AGING METABOLIC CHANGES IN THE MALE ALBINO RAT IN THE KIDNEY TISSUE WITH REFERENCE TO RED GRAPE EXTRACT ON NICOTINE SUBJECTS

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ABSTRACT

Grape (Vitis vinifera L.) presumably originates from Western Asia, from the area between the Caspian Sea region and Asia Minor, where also its cultivation seems to have begun. Red grape is commercial juice products from Concord grapes have been applied in medical research studies, showing potential benefits against the diseases. Nicotine is most biologically active chemical in tobacco smoke. Nicotine has been reported to induce changes both in vivo and in vitro. 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 50mg/kg body weight (after the standardization) via orogastric tube for a period of 2 months); IV) Nicotine + Red Grape (Nt+RGEt) (Rats were received the nicotine with a dose of mentioned for Group II through subcutaneous injection and, red grape extract as mentioned for Group III via orogastric tube for a period of 2 months). The animals were sacrificed after 24 hrs after the last treatment by cervical dislocation and isolated the kidney tissue estimated the activities the levels of Mg2+ - ATPase, Ca2+ – ATPase and Creatine Phosphokinase, (CPK) was decreased in nicotine treated rats in the kidney tissue, increase was observed in the combination (Nt+RGEt), but at 50 mg/kg body weight found to be more effective. This results more improve the energy metabolism.

Key words: Nicotine, Red Grape extract, Mg2+ - ATPase, Ca2+ – ATPase, Creatine Phosphokinase (CPK), Kidney, Male albino rat.

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INTRODUCTION

Nicotine is a naturally occurring alkaloid found primarily in the members of the Solanaceae family, which includes tobacco, potato, tomato, green pepper, and eggplant. Nicotine was first isolated and determined to be the major constituent of tobacco in 1828 (Schevelbein, 1982). In commercial tobaccos, the major alkaloid is nicotine, accounting for about 95% of the total alkaloid content (Jacob et al., 1993). Tobacco use is the leading cause of death in the world today. With 4.9 million tobacco-related deaths per year, no other consumer product is as dangerous or kills as many people as tobacco (WHO An international treaty for tobacco control, 2003).

Nicotine, as most biologically active chemical in tobacco smoke, has been the subject of intense scientific scrutiny. Among the most well characterized chemicals found in tobacco and tobacco smoke, are polycyclic aromatic hydrocarbons (PAHs) and the highly addictive alkaloid, nicotine and its metabolites (Campain, 2004). To further complicate the picture, nicotine is converted, during the production of cigarette and chewing tobacco, into two highly mutagenic nitrosamine, N-nitrosonor nicotin (NNN) and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) and is metabolized into cotinine. These chemicals derivatives also exhibit a wide spectrum of biological activity as compared to parent compound (Campain, 2004). Nicotine has been reported to induce oxidative stress both in vivo and in vitro (Pigeolot et al., 1990). The mechanism of generation of free radicals by nicotine is not clear. But oxidative stress occurs when there are excess free radicals and/or low antioxidant defense, and result in chemical alteration of biomolecules causing structural and functional modification. Oxygen free radicals (OFR) production has been directly linked to oxidation of cellular macromolecules, which may induce a variety of cellular responses through generation of secondary metabolic reactive species (Chiarugi, 2003). Medicinal plants and their active principles have received greater attention as anti-peroxidative agent (Lee and Park, 2002).
Grapes (*Vitis vinifera*) are one of the world’s widely grown fruit crops with an annual production of 58-61 million metric tons (Murthy *et al.*, 2002). Grape growing plays a major role in the worldwide fruit production, with an international acreage of approximately 7.8 million hectares (Oiv 2008). In the 1980s, there was a high rate of global production of fresh grapes, but due to a reduction in the production surface area, there was a drop in the beginning of the 1990s. However, soon after, the production rate plummeted, due to an increase in output trends, favorable climatic conditions and increase in the partial geographical redistribution of vineyards during this period. America saw its 2005 production reach a record high with 142, 6 Mqx (millions of quintals) (Cantos *et al.*, 2002). Anthocyanins tend to be the main polyphenolics in red grapes whereas flavan-3-ols (e.g., catchiness) are the more abundant phenolic in white varieties (Gross, 2007). Total phenolic content, an index of dietary antioxidant strength, is higher in red varieties due almost entirely to anthocyanin density in red grape skin compared to absence of anthocyanins in white grape skin. It is these anthocyanins that are attracting the efforts of scientists to define their properties for human health (Cantos *et al.*, 2002). Phenolic content of grape skin varies with cultivar, soil composition, climate, geographic origin, and cultivation practices or exposure to diseases, such as fungal infections. This study was designed to investigate the effects of red grape extracts on nicotine induced oxidative stress in the kidney of male albino rat.

