



**PROTECTIVE ROLE OF RED GRAPE EXTRACT AND RED GRAPE LEAF EXTRACT
ON NICOTINE IN MALE ALBINO RAT**

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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 and leaf flavonoids has been shown to confer antioxidant protection. In the present study Oxidative and poly enzymes has been assessed in nicotine administered rats to examine the effects of nicotine on the Oxidative enzymes defense systems in lung of male albino rat. Age matched rats were divided into 5 groups of six in each group and treated as follows: i) Normal Control (NC), ii) Nicotine treated (Nt), iii) Nicotine treated + Red Grape Extract treated (Nt+RGEt), iv) Nicotine treated + Red Grape Leaf extract (Nt+RGLet) and v) Nicotine + Red Grape extract + Red Grape Leaf extract (Nt+RGEt+RGLet). The enzymes such as Malondialdehyde (MDA), Xanthine oxidase (XOD), Uric acid were significantly decreased in nicotine treated rats in heart tissue and increase was observed in the combination treatment (Nt+RGEt). This study suggests that improve of red grape extract and leaf extract treatment may be beneficial for nicotine intoxications.

KEYWORDS: Nicotine, Red Grape Extract, Red grape Leaf Extract, Malondialdehyde (MDA), Xanthine oxidase (XOD), Uric acid, Lung tissue and Male albino rats.

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 (1) and the grape byproduct consists 20% of weight from winery process (2). 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 (3,4). 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 (5,6,7). 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 and relapse after abstinence, physical dependence and tolerance (Caponnetto *et al.*, 2012). Nicotine is a natural ingredient acting as a botanical insecticide in tobacco leaves. It is the principal tobacco alkaloid, occurring to the extent of about 1.5% by weight in commercial cigarette tobacco and comprising about 95% of the total alkaloid content. Oral snuff and pipe tobacco contain concentrations of nicotine similar to cigarette tobacco, whereas cigar and chewing tobacco have only about half the nicotine concentration of cigarette tobacco. An average tobacco rod contains 10–14 mg of nicotine (Kozlowski *et al.*, 1998) and on average about 1–1.5mg of nicotine is absorbed systemically during smoking (Benowitz and Jacob 1984). Nicotine in tobacco is largely the levorotary (*S*)-isomer; only 0.1–0.6% of total nicotine content is (*R*)-nicotine (Armstrong *et al.*, 1998). Chemical reagents and pharmaceutical formulations of (*S*)-nicotine have a similar content of (*R*)-nicotine (0.1–1.2%) as impurity since plant-derived nicotine is used for their manufacture.

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

MATERIALS AND METHODS CARE AND MAINTENANCE OF EXPERIMENTAL ANIMALS

Animals

Male pathogenic free wistar albino rats were obtained from the Department of Zoology, Animal House, S.V. University, Tirupati and Andhra Pradesh, India. The animals were housed six to a polypropylene cage and provided with food and water *ad libitum*. The animals were maintained under standard conditions of temperature and humidity with an alternating 12hr light/dark. 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. 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.

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 AND LEAF EXTRACTION

The leaves were dried in shade, powdered and extract by maceration with 70% (v/v) alcoholic for 72 hours in ambient temperature. The extract was filtered and then solvent evaporated to dryness under reduced pressure in a rotary evaporator. The residual extract was used for the study. Grape seeds and skin were removed from the grapes, the grape pulp were crushed for juice and dried in shade, powdered and extract by maceration with 70% (v/v) alcoholic for 72 hours in ambient temperature. The extract was filtered and then solvent evaporated to dryness under reduced pressure in a rotary evaporator. The residual extract was used for the study.

EXPERIMENTAL DESIGN

Age matched rats were divided into 5 groups of six in each group and treated as follows: i) Normal Control (NC), ii) Nicotine treated(Nt), iii) Nicotine treated + Red Grape extract treated(Nt+RGEt), iv) Nicotine treated+ Red Grape Leaf extract (Nt+RGLEt) and v) Nicotine + Red Grape extract +Red Grape Leaf extract (Nt+RGEt+RGLEt).

Group I – Normal Control

Control rats received 0.9% saline.

Group II – Nicotine treated

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

Group III – Nicotine + Red Grape extract

Rats were received the nicotine with 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.

Group IV – Nicotine + Red Grape Leaf extract

Rats were received the nicotine with a dose of 0.6 mg/kg body weight by subcutaneous injection and leaf extract 50mg/kg body weight via orogastric tube for a period of 2 months.

Group V – Nicotine + Red Grape extract +Red Grape Leaf extract

Rats were received the nicotine with a dose as mentioned for Group II through subcutaneous injection and leaf

extract, red grape extract as mentioned for Group III & IV 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°C, washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80° for enzymatic assays. Selected parameters were estimated by employing standard methods.

