PROTECTIVE ROLE OF RED GRAPE EXTRACT AGAINST NICOTINE INDUCED LIPID METABOLISM IN THE SKELETAL MUSCLE FIBERS OF MALE ALBINO RAT

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ABSTRACT

Nicotine is known to induced oxidative stress and depletes antioxidant defense mechanisms; produced reduction in glutathione peroxidase in circulation, lung, liver and skeletal muscle of nicotine-treated animals .The present study aims at evaluating the protective role of red grape extrate on nicotine-induced oxidative stress. Pathogen free, Wistar strain male albino rats were used in the present study. 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 hrs after the last treatment by cervical dislocation and isolated the skeletal muscle fibers, from the hind limbs such as, Soleus (SOL) ,Red vastus(RV) and White vastus(WV), washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80^0C for biochemical analysis and enzymatic assays. In the present study, Total Cholesterol(TC), Triglycerides(TG), and Lipid Peraxidation(LPO) was significantly increased in nicotine treated rats in the all skeletal muscle fibers and the decreased was observed in the RGEt rats.In the combination treatment(Nt+RGEt) upregulation was observed in the all skeletal muscle fibers. 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 antioxidant efficiency and thereby to improve the health status and life span.

Key words: Nicotine, Red grape extract, Cholesterol and Triglycerides, Phospholipids, Lipid peraxidation, Skeletal muscle fibers, Male albino rat.

INTRODUCTION

Nicotine, (Chemical formula for nicotine is $C_{10}H_{14}N_2$) a major toxic component of cigarette smoke, plays an important role in the development of cardiovascular disease and lung cancer in smokers. Nicotine, one of the few natural liquid alkaloids, exerts a number of physiological effects involving the central and peripheral nervous systems, the cardiovascular system, and the endocrine system. In most mammalian species, nicotine is rapidly and extensively metabolized, primarily in the liver (Kyerematen and Vesell, 1991). 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). Oxidative stress generates free radicals that attack on the membrane lipids resulting in the formation of malondialdehyde (MDA), which causes peroxidative tissue damage. Animals studies have shown significantly higher liver and serum levels of MDA, conjugated dienes, hydroperoxides, and free fatty acids in rats induced by cigarette smoke (Zhang *et al.*, 2001).

The major metabolic pathways of nicotine in mammals are *C*-oxidation and *N*-oxidation, *i.e.* cotinine and nicotine-1′-*N*-oxide formation, respectively. In humans, 70–80% of nicotine is converted to cotinine. Cotinine is extensively metabolized, and only about 10–15% of cotinine is excreted unchanged in urine. About 4% of nicotine is converted to nicotine-1′-*N*-oxide, which is largely (if not entirely) excreted in urine without further metabolism (Jacob *et al.*, 1988). In rats, nicotine is excreted as cotinine and nicotine-1′-*N*-oxide, both at about 10% (Kyerematen *et al.*, 1988). It was reported that CYP (cytochrome P450) catalyzes the formation of cotinine from nicotine and FMO (flavin-containing monooxygenase) catalyzes the formation of nicotine-1′-*N*-

oxide in mammals (Cashman *et al*., 1992). Therefore, nicotine is a good probe to simultaneously estimate changes in CYP and FMO after liver injury.

Vitis vinifera L. (common grape) belong to *Vitaceae* family, which fruits have been used as a food and for wine or beverage production. In Ayurvedic (Indian) system, grapes leaves are used as a folk remedy for the treatment of diarrhea and vomiting (Zargari, 1993). Previous reports showed that leaves, fruits and juice of V. vinifera have the hepatoprotective effect on acetaminophen induced hepatic DNA damage, apoptosis and necrotic cell death (Montvale, 2002).It is well known that plant polyphenols act as free radical scavengers *in vitro* (Constantino *et al.,* 1992); tannins occur naturally in plant foods such as tea infusions, wines and fruit juices (Hertog *et al.,* 1993) and exhibit antioxidant effects (Miyara *et al.*, 1993)

