



ROLE OF RED GRAPE EXTRACT AND NICOTINE ON ENERGY METABOLISM WITH REFERENCE TO AGING

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ABSTRACT

In the present study energy metabolism has been assessed in nicotine administered rats to examine the effects of nicotine on the energy metabolism defense systems in heart of male albino rat. Age matched rats were divided into 4 groups of six in each group and treated as follows: Group I, Normal Control (NC) (Control rats received 0.9% saline). Group II, Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months). Group III, Red grape extract treated (RGET). (Red grape extract at a doses of 25 mg/ kg body weight via orogastric tube for a period of 2 months). Group IV. Nicotine + Red grape extract treated (Nt+RGET) (The fourth group of rats were received the nicotine + red grape extract as followed by the second and third group). The energy metabolism profiles such as Mg²⁺-ATPas, Ca²⁺-ATPase, Creatine phosphokinase (CPK) were significantly decreased in nicotine treated rats in heart tissue and increase was observed in the combination treatment (Nt+RGET), This study suggests that red grape extract treatment may be beneficial for nicotine intoxications.

KEYWORDS: Nicotine, Red Grape Extract, Mg²⁺-ATPas, Ca²⁺-ATPase, Creatine phosphokinase (CPK), Heart 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 (Maier *et al.*, 2009) and the grape byproduct consists 20% of weight from winery process (Lafka *et al.*, 2007). In Thailand, grape is usually processed into various products such as wine, juice and raisins. Black queen is one of the grape varieties that is normally processed into wine and juice and the large quantity of byproducts from both processes such as pomace (grape pulp, peels and seeds) were obtained and there has been several studies showing that these kind of by products could be a good source of antioxidants such as polyphenols and flavonoids. Wine is considered to be a high bioactive polyphenol content source. Many studies have revealed the key role played by phenolic compounds from grapes and wine on human health; cardiovascular diseases being the pathologies that have received much attention (Pozo-Bayón *et al.*, 2012, Arranz *et al.*, 2012). Wine is a widely consumed beverage in the world, with thousands of years of tradition. The phenolic compounds in grape berries are responsible for some of the major organoleptic properties of wine, such as color, astringency, bitterness, and aroma

(Minussi *et al.*, 2003; Pérez-Magariño and González-Sanjosé, 2006). During the red winemaking process, phenolic compounds from the skins of red grapes transfer to the must during the fermentation and any maceration steps (Salas *et al.*, 2003). Based on their carbon skeleton, phenolic compounds are divided into two groups: flavonoid (anthocyanins, flavan-3-ols, flavonols) and non-flavonoid compounds (hydroxybenzoic and hydroxycinnamic acids, stilbenes). Different types of phenolic compounds endow grape varieties and wines with specific quality characteristics.

Nicotine is highly addictive (Grana *et al.*, 2014; Holbrook and Bradley, 2016). An average cigarette yields about 2 mg of absorbed nicotine and in lesser doses of that order, the substance acts as a stimulant in mammals, while high amounts (50–100 mg) can be harmful (Mayer, 2014). This stimulant effect is a contributing factor to the addictive properties of tobacco smoking. Nicotine's addictive nature includes psychoactive effects, drug-reinforced behavior, compulsive use, relapse after abstinence, physical dependence and tolerance (Caponnetto *et al.*, 2012). Nicotine is a natural ingredient acting as a botanical

insecticide in tobacco leaves. It is the principal tobacco alkaloid, occurring to the extent of about 1.5% by weight in commercial cigarette tobacco and comprising about 95% of the total alkaloid content. Oral snuff and pipe tobacco contain concentrations of nicotine similar to cigarette tobacco, whereas cigar and chewing tobacco have only about half the nicotine concentration of cigarette tobacco. An average tobacco rod contains 10–14 mg of nicotine (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).

Aging, an unwanted, unavoidable and universal biological phenomenon, is caused by time dependent progressive deleterious and irreversible changes occurring in cells, organs and in the total organism (Patel, 1981). Metabolic machinery of the body deteriorates at an increasing rate after the organism reaches its reproductive maturity (Shock, 1979). Aging may be described as a phenomenon which results from the accumulation of changes in informational biomolecules and is responsible for both the diminished bodily functions with advancing age and associated progressive increase in the chance of diseases and death (Harman, 1992; Masoro, 1993). Numerous definitions has been given by various scientists for aging. Hence this study was designed.

