolic antioxidants can also be used to preserve food because of their protective effects against microorganisms (Shoko *et al*., 1999; Jayaprakasha *et al*., 2003; Vattem *et al*., 2004). Phenolic antimicrobial compounds are found in grape seeds, skins and stem extracts (Jayaprakasha *et al*., 2003).

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

METERIALS AND METHODS

CARE AND MAINTENANCE OF EXPERIMENTAL ANIMALS

Male pathogenic free wistar albino rats were obtained from the Department of Zoology, Animal House, S.V. University, Tirupati, Andhra Pradesh, India. The animals were housed six to a polypropylene cage and provided with food and water add *libitum.* The animals were maintained under standard conditions of temperature and humidity with an alternating 12hr light/dark. The usage of animals was approved by the Institutional Animal Ethics Committee (Resolution No.10/(i)/a/CPCSEA/IAEC/SVU/ZOOL/KC/ Dt.08.07.2012). Animals were fed standard pellet diet (Agro Corporation Pvt. Ltd., Bangalore, India) and maintained in accordance with the guidelines of the National Institute of Nutrition and Indian Council of Medical Research, Hyderabad, India.

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 GRAPE 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 hr 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.

Experimental design

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

Group I – Normal Control

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

Group II – Nicotine treatment (Nt)

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

Group III – Red Grape extract treatment (RGEt)

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

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

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

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

BIOCHEMICAL INVESTIGATIONS

1. SUCCINATE DEHYDROGENASE (SDH):

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

2. ISOCITRATE DEHYDROGENASE (ICDH)

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

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

3. MALATE DEHYDROGENASE (MDH)

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

4. LACTATE DEHYDROGENASE (LDH)

Lactate Dehydrogenase activity was determined by the method described by Nachlas *et al.,*(1960) as suggested by Prameelamma and Swami (1975) with slight modifications. 10% homogenates of the lung tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4^{0}C. The supernatant fraction was used for enzyme assay. The

reaction mixture in a final volume of 2 ml contained 40 µ moles of sodium lactate, 100 µ moles of phosphate buffer (pH 7.4), 0.1 µ mole of NAD and 4 µ moles of INT. The reaction was initiated by the addition of 0.2 ml of homogenate containing 20 mg of tissue and incubated for 30 minutes at 37^0C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. Zero time controls (ZTC) were maintained by addition of 5 ml of glacial acetic acid prior to the addition of the enzyme source to the incubation mixture. The formazan formed was extracted over night into 5 ml of toluene at 5^0C. The color developed was measured at 495 nm in a Spectrophotometer against the toluene blank. The enzyme activity was expressed in µ moles of formazan formed / mg protein / hour.

STATISTICAL ANALYSIS

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

RESULTS

Lactate Dehydrogenase (LDH)

In the present study the Lactate dehydrogenase activity was increased in both (young and old) nicotine treatment rats (young by 9.09%; old by 16.77%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increase (young by 4.85 %; old by 8.59 %) was observed when compared to control rats. In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table.1).

Table 1: Changes in Lactate Dehydrogenase (LDH) activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as μ moles of formazan formed/mg protein/hour.

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

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
*Values are significant at P < 0.001
**Values are significant at P < 0.01
@ Values are not significant.

Isocitrate Dehydrogenase (ICDH):

In the present study in iso-citrate dehydrogenase content was decreased in both (young and old) nicotine treatment rats (young by -43.32 %; old by (-25.95%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 8.99 %; old by 5.09 %). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table.2).

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

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

All the values are ± SD of six individual observations
Values in parentheses denote per cent change over respective control.
*Values are significant at P < 0.001
**Values are significant at P < 0.01
@ Values are not significant.

Succinate Dehydrogenase (SDH)

In the present study the succinate dehydrogenase activity was decreased in both (young and old) nicotine treatment rats (young by -47.26 %; old by -28.79%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 14.06 %; old by 21.11 %). 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 Succinate dehydrogenase (SDH) activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as μ moles of formazan formed/mg protein/hour.

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

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
*Values are significant at P < 0.001
[@]Values are not significant.

Malate Dehydrogenase- (MDH)

In the present study the malate dehydrogenase activity was decreased in both (young and old) nicotine treatment rats (young by -62.70 %; old by -51.50%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 40.06 %; old by 39.96 %). 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 Malate dehydrogenase (MDH) activity due to Nicotine treatment (Nt), Red Grape Extract treatment (RGEt) and interaction of the both (Nt+RGEt) for a period of 2 months over the control in Lung tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as *μ* moles of farmazan formed/mg protein/hour.

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

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
*Values are significant at P < 0.001
** Values are significant at P < 0.01

DISCUSSION

The rats witch, received nicotine showed an elevation of LDH activity in the lung tissue in both the age groups. Moreover, the high per cent elevation of LDH was noticed in old (by 16.77%) group than the young group (9.09%) compared to their respective control rats. Several authors have reported increased LDH activity in afferent tissues with reference to different toxic conditions. LDH is a cytosolic enzyme, which allows the assessment of the process of anaerobic energy production by the cell. This enzyme is a marker of metabolic activity of renal glomeruli. A dose-dependent increase in LDH activity, evident after 24 weeks of cadmium (Cd) exposure, in the main tubules and glomeruli reflects intensification of anaerobic respiration (Malgorzata *et al*., 2004). Cunningham and Ivester, (1999) reported that significant ethanolrelated increase in lactate dehydrogenase activity released from both periportal and perivenous cell that occur under toxic conditions or at low oxygen tensions. The release of LDH was greatest in perivenous-ethanol hepatocyte, but was significantly different from control hepatocytes in both cell types. Strubelt *et al.*, (1999). According to Yildiz D *et al.*, (1999) LDH activity was increased due to nicotine induced oxidative stress. In our present investigations, the increased levels of LDH activity in nicotine treatments. This is due to the increased generation of ROS by nicotine that leads to cell damage and also indicated the low capacity to combat against ROS.