Polyphenols, a ubiquitous group of secondary plant metabolites, represent a large group of natural antioxidants abundant in fruits, vegetables, and beverages such as grape juice, wine, and tea. Several studies have linked wine consumption to a reduction in cardiovascular diseases (Hertog *et al.,* 1993) attributing some of the cardio protective effects to the polyphenols present in red wine and grape products (Folts , 2002) Polyphenols were shown to exhibit strong antioxidant properties that could protect LDL from oxidation (O Byrne *et al*., 2002). In addition to their antioxidant activity, polyphenols also possess many different biological properties that may contribute to their cardio protective effects, including the ability to inhibit platelet activity and thrombosis (Freedman *et al*., 2001) and the potential to reduce plasma lipids. Epidemiologic and clinical studies suggest that polyphenols are good candidates to explain the protective effects of vegetables, fruits, and red wine against certain cancers and cardiovascular diseases (Folts ,2002). In addition to their antioxidant and anti-inflammatory

(Zern, 2005) properties, the effects on cholesterol homeostasis shown here may contribute to the cardioprotective effects of *Vitis vinifera* polyphenols.

Histochemical myosine ATPase fiber typing used to classify muscle fibers as Type I and Type II, which are known to correspond to slow and fast muscle fibers, respectively(Benowitz *et al*., 2002).The enzymes that are analyzed reflect metabolic pathway that are either aerobic/oxidative or anaerobic/glycolytic (Pausova *et al.*, 2003). This classification leads to 3 fiber types; Slow-twich oxidative (SO) soleus, (Type I); fast-twitch oxidative glyoglytic (FOG) red vastus, (Type II A); and Fast-twich glycolytic (FG) white vastus, (Type II B). (Benowitz *et al*., 2002; Pausova *et al.*, 2003; Valenca *et al.*, 2004). This study was designed to investigate the effects of red grape extracts on nicotine induced oxidative stress in the skeletal muscle fibers of male albino rat.

METHODOLOGY

ANIMALS:

Male pathogenic free wistar albino rats were obtained from the Department of Zoology, Animal House, S.V. U PG Centre, Kavali, Andhra Pradesh, India. The animals were housed six to a polypropylene cage and provided with food and water *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.

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 surrounding fields in Bangalore and identified as *Vitis vinifera* L. (Family *Vitaceae*) The red grape were crushed (whole fruit) 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 red grape extract was used for the study.

EXPERIMENTAL DESIGN:

Age matched rats divided into 4 groups of six in each group and treated as follows: I) Normal Control (Control rats received 0.9% saline) , II) Nicotine treated (Rats were received the nicotine at a dose of 0.6 mg/kg body weight by subcutaneous injection for a period of 2 months). , III) Red grape extract treated (Rats were received red grape extract 50mg/kg body weight via orogastric tube for a period of 2 months) and IV) Nicotine + Red grape extract treated (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 be sacrificed after 24 hrs after the last treatment session by cervical dislocation and the skeletal muscle fibers were be isolated at 4^0C, 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 INVESTIGATIONS:

The total cholesterol content was estimated using Liebermann-Burchard reaction as described by Natelson (1971). The skeletal muscles were homogenized in 4 ml of 1N sulphuric acid . To this 4 ml of chloroform was added. The contents were transferred into a tube having a tight stopper and shaken vigorously for 20 min . The contents were centrifuged . The supernatant acid the protein at the interface were then aspirated off. From the remaining chloroform, 2 ml of aliquot was taken and to it 1 ml of acetic anhydride mixture (acetic anhydride: conc. Sulphuric acid – 20:1.5) was added. The colour was developed for 9 min at 25^{o}C in dark and then read in a spectrophotometer at 625 nm. The standard consisted of 2 ml of working standard (0.05 µg/ml) and 1 ml of acetic anhydride mixture. The cholesterol content was expressed as mg cholesterol per gm wet wt. of tissue.

Triacylglycerol was estimated by the method of Natelson(1971) with slight modification as given by below. Triacylglycerol was assayed by hydrolysing them to glycerol and the liberated glycerol was determined. An aliquot of 4 ml from the total lipid extract was collected and 200 mg of dried sialic acid was added. The contents were vigorously shaken and supernatant was evaporated to dryness. To each tube, while it was still hot, 0.4 ml of absolute ethanol plus 0.01ml of alkaline barium solution were added and heated for 30 min at 80^{0}C to ensure complete hydrolysis. The reagent blank was prepared by taking 0.4 ml of ethanol and 0.1 ml of alkaline

barium solution and proceeded as mentioned below. After heating, the tubes were cooled and 1 ml of 2N sulphuric acid was added and shaken well. To this 0.1 ml of sodium peroxide solution was added and mixed well. The contents were kept aside for 10 minutes and 0.1 ml of arsenite solution was added. To this 5 ml of chromotropic acid was added and the contents were heated. The colour was read at 575 nm againtest the reagent blank. Standard graph was prepared by taking various concentrations of glycerol. The triacylglycerol content was represented as mg/g wet wt. of tissue.