RESEARSH MEHTODS

Pathogen free, wistar strain male albino rats of two age groups (3 months and 18 months) 3 months age group

considered as ‘Young age’ and 18 months age group considered as ‘Old age’ as per the life span of Wistar strain, (Jang *et al.*, 2001) were used in the present study. The usage of animals was approved by the Institutional Animal Ethics Committee (Regd.No. 438/01/a/CPCSEA/dt.17-2-2001) in its resolution number 9/IAEC/SVU/Zool/dt.4-3-2002. The rats were housed in clean polypropylene cages under hygienic conditions with photoperiod of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai) and water *ad libitum*.

Age matched rats were divided into 4 groups of six in each groups. i) Narmal Control (NC) (Six rats were put on a six-channel, the rats were treated with normal saline (0.9%) orally via orogastric tube for a period of 2 months), ii) Nicotine treatment (Nt) (Rats were received the nicotine at a dose of 0.6 mg/kg body weight (0.5ml) by subcutaneous injection for a period of 2 months), iii) Red Grape extracts treatment (RGEt) (Rats were received red grape extract 25mg/kg body weight via orogastric tube for a period of 2 months) and iv) Nicotine + Red Grape extract treatment (Nt+RGEt), (Rats were received the nicotine at a dose of 0.6 mg/kg body weight (0.5ml) by subcutaneous injection and red grape extract 25mg/kg body weight via orogastric tube for a period of 2 months). The animals were sacrificed after 24 hrs after the last treatment session by cervical dislocation and the heart tissue, were isolated at -40, washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -800 for biochemical analysis and enzymatic assays. Before assay, the tissues were thawed, sliced and homogenized under ice-cold conditions. Selected parameters were estimated by employing standard methods.

BIOCHEMICAL ANALYSIS

ATPases: (ATP Phosphohydrolase) (E.C: 3.6.1.3)

Mg²⁺ - ATPase

ATPase activity was assayed by the method of Fritz and Hamrick (1966) as modified by Desai and Ho (1979). Tissue homogenates were prepared in ice cold 0.32 mM sucrose containing 1.0 mM EDTA and 10M imidazole (pH 7.5). The homogenates were centrifuged at 1000g for 15 minutes at 4⁰C and the supernatant obtained was used as the enzyme source. The reaction mixture in a volume of 3 ml contained 3 mM ATP, 3 mM MgCl₂, 100 mM NaCl, 20 mM KCl, 135mM imidazole hydrochloric acid buffer (pH 7.5) and 10-30 μgm of protein as enzyme source. The reaction mixture was incubated at 37⁰C for 30 minutes and the reaction was stopped by the addition of 0.1 ml of 50% TCA. Samples were then assayed for inorganic phosphate using the method of Fiske and Subba Row (1925). The colour was read at 660 nm in a spectrophotometer against the reagent blank. The Mg²⁺ - ATPase activity was measured in the presence of 1 mM ouabain, a specific inhibitor of Na⁺ K⁺ - ATPase (McIl Wain, 1963). Ouabain sensitive Na⁺K⁺ - ATPase activity was obtained by the difference between total ATPase and

Mg²⁺-ATPase activity. The enzyme activity was expressed as μ moles of inorganic phosphate formed /mg protein/hour.

Ca²⁺ – ATPase

Ca²⁺ – ATPase activity was determined by measuring the inorganic phosphate liberated during the hydrolysis of ATP. The reaction medium contained 135 mM imidazole-HCl buffer (pH 7.5), 5 mM MgCl₂, 0.05 mM CaCl₂, 4 mM ATP and 30-40 μ gm of protein. The mixture was incubated at 37°C for 30 minutes and the reaction was stopped by the addition of 0.1 ml of 50% TCA. The inorganic phosphate formed was estimated by the method of Fiske and Subba Row (1925). The colour was read at 660 nm against the blank in a spectrophotometer. Mg²⁺-ATPase activity was measured in the presence of 0.5 mM EDTA and this value was subtracted from total ATPase activity to get Ca²⁺-ATPase activity. Enzyme activity was expressed as μ moles of inorganic phosphate formed/mg protein/hour.

Creatine phosphokinase (CPK) (ATP creatine N-phosphotransferase: E.C:2.7.3.2)

Creatine phosphokinase activity was estimated by the method of Kuby *et.al.*, (1954), with slight modifications as given in the Sigma Technical Bulletin (1977) No.661. Ten percent homogenate of the muscle was prepared in ice cold distilled water and centrifuged at 1000g for 15 minutes. The supernatant fraction was used for enzyme

assay. The reaction mixture in a final volume of 2.4 ml contained 60 μ moles of creatine, 100 μ moles of tris buffer (pH 9.0), 0.3 ml of the homogenate supernatant and remaining quantity of distilled water. The contents were thoroughly mixed and the tubes were placed in a water bath in 37°C for a few minutes to warm up. The reaction was initiated by adding 5 μ moles of ATP and the contents were incubated for 30 minutes at 37°C.