BIOCHEMICAL ANALYSIS

1. Malondialdehyde (MDA) - Ohkawa *et al.*, 1979
2. Uric acid - Martinek (1970)
3. Xanthion oxidase (XOD) - Srikanthan and Krishnamurthi, 1955

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

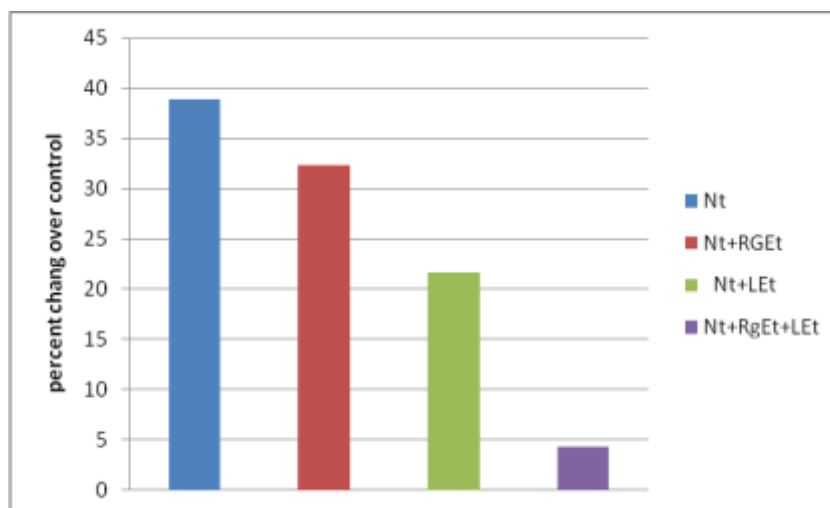
Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance; the results were presented with the P-values.

RESULTS AND DISCUSSION

MALONDIALDEHYDE (MDA)

In the present study a significantly increase (+38.94%) activities of MDA were observed in the lung tissue of nicotine treated rats when compared to normal rats. In all the experimental animals slightly hike (Nt+RGEt by -32.37%, Nt+LEt by -21.66% and Nt+RGEt+LEt by -4.35%) activity of enzyme was observed in the lung tissue, especially more hike was observed in the Nt+RGEt+LEt.(Fig:1).

Fig.1: Per cent change over respective control in Malondialdehyde the Lung tissue of i) Nicotine treated (Nt), ii) Nicotine treated + Red Grape extract treated (Nt+RGEt), iii) Nicotine treated + Red Grape Leaf extract treated (Nt+RGLEt) and iv) Nicotine treated + Red Grape extract treated +Red Grape Leaf extract treated (Nt++RGEt+ RGLEt) in the male albino rats. Values are expressed in μ moles of Malondialdehyde / gm Wet wt of tissue.



Nicotine produces Oxidative stress result of an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant system infavour of the former. So, intensity of oxidative stress is determined not only by the free radicals production but also by antioxidants (enzymatic and non-enzymatic) defense (32). Nicotine, a major toxic component of cigarette smoke, is a well established procarcinogen (37) In Cigarate smokers the MDA levels were increased reported by Santanu Kar Mahapatra *et all* 2008.In our study the MDA levels were increased. In the combination treatment (Nt+RGEt+ RGLEt) observed decrease of MDA levels, more decrease was observed in the Nt+RGEt+RGLEt. During lipid peroxidation poly unsaturated fatty acids in membranes are degraded to a great variety of aldehydes (Anuradha and Balakrishan, 1998) of which the amount of MDA is most frequently

used as a measure/ marker for the extent of lipid peroxidation in tissues (Somani,1996).

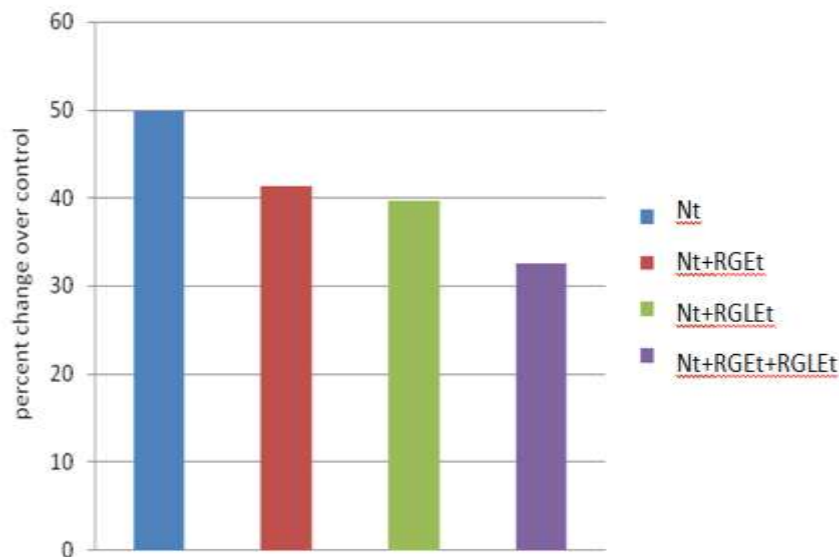
URIC ACID

In the present study a significantly increased (+46.84%) activities of uric acid were observed in the lung tissue of nicotine treated rats when compared to normal rats. In all the experimental animals decreased (Nt+RGEt by -41.31%, Nt+RGLEt by -39.65% and Nt+RGEt+RGLEt by -32.57%) the activity of enzyme was observed in the lung tissue, especially more decreased was observed in the Nt+RGEt+RGLEt.(Fig.2).