Phospholipids were estimated by the method of Zilversmidth and Davis (1950). The skeletal muscles were homhgenized with 3 ml of 10% TCA and centriuged at 1500 rpm for 10 min. The supernatants were discarded and to the residues 1 ml of 60% perchloric acid was added and digestion was continued on a heating mantle to obtain a clear and colourless solution. Then the contents were cooled and 5 ml of distilled water was added to each tube.To each sample, 1 ml of 4%(W/V) ammonium molybdate was added and mixed.Finally 1 ml of ANSA (1-amino-2-naphthalo-4-sulphonic acid) reagent was added and the contents were made upto 10 ml with glass distilled water.The time of addition of ANSA reagent was noted. The colour developed was read at 660 nm against the blank within 6 min. Blank contained 0.8 ml of 60% perchloric acid and 2 ml of phosphate standards (containing 0.01 mg phosphorus/ml). The blanks and standards were also diluted with distilled water to a volume of 10 ml .The colour developed was read in the manner same as above. The results were finally expressed as mg phospholipids/g wet wt. of tissue.

The lipid peroxides were determined by the TBA method of Hiroshi et al. (1979). The isolated skeletal muscles were homogenized in 1.5% KCl (10% W/V). To 1 ml of tissue

homogenate 2.5ml of 20% TCA and 2.5 ml of 0.05 M sulphuric acid were added. To this 3 ml of thiobarbituric acid (TBA) was added and the samples were kept in a hot water bath for 30 minutes. The sample were cooled and malondialdehyde (MDA) was extracted with 4 ml of n-butanol and read at 530 nm in a spectrophotometer against the reagent blank. The trimethoxy pentane (TMP) was used as external standard.

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

The levels of TC, TG and LPO were significantly increased in animals treated with nicotine when compared to controls in all muacle fibers. Administration of red grape extract to nicotine-treated animals significantly decreased the level of TC, TG and LPO when compared with nicotine treated animals. The enhancing effect was observed in nicotine treated+ red grape extract treated animals in all muscle fibers (Table-1, 2, 4). The levels of phospholipids were signifi cantly decreased in nicotine treated rats when compared to controls rats and the upregulation was observed (Table-3) in red grape extract treated (RGEt) rats. In the combination treatment (Nt+RGEt), the protective effect was significant ($p<0.01$) in nicotine+ combination treatment (Nt+RGEt) when compared to nicotine treated tars.

DISCUSSION

Knowledge of the toxicity of nicotine is important to understand tobacco-induced human diseases, as well as to assess the potential risks associated with the therapeutic use of nicotine as an aid to assist

smoking cessation. Many drugs and chemicals have been shown to induce toxic side effects, and adverse or beneficial effects on multiple enzymes and metabolic processes. For example, a diuretic drug, acetazolamide inhibits carbonic anhydrase. Inhibition of specific enzymes due to drug use may exert pathological states in experimental models, for example, chronic nicotine administration inhibits cytochrome P450 (CYP2Abin liver, CYP1A1 in lungs) as well as generates free radicals, and exerts oxidative tissue injury (Yamazaki *et al.*, 1999). The generation of oxygen free radicals can be prevented or scavenged by host antioxidant defense mechanism (Wetshcer *et al*., 1995)

Cholesterol is presnt in tissues and in palsma lipoprotilns eilther as free cholesteryl or as cholesteryl ester.It is syntesized in many tissues from acetl Co-A and is the precursor of all other steroids in the body such as corticosteroids, sex hormons ,bile acid and vitaminD.Cholesterol and its ester are used for biosynthesis of membrans lipopritein layers and several metabolically active compounds.The localization of cholestrol may also be attirbuted to the architectural and physilogical functions of the tissue (Gurr and James , 1980).Anti-inflammatory and anti-allergic derivatives of Cholestrol are essential for increasing the survivability of animals under adverse stress conditions (Swami *et al*., 1983).