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

An age-dependent decrease was noticed in the activities of heart tissue ATPases. The drop in the activity caused by aging was elevated by red grape extract in both young and old rat heart. However a decrease in the activity of Mg²⁺ and Ca²⁺ -ATPases due to nicotine treatment was observed, (Table.1 and 2). CPK was assayed in heart tissue and were expressed as μ moles of pi/mg protein/hour. The CPK was considerably decreased in heart tissue of old rats. The heart tissue of red grape extract treatment rats of two age groups showed an increase in the CPK activity as compared to age-matched controls. However, due to nicotine treatment the CPK activity was decreased in the present study (Table- 3).

Table-1: Changes in Mg²⁺-ATPase activity in Heart tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in units Mg²⁺-ATPase reduced/ mg proteins.

Name of the tissue	Young				Old			
	Control	RGEt	Nt	Nt+RGEt	Control	RGEt	Nt	Nt+RGEt
Heart	9.24 ±4.47	11.79* ±1.49 (+27.59)	8.61** ±1.94 (-6.81)	10.39 [@] ±1.83 (+12.44)	8.23 ±1.44	9.29* ±3.95 (+12.87)	7.43* ±1.97 (-9.72)	8.69 [@] ±2.30 (+5.58)

All the values are \pm SD of six individual observations.

Values in parentheses denote per cent change over respective sedentary control.

* Values are significant at P < 0.001

** Values are significant at P < 0.05

[@] Values are non significant.

Table-2: Changes in Ca²⁺ – ATPase activity in Heart tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in units Ca²⁺ – ATPase reduced/ mg proteins.

Name of the tissue	Young				Old			
	Control	RGEt	Nt	Nt+RGEt	Control	RGEt	Nt	Nt+RGEt
Heart	11.03 ±4.15	13.96** ±2.71 (-26.56)	11.53 [@] ±2.66 (+4.53)	15.73* ±3.79 (+42.61)	11.50 ±4.18	12.17 [@] ±2.56 (+5.82)	10.08 [@] ±3.40 (-12.34)	13.75* ±3.43 (+19.82)

All the values are \pm SD of six individual observations.

Values in parentheses denote per cent change over respective sedentary control.

* Values are significant at P < 0.001

** Values are significant at P < 0.05

[@] Values are non significant.

Table-3: Changes in Creatine phosphokinase (CPK) activity in Heart tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in units Changes in Creatine phosphokinase (CPK) reduced/ mg proteins.

Name of the tissue	Young				Old			
	Control	RGEt	Nt	Nt+RGEt	Control	RGEt	Nt	Nt+RGEt
Heart	19.17 ±3.92	23.88** ±4.60 (+24.56)	14.75* [@] ±5.36 (-23.05)	16.01* ±4.27 (-16.48)	17.17 ±3.95	17.54 [@] ±4.48 (+2.15)	13.77 [@] ±5.90 (-19.80)	15.47* ±4.39 (-9.90)

All the values are ± SD of six individual observations.

Values in parentheses denote per cent change over respective sedentary control.

* Values are significant at P < 0.001

** Values are significant at P < 0.05

[@] Values are non significant.

DISCUSSION

The decrease in the Mg²⁺ - ATPase during aging (Table1) may be attributed to reduced energy metabolism and free energy formation. Aging process reduces ATP synthesis by affecting TCA cycle oxidation and respiratory chain. In the similar studies Decrease in Mg²⁺ activated (Rockstein and Brandt, 1962) and Ca²⁺ activated ATPase activities in gastrocnemius muscle of old rats (Syrový and Gutmann, 1970) suggest more profound metabolic disturbances in the contractile machine. A decrease in oxidative enzyme activities and ATPase activities with a concomitant decrease in phosphorylase and glycogen content of the tissues in old age was reported by Talesara and Mohini (1978). They also reported a large decrease in the activities of all the enzymes in the aged rat heart tissue. Thus, the changes in the energy metabolism in heart tissue particularly with advancing age, may have an effect on its working capacity of the heart tissue ultimately. Decrease in the ATPase may be the result of age related decrease in number of contractile elements as revealed by low content of electrophoretically analysed myofibrillar proteins (Talesara and Rajni Arora, 1994).

