MODULATIONS IN THE CARBOHYDRATE METABOLISM BY NICOTINE AND RED GRAPE EXTRACT IN THE LIVER TISSUE OF MALE ALBINO RAT WITH REFERENCE TO AGING

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ABSTRACT

The grape (Vitis vinifera) has been well recognized worldwide for over 2,000 years as one among the edible sweet fruits and recognized for its wide spectrum of biological properties. In vitro studies showed that red grape has significant antioxidant activity, antimicrobial, anti-inflammatory action and protection against hepatic damage. Tobacco plants that have been domesticated and used to obtain the alkaloid nicotine. Nicotine also induces oxidative stress both in vivo and in vitro that causes a peroxidant/antioxidant imbalance in tissues. 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 treatment at a doses of 50 mg/ kg body weight via orogastric tube for a period of 2 months). Group IV. Nicotine treated + 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 liver tissue such as the activities of the levels of Total Carbohydrates, Glycogen and Total free amino acids, were significantly decreased in nicotine treated rats in the liver tissue and enhance was observed in the combination treatment (Nt+RGEt), but Red grape extract treatment at a dose of 50 mg/kg body weight found to be more effective. This results stating that red grape extract treated rats were beneficial, especially for the nicotine subjects to improve the carbohydrate metabolic profile.

Key words: Nicotine, Red Grape Extract, Total Carbohydrates, Glycogen, Total free amino acids, Liver tissue and Male albino rats.

INTRODUCTION

Grape (Vitis vinifera L.) is one of the world’s largest fruit crops (Shaker, 2006). With nearly 70 million tones currently produced worldwide grapes are possibly the world’s largest cultivated fruit, most of which are used in wine making, while the rest are consumed as table grapes or processed into raisins, juices, jams or other products, of all grapes, cultivars of the Vitis vini-fera L. species are the most important throughout world, but especially in Europe (Mazza, 1995). There are dozens of other less important species of grapes that belong to Vitis genus (Chalker-Scott, 1999; Zhao, et al., 2010). Several epidemiological studies have indicated that regular intake of red wine, vegetables, fruit, and green tea, are associated with a decreased global mortality due to a reduced number of cancer and coronary diseases (Hertog et al., 1993; Renaud et al., 1992). The protective effect
has been attributable, at least in part, to polyphenols (Hertog et al., 1995; Knekt et al., 1996). There is extensive epidemiological evidence suggesting that red grapes having antioxidant activity, antimicrobial, anti-inflammatory action and protection against hepatic damage and anti cancer.

Nicotine is a naturally occurring alkaloid found in the nightshade family (Solanaceae) of plants, predominantly in tobacco plant (Nicotiana tabaccum) (Wu et al., 2002). Hellermann et al., (2002). Tobacco generally refers to the leaves and other parts of plants that have been domesticated and used to obtain the alkaloid nicotine. There are 64 Nicotiana species; the two are only cultivated for tobacco are Nicotiana tabaccum and Nicotiana rustica, these two are containing higher levels of nicotine. Nicotiana tabaccum is the major source of commercial tobacco. In most mammalian species, nicotine is rapidly and extensively metabolized, primarily in the liver (Kyerematen and Vesell, 1991).

The major metabolic pathways of nicotine in mammals are C-oxidation and N-oxidation, i.e. cotinine and nicotine N′-oxide formation, respectively. Cotinine formation from nicotine is a two-step reaction in mammals. The first step is the conversion of nicotine to nicotine-Δ1(5′)-iminium ion by CYP. The second step, the conversion of the iminium ion to cotinine, is mediated by cytosolic aldehyde oxidase (Kyerematen and Vesell, 1991). Other pathways of nicotine metabolism include glucuronidation and N-demethylation, though these may only play a small role in humans (< 5%, Hukkanen et al., 2005) and rats (Crooks et al., 1997; Ghosheh and Hawes, 2002). Some of nicotine’s 12 metabolites may have pharmacological activities of their own, e.g., nornicotine (Bardo et al., 1999b; Green et al., 2000). Cotinine and other nicotine metabolites have longer half-lives than nicotine and more readily accumulate in plasma and brain (Sastry et al., 1995), possibly resulting in extended pharmacological actions of smoking.

Liver is an important organ that has many tasks, and is responsible for processing drugs, alcohol and other toxins to remove them from the body. Nicotine from heavy smoking increases the risk of developing hepatocellular carcinoma (HCC), (El –Zayadi, 2006). Smoking increases the production of pro-inflammatory cytokines involved in liver cell injury (Moszczynski et al., 2001). It has been reported that smoking increases fibrosis score and histological activity index in chronic hepatitis C (CHC) patients (Pessione et al., 2001). El-Zayadi et al., (2002) have reported an association between heavy smoking and liver cell injury in the form of necroinflammation, apoptosis and excess iron deposition in the liver. These effects are attributed to iron overload with consequent iron deposition in hepatocytes (El-Zayadi et al., 2002; Bonkovsky et al., 1997). Like other organs of the body, liver structure and functions are also altered with age advances. Nevertheless, an age related decrease in the expression of several genes involved in mitochondrial bioenergetics and mitochondrial biogenesis occurs in aging mice, the largest age associated alteration affects the matrix enzyme Lon protease (Lee et al., 1999). Old age is associated with the accumulation of the aging pigment lipofusion, which consists of the end products of lipid peroxidation in lysosomes (Tauchi and Sato, 1978) and does not appear to influence hepatic function (Schmucker, 2001).