In the present study in all muscle fiber TC levels was increased due to nicotine toxicity.Regarding the skeletal muscle fibers and nicotine toxicity literature not available. In another studies chronic administration of nicotine was found to produce enhanced synthesis of cholesterol levels in the tissues of rats (Kavitharaj and Vijayammal, 1999).Our result also agreement with this. In the preset study administration of RGEt the cholesterol levels was decreased (Table-1) in all the muscle fibers then the control, in the combination treatment (Nt+RGEt) the upregulation was observed due to nicotine toxicity. In *vivo* studies showed that

administration of dealcoholized red wine (Vinson *et al*., 2001), grape seed procyanidins (Del Bas *et al*., 2005), polymethoxylated flavones (Kurowska *et al*., 2004) lowered plasma cholesterol in laboratory animals with diet-induced hypercholesterolemic. Recently, reduction of cholesterol LDL plasma concentration was reported in women consuming a lyophilized grape powder (Zern *et al*., 2005) or red wine (Naissides *et al*., 2006).

In this context, many studies investigating beneficial mechanisms of grape polyphenols have focused on their antioxidant properties. Flavonoids and other polyphenols found in grapes have the capacity to scavenge reactive oxygen species (Rice-Evans *et al*., 1996). Hayek *et al*.,(1997) observed that hypercholesterolemic mice consuming wine polyphenols for 6 wk had markedly less atherosclerosis than control animals. These treatments were associated with protection against LDL oxidation. Several animal models and human intervention studies showed that consumption of beverages rich in polyphenols, including red wine and dealcoholized red wine, decreases circulating levels of LDL cholesterol (Naissides *et al.*, 2006). However, the mechanism u nderlying this effect and the nature of the active compound have yet to be elucidated. Pal *et al.,* (2003) demonstrated that red wine polyphenols increase LDL receptor expression and activity in human cells in vitro. In the present work, we report that grape polyphenols are efficiently taken up by cells, as shown for quercetin, and interfere with the LDL endocytic pathway, counteracting the repression of LDL receptor expression otherwise generated by LDL cholesterol.

The triglycerides or so called neutral fats are esters of the alcohol glycerol and fatty acids. Acylglycerols in the form of triacylglycerols constitute the majority of lipids in the body .They are the major lipids in the fat deposits and in the food. They play a major role in lipid transport

and storage and in various physiological condition such as obesity, diabetes, and hyperlipo – proteinemia . Free fatty acids (FFA) a predominant substrate for oxidation in the muscle (Holloszy and Booth , 1976) ,is derived from the hydrolysis of triglycerol (TG) in the circulating chylomicrons and very low density lipoproteins ,TG stored in adipose tissue and TG stored within muscle cell (Palmer ,1983). Reports have indicated that circulating FFA originating from adipose tissue TG hydrolysis is the primary source of lipid fuel. However lipid in the form of free fatty acid (FFA), is a primary fuel for skeletal muscle during sub maximal work (Zierker, 1976). Chronic administration of nicotine was found to produce enhanced synthesis of TG levels in the tissues of rats (Kavitharaj and Vijayammal, 1999). Our studies also confirmed that, increased concentration of Triglycerides in the nicotine treated rats in all muscle fibers (Table-2). In the preset study administration of RGEt the TG levels was decreased (Table-2) in all the muscle fibers then the control, in the combination treatment (Nt+RGEt) the upregulation was observed due to nicotine toxicity.

Phospholipids are the major structural component of biological membranes and, as such, have significant influence on their physical properties. Phospholipids are compound lipids,they contain in addition to fatty acides glycerol/or other alcohol, a phosphoric acid residue, nitrogen containing base and other substiuents. Phospholipids parcitate in lipoprotein complexes which are thought to constitute the matrix of cell walls and membranes,the myelin sheath,and such structures as mitochondria and microsomes. Phospholipids play an essential role in the blood coagulation process (Chattergea and Ranashinde, 1993).In the present study phospholipids was decreased in nicotine treated rats. The increase was observed in RGEt rats,in all the muscle fibers .In the combination treatment (Nt+RGEt) the decrease was observed then

the RGEt due to nicotine oxidative stress (Table-3).Wiswedel et al (2010) reported oxidative stress produces by the free radicals,these are causes for degradation of phospholipids by oxidation, resulting in mitochondrial dysfunction.