Aging is associated with spontaneous degenerative changes of renal function and structure. Renal aging in humans and rodents is associated with a spontaneous and progressive decline of kidney function and structural changes and cortex (Thomas Lattmann *et al.*, 2005). Aging is associated with a degenerative effect on many organs including the kidney. Blood vessels play a key role in the progression of renal damage in aging, with reductions in glomerular filtration rate and renal blood flow. Aging is a natural process that occurs in all species and has a degenerative effect on many organs. Interestingly for nephrologists, one of the most prominent organs affected by aging is the kidney. Aging can cause histological, functional and molecular changes in the Kidney (David Long *et al.*, 2005). Aging is associated with a loss of renal reserve; and increased either xenobiotic or physiologic insult (Kl-Yoon Jung *et al.*, 2004). This work carried out aging changes in the kidney tissue.
MEHTODOLOGY

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.

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 GGRAPE EXTRACTION:

Red Grapes, as large clusters with red berries, were brought from a local supermarket in Bangalore and identified as *Vitis vinifera* L. (Family *Vitaceae*). The grape were crushed (whole fruit) for juice and dried in shade and extract by maceration with 70% (W/V) alcoholic for 72 h 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 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 orogastic tube for 5 days / week 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 50mg/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 50mg/kg body weight via orogastric tube for a period of 2 months).

The animals were sacrificed after 24 hrs after the last treatment session by cervical dislocation and the kidney tissue, were isolated at -40°C, washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80°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 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 Desaiah 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 4°C 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 37°C 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 (Mcll 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 µmoles of inorganic phosphate formed /mg protein/hour.

**2. 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 µgm 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. Mg$^{2+}$-ATPase activity was measured in the presence of 0.5 mM EDTA and this value was subtracted from total ATPase activity to get Ca$^{2+}$-ATPase activity. Enzyme activity was expressed as $\mu$moles of inorganic phosphate formed/mg protein/hour.

**3. 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 $\mu$moles of creatine, 100 $\mu$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$^\circ$C for a few minutes to warm up. The reaction was initiated by adding 5 $\mu$ moles of ATP and the contents were incubated for 30 minutes at 37$^\circ$C.

The reaction was arrested by the addition of 1.6 ml of ice cold 20% (W/V) TCA and centrifuged. The inorganic phosphate formed was estimated by the method of Fiske and Subba Row (1925). To the supernatant 4.0 ml of distilled water, 1.0 ml of acid molybdate solution was added followed by the addition of 0.25 ml of amino-naptho sulphonic acid (ANSA). The contents were mixed well and allowed to stand for 30 minutes at room temperature to hydrolyse phosphocreatine and the colour developed was read at 660 nm in a spectrophotometer against the reagent blank. CPK activity was expressed as $\mu$moles of inorganic phosphate liberated/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:

In the present study the, \(Mg^{2+}\)- ATPase activity was decreased in both (young and old) nicotine treatment rats (young by -11.21%; old by -19.49%) 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 13.01%; old by -7.20%). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups. (Table-1). In this investigation the, \(Ca^{2+}\)- ATPase, activity was decreased in both (young and old) nicotine treatment rats (young by -6.74%; old by -16.22%) 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 7.22%; old by 7.85%). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups. (Table-2).

In our knowledge the, Creatine Phosphokinase, activity was decreased in both (young and old) nicotine treatment rats (young by -31.05%; old by -21.75%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased was observed when compared to the control rats (young by 5.59%; old by 2.27%). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups. (Table-3).

DISCUSSION:

The decrease in the \(Mg^{2+}\)- ATPase during aging (Table.1) may be attributed to reduced oxidative metabolism and free energy formation. Aging process reduces ATP synthesis by affecting TCA cycle oxidation and respiratory chain. Decrease in \(Mg^{2+}\) activated (Rockstein and Brandt, 1962) and \(Ca^{2+}\) activated ATPase activities in gastrocnemius muscle of old rats (Syrovy 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 kidney. Thus, the changes in the energy metabolism in kidney tissue particularly with advancing age, may have an effect on its working capacity of the kidney 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 Mg\(^{2+}\) - ATPase and Ca\(^{2+}\) - ATPase were elevated in both the kidney tissue after acute exercise (Table.1, 2). The increase in Mg\(^{2+}\) and Ca\(^{2+}\) - ATPases implied stimulation of a series of energy consuming reactions in intermediary metabolism and increased transport of Mg\(^{2+}\) and Ca\(^{2+}\) across cell membranes. Increase in Mg\(^{2+}\) and Ca\(^{2+}\) - 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 tissues (i.e., the oxygen environment) can influence the concentrations of ATP in the kidney tissue, particularly in animals that chronically consume nicotine. After a period of oxygen deficiency, the muscles of alcohol-treated animals showed greater reductions in ATP levels than did those of sedentary control animals. These findings indicate that nicotine consumption increases the sensitivity of kidney tissue cells to oxygen deficits, resulting in decreased ATP concentrations in the cells.