**MATERIALS AND METHODS**

**Animals:**
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 male albino rats (Jang et al., 2001) were used in the present study. The usage of animals was approved by the Institutional Animal Ethics Committee (No:19/2012-2013/(i)/a/CPCSEA/IAEC/SVU/KC/SVU/KC/RSS dt.01.07.2012).

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.
Dosage of nicotine:
The dose administration of nicotine was followed as per the protocol given by (Shoaib and Stolerman, 1999; Helen et al., 2003) 0.6 mg / kg body weight (0.5ml) was chosen as the dose, for this study.

Procurement of chemicals:
All the chemicals used in the present study were Analytical grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Red Grape Collection and Extraction
Red Grapes, as large clusters with red berries, were brought from local surroundings in Bangalore and identified as Vitis vinifera L. (Family Vitaceae). The grape were crushed (whole fruit) for juice and dried in shade, powdered and extract by maceration with 70% (v/v) alcoholic for 72 hours 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.

Treatment schedule:
Age matched rats divided into 4 groups of six in each group and treated as follows:
Group: i) Control rats (Rats received 0.9% saline). Group:ii) Nicotine treatment(Nt) (Rats were received the nicotine at a dose of 0.6 mg/kg body weight by subcutaneous injection for a period of 2 months). Group:iii) Red Grape Extract treatment(RGET) (Rats were received red grape extract 50mg/kg body weight via orogastric tube for a period of 2 months). Group:iv) Nicotine + Red grape extract(Nt+RGET) (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 hours after the last treatment session by cervical dislocation and the skeletal muscle fibers were be isolated at 4°C, washed with ice-cold saline, immediately immersed in liquid nitrogen and

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Figure-1. Major Pathways of nicotine metabolism (Source: Perterson, L.A et al, 1987)
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 Investigation:**
In the present study Corbohydrate metabolic profiles such as Total Carbohydrates, Glycogen, Total Free Amino acids were analyzed. Total Carbohydrates was measured by the method of Carroll et al., (1956). The total carbohydrate content was expressed as mg of glucose/gm wet weight of the tissue. Glycogen was estimated by the method of Kemp and Van Heijnigen (1954). The glycogen content was expressed in mg of glucose/gram wet weight of the tissue. The total free amino acids were estimated by the method of Moore and Stein (1954). The total free amino acid content was expressed in mg of free amino acids per gram wet weight of the tissue.

**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-value

**RESULTS AND DISCUSSION**

**Total Carbohydrates:**
In the present study the total carbohydrates content was significantly decreased in both age groups (young and old) of nicotine treated rats (young by -18.91 %; old by -13.64 %) when compared to the control rats. In Red Grape Extract treated rats of both age groups (young and old) significantly an increase was observed when compared to the control rats, (young by 10.27 %; old by 7.82 %). In the combination treatment (Nt+RGEt) non significantly increase was observed when compared to the control rats of both age groups. (Table.1)

In the present investigation it was observed that the age induced slight elevation in total carbohydrate content in the liver, which may be due to decreased metabolic utilization in the old animals. The impaired alterations in the activities of enzymes involved in the carbohydrate metabolism contribute to the reduction of carbohydrate catabolism and elevation in age-related accumulation of tissue carbohydrates. The age-related slowing down and impairment in carbohydrate metabolism appears to play a role in the expression of cellular senescence (Tollefsbol, 1987). The decrease in total carbohydrate levels in the liver of both young and old rats after nicotine treatment, suggest possible utilization of carbohydrates to meet the energy demand during nicotine toxicity. Nicotine produces stress in the body both in vivo in vitro (suleyman et al., 2002). Barry and Mizock (1995) reported stress causes to the alteration in the carbohydrate metabolism. These alterations include enhanced peripheral glucose uptake and utilization, hyperlactatemia, increased glucose production, depressed glycogenesis, glucose intolerance, and insulin resistance. The hyper-metabolic state is induced by the area of infection or injury as well as by organs involved in the immunologic response to stress; it generates a glycemic milieu that is directed toward satisfying an obligatory requirement for glucose as an energy substrate.