Lipid peroxidation is an oxidative process in which lipid molecules undergo a series of chemical alterations initiated by free radical and oxygen (Kappus,1991).In this process in which molecular oxygen interacts with unsaturated membrane lipids (Cholesterol , Phospholipids) through a series of complex multistage reaction . For lipid peroxidtion to occur activation of lipid molecules and pro-oxidants are both necessary. This process can become autocatalytic after initiation and will yield lipid peroxide, lipid alcohol and aldehyde by products. In the present study in all muscle fiber lipid peroxidation was increased in the nicotine treated rats. Kavitharaj and Vijayammal (1999) reported chronic administration of nicotine was found to produce enhanced synthesis of lipid peroxidation in the tissues of rats.Our result also agreement with this. In the preset study administration of RGEt the levels was lipid peroxidation decreased (Table-4) in all the muscle fibers then the control, in the combination treatment (Nt+RGEt) the upregulation was observed due to nicotine toxicity.

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: Levels of total cholesterol in the skeletal muscle fibers (mean ±SD; n=6).Values are expressed in mg of total cholesterol /gm wt weight of tissue.

S.No	Name of the tissue	NC	Nt	RGEt	Nt + RGEt
1	SOL	3.29±0.19	3.95±0.09* (+20.06)	2.92±0.06* (-11.24)	3.36±0.06** (+2.12)
2	RV	2.86±0.10	3.71±0.04 * (+29.72)	2.44±0.07* (-14.68)	3.23±0.05** (+12.93)
3	WV	2.29±0.07	3.33±0.06 * (+45.41)	2.13±0.06* (-6.98)	3.09±0.07** (+34.93)

*Values are significant at $P < 0.001$,** Values are significant at $P < 0.01$

Table-2: Levels of triglycerides in the skeletal muscle fibers (mean ±SD; n=6).Values are expressed in mg of triglycerol/gm wt weight of tissue.

S.No	Name of the Tissue	NC	Nt	RGEt	Nt + RGEt
1	SOL	1.83±0.06	2.32±0.06 * (+26.77)	1.62±0.06 * (-11.47)	2.13±0.05** (+16.39)
2	RV	1.43±0.06	2.16±0.06* (+51.04)	1.36±0.06* (-4.89)	1.66±0.06** (+16.08)
3	WV	1.06±0.06	1.95±0.07* (+83.96)	0.96±0.05* (-9.43)	1.26±0.06** (+18.86)

*Values are significant at P < 0.001,** Values are significant at P < 0.01

Table-3: Levels of phospholipids in the skeletal muscle fibers (mean ±SD; n=6). Values are expressed in mg of triglycerol/gm wt weight of tissue.

S.No	Name of the Tissue	NC	Nt	RGEt	Nt + RGEt
1	SOL	2.46±0.06	2.26±0.06 * (-8.13)	2.89±0.05* (+17.47)	2.63±0.08** (+6.91)
2	RV	2.30±0.08	2.19±0.06* (-4.78)	2.70±0.07* (+17.39)	2.48±0.08** (+7.82)
3	WV	2.15±0.06	2.04±0.08* (-5.11)	2.43±0.08* (+13.02)	2.19±0.05** (+1.86)

* Values are significant at P < 0.001,** Values are significant at P < 0.01

Table-4: Changes in Lipidperoxidation in the skeletal muscle fibers (mean ±SD; n=6) .Values are expressed in nano moles of malondialdehyde/gm wt weight of tissue.

S.No	Name of the Tissue	NC	Nt	RGEt	Nt + RGEt
1	SOL	2.98±0.05	3.46±0.10* (+16.10)	2.69±0.06 * (-9.73)	3.32±0.06** (+11.40)
2	RV	2.41±0.05	2.94±0.06* (+21.99)	2.33±0.05 * (-3.31)	2.70±0.06** (+12.03)
3	WV	2.19±0.06	2.36±0.05* (+7.76)	2.13±0.05 * (-2.73)	2.25±0.06** (+2.73)

* Values are significant at P < 0.001,** Values are significant at P < 0.01

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)