In general the specific activities of Mg²⁺ - ATPase and Ca²⁺ -ATPase were elevated in both the heart tissue after red grape extract (Table.1and 2). In the similar studies the increase in Mg²⁺ and Ca²⁺ -ATPases implied stimulation of a series of energy consuming reactions in intermediary metabolism and increased transport of Mg²⁺ and Ca²⁺ across cell membranes. Increase in Mg²⁺ and Ca²⁺ -ATPases enhances resistance to fatigue of low frequency stimulated muscle prior to elevations in aerobic oxidative capacity (Green *et al.*, 1992). The increase in the specific activity of ATPases in general results in the hydrolysis of ATP which is utilized to overcome the energy demands during endurance red grape extract treatment. Oxygen levels in the tissue (i.e., the oxygen environment) can influence the concentrations of ATP in the heart tissue, particularly in animals that nicotine treatment. When the cells or tissues had oxygen deficits, however, ATP levels in the heart tissue were reduced much more dramatically in the nicotine-consuming than in the control animals. These findings indicate that nicotine consumption increases the sensitivity of heart cells to oxygen deficits, resulting in

decreased ATP concentrations in the cells. Excessive nicotine can have profound negative effect on heart tissue and their functions in maintaining the electrolyte balance. Nicotine has been shown to reduce ionic transfer through alterations in the monovalent cation pump and the antiport system. The combination treatment upregulates the ATPases in both the heart tissue clearly indicates set right conditions of energy metabolism with the influence of red grape extract treatment.

The enzyme CPK catalyses reversible rephosphorylation of ADP by phosphocreatine to form ATP and creatine. The CPK activity was decreased in the heart tissue of old rats when compared to young ones. The clinical biochemistry of neuromuscular disease concerns mainly with serum enzymes originating from heart tissue. In the similar study amongst the serum enzymes, CPK has proved to be the most valuable and useful diagnostic tool for the detection of muscle damage, since it is generally considered highly sensitive and relatively specific to muscle (Dioszeghy, 1992). In the present investigation CPK activity was decreased in both the heart tissue during aging which may be due to leakage of the enzyme from the heart tissue into serum as a consequence of heart tissue damage or loss of heart tissue mass or due to its decreased synthesis with advancement of age.

High ammonia concentration (Banister *et al.*, 1985), pH decline (Hogan and Welch, 1984; Jones *et al.*, 1985), rapid ATP hydrolysis (Green *et al.*, 1983; Dudley and Terjung, 1985), the altered intra cellular metabolism and changes in the permeability of the membranes are also responsible for low CPK activity in heart tissue of old rats. CPK has been shown to be sensitive to the levels of ADP (Bessman and Fanyo, 1966). Hence, it is presumed that elevated ADP levels inactivate CPK in the senile heart tissue. The decreased phosphorylation of creatine may also be one of the causes for the low CPK activity in heart tissue during aging. In order to understand the metabolic efficiency in terms of ATP hydrolysis and its replenishment, CPK activity levels were estimated after endurance red grape extract treatment. The elevated CPK activity of heart tissue by red grape extract treatment in the present study suggests utilization of CPK to rephosphorylate ADP to reimburse the depleted ATP

levels. The ATP so formed is utilized for the red grape extract contraction. The combined treatment of nicotine and red grape extract treatment and also due to nicotine treatment alone, the CPK activity decreased in both heart tissue, which elucidates the possible inhibition of CPK enzyme by the nicotine metabolic products, thereby the ATP turnover is decreased under nicotine induced stress condition. In the present investigation the impact of interaction of red rape extract treatment and nicotine on energy metabolism has been studied in selected heart tissue with reference to aging and enzyme systems of energy metabolism by taking male albino rat as an experimental model.

Aging is the sum total of changes during an individual's life span which are common to all members of the species. If energy metabolism is infarct the causative factors responsible for senescence, the maintenance of energy metabolism would minimize damage to physiological system and consequently the process of aging could be delayed. Red grape extract treatment enhances the ability to release energy by effective utilization of various metabolic fuels including stored ones, due to improved energy metabolism. The red grape extract treatment was selected for the study because it is exclusively used to evaluate the physiology of red grape. The advantage of red grape over other types is, it brings about a greater involvement of heart tissue mass which leads to higher maximum aerobic capacity. The survey of literature revealed that the reports on the effect of red grape, nicotine treatment and aging rats in functionally the heart tissue are limited. Hence, the present investigation was programmed to elucidate the adaptive changes if any, induced by red grape (RGEt), nicotine treatment (Nt) and both RGEt +Nt on energy metabolism and the fate of some products of energy metabolism in aging rat heart tissue.

CONCLUSION

In the present study all the energy metabolism (Mg^{2+} , Ca^{2+} and Creatine phosphokinase (CPK)), the upregulation was found with the response of combination (Nt+RGEt) in both age groups of rats. The present study suggesting that RGEt may help to develop a resistance in the heart to cope with nicotine induced maintains the antioxidant system.

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