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

However, the direction of LDH is determined by the lactate / pyruvate ratio multiplied by the NAD / NADH ratio' Lactate oxidation only occurs if this mass action ratio is larger than the equilibrium constant (Rasmussen *et al.*, 2002). These evidences support the age related increased in LDH activity in old age rat of lung tissue,

however in the combination treatment (Nt+RGEt) the LDH activity was increased in both age groups.

In the present study a decrease of ICDH activity was observed in lung tissue of both the age groups of nicotine treatment rats. The decrease in specific activity of NAD/NADP-ICDH as a consequence of induced nicotine toxicity suggests reduced conversion of isocitrate to α-Ketoglutarate. Similar changes in ICDH activity was reported in different animals treated with various toxic compounds (Joseph and Rao, 1990; Reddy and Rao, 1991). The changes in NADP-ICDH could be attributed to the mitochondrial damage caused by nicotine treatment. Interaction of enzyme with NADPH may result in an unfavorable conformation of the enzyme molecule (Plaut, 1963). The lung NADP-ICDH was decreased, suggesting reduced mitochondrial oxidation of isocitrate in lung with advancement of age. This could be credited to diminished supply of keto acids into citric acid cycle (Thalwar *et al.*, 1989). Sanadhi, (1967) reported that the transfer of substrate by the mitochondrial membrane is altered in old cells because of rupture of the membrane. Age dependent damage in individual process has been reported for several enzymes including mitochondrial oxidoreductases (Thalwar *et al.*, 1989).

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

The decrease in SDH activity due to the nicotine stress condition indicates reduction in the conversion of succinate to fumarate resulting in decreased oxidative metabolism. Similar inhibition of SDH activity was reported in animals under induced different toxic conditions (Hamilton and Gould, 1987; Veerababu, 1988; Gupta *et al.*, 1991). Chennaiah *et al.*, (2011) reported the decreased SDH activity was observed in all skeletal muscle fibres of rats treated with nicotine, indicating depressed oxidative metabolism in mitochondria. Since the activity of SDH is reduced, it is evident that this might affect the conversion of malate to oxaloacetate by MDH because of low succinate oxidation. A decrease in oxygen consumption in stress condition also leads to inhibition of mitochondrial oxido-reductases (Moorthy *et al.*, 1985). The reduced availability of oxidized form of flavoproteins needed for succinate oxidation results in decreased activity of SDH (Swami *et al.*, 1983).

The SDH activity was increased in the lung tissue of both the age groups supplemented with RGEt when compared to the control rats. The increase in maximal and specific activity of SDH by RGEt suggests the increased mitochondrial oxidative potential and energy synthesis utilizing carbohydrates and fats as substrates. In the present study an increase was observed in the RGEt rats of both the age groups. The increase in specific activity of SDH in old age rats with response to RGEt suggests the increased mitochondrial oxidative potential and energy synthesis utilizing carbohydrates and fats as substrates function of mitochondria is energy production, isolated mitochondria generate reactive oxygen species during oxidative phosphorylation.

There is surprisingly little direct evidence for the generation of reactive species by mitochondria in intact cells of tissue (Leeuwenburgh and Heinecke, 2001). In the combination treatment with (Nt+RGEt) upregulation was observed in the lung tissue of both age groups. Thus differential response of SDH activity was observed in the lung tissue of both age groups in the present study.

A significant decrease in the specific activity of NADP-ICDH (Table.4) and as a consequence of nicotine-treatment observed in the present study indicates reduced formation of malate. The decrease in activity levels of dehydrogenases is consistent with the decreased CO2 formation (Cederbaum *et al.*, 1976). An increase in proteolytic activity during nicotine intoxication may also be responsible for the decreased MDH activity. A similar study Chennaiah *et al.*, (2011) reported a decrease in specific activity of MDH was observed in the muscle fibers of rats treated with nicotine, suggesting decreased utilization of malate. The decreased MDH activity could be attributed to low availability of substrate, lesser conversion of succinate-fumarate-malate, and the changes in the structural integrity of mitochondria. Similar inhibition of MDH activity was reported in animals under different toxic conditions (Veerababu, 1988; Tripathi and Shukla, 1990; Reddy and Yellamma, 1991). The decrease in specific activity of MDH in lung tissue of both age groups of rats as a consequence nicotine treatment suggests decreased utilization of malate. The reduced levels of TCA cycle intermediates may also be due to the decrease in MDH activity during nicotine-treatment.

Mitochondrial disfunction and accumulation of protein damage have been proposed to contribute to aging process (Bakala *et al.*, 2003). It has been recently demonstrated that impairment in mitochondrial respiration and oxidative phosphorylation elicits an increase in oxidative stress (Sateesh Pujari and Estari Mamidala, 2015; Yan and Sohal, 1998 and Sivasankar *et al*,. 2014). In recent years, much data has been accumulated to suggest that mitochondria act like a timer that ticks all the way through the aging process (Wei and Lee, 2002). From the present study we report that the combination treatment (Nt+RGEt) exhibits a beneficial recovery of MDH activity in both the age groups of lung tissue. This suggests that red grape extract treatment is

very much useful for the nicotine subjects to upregulate the decreased oxidative metabolism.

CONCLUSION

This investigation draw a conclusion stating that this much of red grape 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. The activities were inhibited in lung tissues of rats treated with Nicotine. In conclusion, the present study shows that red grape extracts treatment mitigates nicotine intoxication-induced oxidative damage, which could be due its antioxidant nature that combines free radical scavenging and metal chelating properties.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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