Fig.2: Per cent change over respective control in Uric acid in the Lung tissue of i) Nicotine treated (Nt), ii) Nicotine treated + Red Grape extract treated (Nt+RGEt), iii) Nicotine treated + Red Grape Leaf extract treated

(Nt+RGLEt) and iv) Nicotine treated + Red Grape extract treated +Red Grape Leaf extract treated (Nt++RGEt+ RGLEt) in the male albino rats. Values

are expressed in μ moles of Uric acid/ gm Wet wt of tissue.



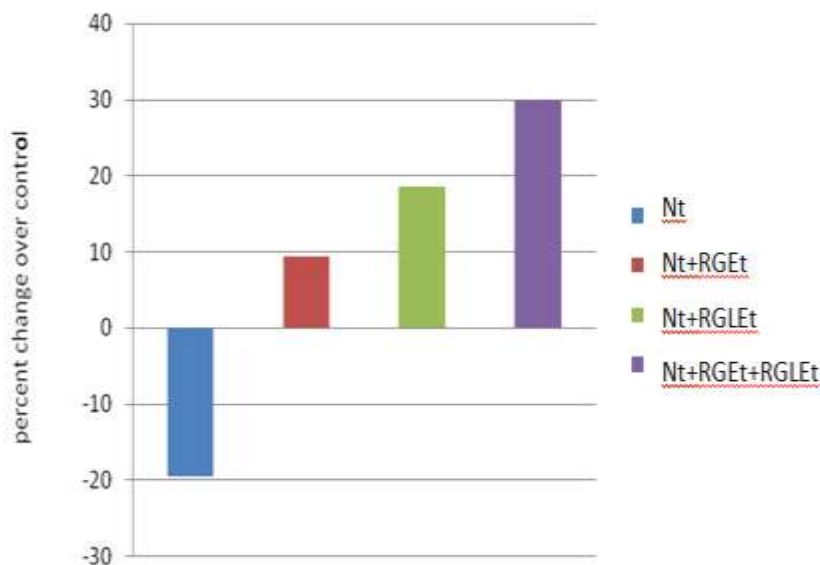
Uric acid helps the tissues to protect from oxidative damage. It also acts as aqueous antioxidant (Ames *et al.*, 1991; Floyd, 1993). The site of uric acid formation is liver, from where it is transported to kidneys. Uric acid is known to play a significant role as free radical scavenger and singlet oxygen quenchers. The decrease was observed in the red grape extract and leaf extract administrated rats in the lung tissue, this due to the reduction of free radical scavengers by the red grape extract and red grape leaf extract.

XANTHINE OXIDASE (XOD)

In the present study a significantly decreased (-19.48%) activities of XOD were observed in the lung tissue of nicotine treated rats when compared to normal rats. In all

the experimental animals increased (Nt+RGEt by +9.41%, Nt+RGLEt by +18.54% and Nt+RGEt+RGLEt by +29.97%) the activity of enzyme was observed in the lung tissue, especially more increase was observed in the Nt+RGEt+RGLEt.(Fig.3).

Fig.3: Per cent change over respective control in Xanthion oxidase in the Lung tissue of i) Nicotine treated (Nt), ii) Nicotine treated + Red Grape extract treated (Nt+RGEt), iii) Nicotine treated + Red Grape Leaf extract treated (Nt+ RGLEt) and iv) Nicotine treated + Red Grape extract treated +Red Grape Leaf extract treated (Nt++RGEt+ RGLEt) in the male albino rats. Values are expressed in μ moles of formazan formed / mg protein/hour.



Different metabolic states (hypoxia, ischemia) lead to the conversion of the dehydrogenase form of xanthine oxidoreductase to an oxidase form (XOD), which relates the metabolism of oxypurines with the generation of reactive oxygen radicals, conversion of dehydrogenase form of xanthine oxidoreductase to an oxidase form in hypoxic condition and generation of reactive oxygen radicals. This decreased in enzyme activity, most probably reflects the increased oxidative stress through the nicotine toxicity by producing the free radicals. The increase was observed in the red grape extract and red grape leaf extract administrated rats in the lung tissue. In the combination treatment (Nt+RGEt+ RGLEt) observed more increase due to red grape extract and leaf extract.

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