Soleus (SOL) ,Red vastus(RV) and White vastus(WV).Total Cholesterol(TC), Triglycerides(TG), and Lipid Peraxidation(LPO)

REFERENCE

1. Kyerematen, GA,and Vesell ES. (1991). Metabolism of nicotine. *Drug Metab Rev*; 23:3–41.
2. Suleyman, H., Gumustekin, K., Taysi, S., Keles, S., Oztasan, N.,Aktas, O., *et al.*, (2002) Beneficial effects of Hippo phae rhamnoidesL. On nicotine induced oxidative stress in rat blood comparedwith vitamin E. *Biol Pharm Bull*; 25: 1133-6.
3. Zhang, J., Jiang, S., Watson, R.R., (2001). Antioxidant supplementation prevents oxidation and inflammatory responses induced by sidestream cigarette smoke in old mice. Environ Health Perspect; 109 : 1007-9.
4. Jacob, P. III., Benowitz, N.L.,Shulgin, A.T., (1988). Recent studies of nicotine metabolism in humans. *Pharmacol Biochem Behav*; 30:249–253.

5. Kyerematen, G.A., Owens, G.F., Chattopadhyay, B., deBethizy, J.D., Vesell, E.S., (1988). Sexual dimorphism of nicotine metabolism and distribution in the rat: studies in vivo and in vitro. *Drug Metab Dispos* ; 16:823–828.

6. Cashman, J.R., Park, S.B., Yang, Z.C.,Wrighton, S.A., Jacob, .P III., Benowitz, N.L., (1992).Metabolism of nicotine by human liver microsomes: stereoselective formation of. trans-nicotine N′-oxide. *Chem Res Toxicol*; 5:639–646.

7. Zargari, A., (1993) In: Medicine Plant. Tehran University Publication, Tehran;1: 362–365.

8. Montvale, N.J., (2002). PDR for Herbal Medicines, second ed. MedicalEconomics: 362–363.

9. Constantino, L., Albasini, A., Rastelli, G., Benvenuti, S., (1992). Activity of polyphenolic crude extracts as scavengersof superoxide radicals and inhibitors of xanthine oxidase.*Planta Med*; 58: 342-344.

10. Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M.B., Kromhout, D., (1993) Dietary antioxidantflavonoids and risk of coronary heart disease, the Zutphen elderly study. *Lancet*; 342: 1007-1011.

11. Miyara, M., Ohtaka ,H., Katayama, H., Tatsumi,Y., Miyaichi, Y., Tomimori, T., (1993). Structure-activity relationship of flavonoids in suppressing rat liver lipid peroxidation. Yukug. Zuss;113: 133-154.

12. Folts, J.D., (2002). Potential health benefits from the flavonoids in grape products on vascular disease. *Adv Exp Med Biol*; 505:95–111.

13. OByrne, D.J., Devaraj, S., Grundy, S.M., Jialal, I.,(2002). Comparison of antioxidant effects of Concord grape juice flavonoides and a-tocopherol on markers of oxidative stress in healthy adults. *Am J Clin Nutr*.; 76:1367–74.

14. Freedman, J.E., Parker, III. C., Li, L., Perlman, J.A., Frei, B., Ivanov, V., Deak, L.R., Iafrati, M.D., Folts, J.D., (2001).Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation; 103: 2792–98.

15. Folts, J.D. (2002).Potential health benefits from the flavonoids in grape products on vascular disease.*Adv Exp Med Biol*.; 505:95–111.

16. Zern, T.L., Wood, R.J., Greene, C., West, K.L., Liu, Y., Aggarwal, D., Shachter, N.S., Fernández, M.L., (2005).Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *J Nutr*.; 135:19.

17. Benowitz, N.L., Hansson, A., Jacob, P., (2002).Cardiovasculare ffects of nasal and transdermal nicotine and cigarette smoking. Hypertension; 3rd 39: 1107-12.

18. Pausova, Z., Paus, T., Sedova, L., Berube, J., (2003). Prenatalexposure to nicotine modifies kidney weight and blood pressure in genetically susceptible rats: a case of gene environment interaction. Kidney Int; 64: 829-35.

19. Valenca, S.S., de Souza da Fonseca, A., da Hora K, Santos, R., and Porto, L.C.,(2004). Lung morphometry and MMP-12 expression in rats treated with intraperitoneal nicotine. *Exp. Toxicol. Pathol*; 55: 393-400.