The enzyme CPK catalyses reversible rephosphorylation of ADP by phosphocreatine to form ATP and creatine. The CPK activity was decreased in the CPK was assayed in kidney tissue and were expressed as \(\mu\) moles of pi/mg protein/hour. The CPK was considerably decreased in both kidney tissue of old rats. The kidney tissue of red grape extracted rats of two age groups showed an increase in the CPK activity as compared to controls. However, due to nicotine treatment the CPK activity was decreased in the present study (Table.3), of old rats when compared to young ones. A decrease in CPK activity was reported in several pathological conditions of muscles (Murray and Hoffmann, 1990).

The clinical biochemistry of neuromuscular disease concerns mainly with serum enzymes originating from kidney tissue. 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 kidney tissue during aging which may be
due to leakage of the enzyme from the kidney tissue into serum as a consequence of kidney tissue damage or loss of kidney 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 kidney 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 kidney tissue. The decreased phosphorylation of creatine may also be one of the causes for the low CPK activity in kidney 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 training. Creatine phosphate is the main source of energy in muscle to supplement the depleted ATP levels during sustained contractions (Martin et al., 1985). The elevated CPK activity of kidney tissue by red grape extract 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 kidney tissue contraction. The increased CPK activity shows improved functional efficiency of the kidney tissue after red grape extract. Yamashita and Yoshitada (1992) also reported a significant increase in two isozymes of CPK, CPK-MB and CPK-BB in kidney tissue in rats after red grape extract. The combined treatment of nicotine and red grape extract and also due to nicotine treatment alone, the CPK activity decreased in both kidney 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.  

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.  

ACKNOWLEDGEMENTS  

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Table-1: Changes in \( \text{Mg}^{2+} - \text{ATPase} \) 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 Kidney tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in mg/gram wet weight of the tissue.

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Young</th>
<th></th>
<th>Old</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nt</td>
<td>RGEt</td>
<td>Nt+RGEt</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.91 ±6.40</td>
<td>12.30** ±4.86 (-11.21)</td>
<td>15.72** ±3.81 (+13.01)</td>
<td>16.21* ±4.74 (+16.21)</td>
</tr>
</tbody>
</table>

All the values are ± SD of six individual observations. Values in parentheses denote per cent change over respective control.

* Values are significant at \( P < 0.05 \)

** Values are significant at \( P < 0.01 \)

@ Values are non significant.

Table-2: Changes in \( \text{Ca}^{2+} - \text{ATPase} \) 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 Kidney tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in mg/gram wet weight of the tissue.

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Young</th>
<th></th>
<th>Old</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nt</td>
<td>RGEt</td>
<td>Nt+RGEt</td>
</tr>
<tr>
<td>Kidney</td>
<td>12.60 ±4.95</td>
<td>11.75** ±2.38 (-6.74)</td>
<td>13.51** ±2.34 (+7.22)</td>
<td>14.57** ±3.05 (+15.63)</td>
</tr>
</tbody>
</table>

All the values are ± SD of six individual observations. Values in parentheses denote per cent change over respective control.

** Values are significant at \( P < 0.01 \)

@ Values are non significant.
Table 3: Changes in Creatine Phosphokinase 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 Kidney tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in mg/gram wet weight of the tissue.

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nt</td>
</tr>
<tr>
<td>Kidney</td>
<td>17.87±2.77</td>
<td>12.32*±1.93</td>
</tr>
<tr>
<td></td>
<td>(31.05)</td>
<td>(+5.59)</td>
</tr>
</tbody>
</table>

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at P < 0.05
** Values are significant at P < 0.01
@ Values are non significant.

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 5 days / week for a period of 2 months). 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). Red Grape extracts treatment (RGEt) (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 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 50mg/kg body weight via orogastric tube for a period of 2 months).

Kindy Tissue (Yong and Old age), of Mg²⁺ - ATPase , Ca²⁺ – ATPase and Creatine Phosphokinase, (CPK).

REFERENCES