20. Nateson,S.,(1971). Triglycerides.In: Techniques of Clinical Chemistry. Charles,C.Thomas,Publi. Spring Field, Illinos (USA), 3rd edition; 720-723.

21. Zierelrsimidht, D.B., Daviz, A.K., (1950). Micro determination of plasma phospholipid by trichloroacetic acid precipition. *J. Lab. Clin. Med*; 35:155-159.

22. Hiroshi, O., Ohisi, N., Yagi, K., (1979).Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*; 95: 351-358.

23. Yamazaki, H., Inoue, K., Hashimoto, M., Shimada, T., (1999).Roles of CYPE2 A6 and CYP2B6 in nicotine C-oxidation by human by CTmicrosomes. *Arch Toxicol*; 73: 65-70.

24. Wetshcer, G. J., Bagchi, M., Bagchi, D., Perdikis, G., Hinder, P.R., Glaser, K., Hinder, R.A., (1995). Free radical production in nicotine-treated pancreatic tissue. *Free Radical Biol Med*; 18: 877-882.

25. Gurr ,M. , James, A.T., Lipid Biochemisrty. An Introduction University press, Combridge 1980.

26. Swami, K. S., Jagannadha Rao, K.S., Sathyavelu Reddy, K., Srinivasa Moorthi, G., Chetty, C.S., Indira, K., (1983).The possible metabolic diversions adapted by the fresh water muscle to counter the toxic metabolic effects of selected pesticides. *Ind. J. Comp.Anim*. Physiol; 1: 95-106.

27. Kavitharaj, N.K., Vijayammal, P.L., (1999). Nicotine administration induced changes in the gonadal functions in male rats. *Pharmacolog*; 58: 2–7. (2010).

28. Vinson, J.A., Teufel, K., Wu ,N., (2001). Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in hamster model. Atherosclerosis; 156: 67–72.

29. Del Bas, J.M., Fernandez-Larrea ,J., Blay, M., Ardevol, A., Salvado, M.J., Arola, L., Blade, C., (2005).Grape seed procyanidins improve atherosclerotic risk index and induce liver CYP7A1 and SHP expression in healthy rats. *FASEB J*; 19: 479–81.

30. Kurowska. E.M., Manthey, J.A., (2004).Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia. *J Agric Food Chem*; 52: 2879–86.

31. Naissides, M., Mamo, J.C.L., James, A.P., Pal, S., (2006). The effect of chronic consumption of red wine on cardiovascular disease risk factors in postmenopausal women. Atherosclerosis; 185:438–45.

32. Rice-Evans, C.A., Miller, N.J., Paganga, G., (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med*; 20:933–56.

33. Hayek, T., Furman, B., Vaya, J.R., Rosenblat, M., Belinky, P., Coleman, R., Elis, A., Aviram, M., (1997). Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler Thromb Vasc Biol; 17:2744–52.

34. Pal, S., Ho, N., Santos, C., Dubois, P., Mamo, J., Croft, K., Allister, E., (2003) Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of apoB100 from human HepG2 cells. *J Nutr*; 133: 700–6.

35. Holloszy, J.O., Booth, F.W., (1976). Biochemical adaptations to endurance exercise in muscle. *Annu. Rev. Physiol*; 38:273-291.

36. Palmer, W. K., (1983). Hormonal regulation of myocardial lipolysis. *Med. Sci. Sports. Exercise*; 15: 331-335.

37. Zierler ,K. L., (1976). Fattyacids as substrates for heart and skeletal muscle. *Circ. Res*; 38: 459-463.

38. Chattergia, M.N., Ranashinde, Arjuna, Y.N., Jaypee.(1993). In:text book of medical biochemistry , Brothders Medical Publishers Pvt. Lid,. New Delhi; 445-531.

39. Wiswedel, I., Gardemann, A., Storch, A., Peter, D., Schild, L., (2010). Degradation of phospholipids by oxidative stress--exceptional significance of cardiolipin. *Free Radic Res* ; 44(2):135-45 .

40. Kappus, H., (1991).Biochemical mechanisms of chemical - induced lipid peroxidation. In: Membrane lipid oxidation, Vig-Perfrey C, ed CRC press Boca Raton FL, II: 104.