The ability to metabolize carbohydrates is reduced with advancement of 52-age. An age related decrease in respiratory activity and metabolic utilization of carbohydrates has been observed in heart tissue slices (Bilwanath, 1996). Kidney, liver and muscle (Sailaja, 1997; Gurumurthy, 2001). Enzymes of Kreb's-citric acid cycle show diminished activities with age (Ermini, 1972). Cartee et al.,(1993) reported decreased activity levels of glucose-6-phosphate dehydrogenase and glucose-6-phosphofructokinase with advancement of age. Glycolytic enzymes, pentose phosphate shunt enzymes are decreased with age. Young rats can more readily maintain high levels of oxygen consumption accompanied by a more efficient use of fats, carbohydrates as an energy source compared to old ones (Somani et al., 1992). The decreased glycolytic and Kreb's-citric cycle enzymes which are necessary for the catabolic process of carbohydrates, may lead to increase the total carbohydrate content in the liver of old age rat.
Several authors have been reported decreased total carbohydrate in afferent tissue with reference to different treatments. Subramanyam, (1984) reported decreased total carbohydrate content in different tissues with acetaldehyde toxicity. The decreased levels of total carbohydrates suggest the greater utilization of the substrate which is known to energy demand (Heilmayer et al., 1970). It also explains a biochemical situation where in much of the metabolic functions of glycolysis carbohydrate interconversions would be high. This leads to the greater availability of transporting monosaccharides which are the immediate source, for energy supply. The decrease in the total carbohydrates under nicotine treatment clearly suggests that the substrates derived from the total carbohydrates constitute the important functional role in the supply of energy. Similar sort of results were obtained under several stress conditions (Nihira, 1982; Kabeer Ahammad et al., 1978) and Linda and Charles, (1983). Another factor which may be responsible for the depleted levels of total carbohydrates was the inhibition of glycogen synthesis as reported by

### Table 1: Changes in Total Carbohydrates content 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 Liver 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 NC</th>
<th>Nt</th>
<th>RGEt</th>
<th>Nt+RGEt</th>
<th>Old NC</th>
<th>Nt</th>
<th>RGEt</th>
<th>Nt+RGEt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>101.57±5.30</td>
<td>82.36**±5.13</td>
<td>112.01**±4.72</td>
<td>104.77**±5.70</td>
<td>118.77±7.54</td>
<td>81.19**±6.04</td>
<td>128.06**±5.74</td>
<td>119.72**±7.54</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 Glycogen content 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 Liver 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 NC</th>
<th>Nt</th>
<th>RGEt</th>
<th>Nt+RGEt</th>
<th>Old NC</th>
<th>Nt</th>
<th>RGEt</th>
<th>Nt+RGEt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>135.69±3.20</td>
<td>99.51**±4.81</td>
<td>138.65**±7.91</td>
<td>136.74**±11.59</td>
<td>117.23±5.27</td>
<td>97.32**±3.81</td>
<td>129.65**±3.93</td>
<td>119.42**±7.41</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.
decreased gluconeogenesis in liver (Prasanna and Ramakrishnan, 1983).

We observed in the present study the induced effect of Red Grape Extract treatment increase the total carbohydrates content in both age groups at the same time decrease was observed in the nicotine treated rats when compare to control rats. Interestingly, in the present investigation with combination treatment an increase in the levels of total carbohydrate content was found in the liver of both age groups of rats. Thus, these results clearly suggest that, the beneficial role of Red Grape Extract treatment under nicotine induced subjects.

**Glycogen:**
In the present study the glycogen content was significantly decreased in both age groups (young and old) of nicotine treated rats (young by -26.66 %; old by -16.98 %) when compared to the control rats. In Red Grape Extract treated rats of both age groups (young and old) significantly an increase was observed when compared to the control rats (young by 2.18%; old by 10.59%). In the combination treatment (Nt+RGEt) non significantly increase was observed when compared to the control rats of both age groups. (Table.2).

From the present investigation it was observed that the liver glycogen levels were decreased in the nicotine treatment in both age groups. Several authors have been reported decreased glycogen content in afferent tissues with reference to different toxic conditions. Vijayakumar Reddy, (1990) reported decreased glycogen levels in the liver, muscle, and kidney under guanidine toxicity. Hariprasad, (1996) observed decrease Glycogen content in fish with ammonium toxicity. Decrement in tissue glycogen levels has been reported during ammonia stress (Santhi 1991; Nadhamuni Cherry, 1992 and Obula Reddy, 1994). The decreased glycogen content in the liver tissue with nicotine treatment rats observed in the present study indicates its greater metabolic utilization possibly to meet higher energy demands to mitigate nicotine toxicity (or) decreased rate of its synthesis. This could be accomplished either through glycolysis (or) the alternative pathway namely the Hexose Monophosphate Pathway (HMP). The decrease in glycogen level in the liver tissue in the present study indicate the possibility of active glycogenolysis (or) the depletion in the glycogen content might be due to the activation of glucokinase (or) inactivation of the enzymes involved in glycogen synthesis.

In our present findings, it is observed that the glycogen content was more in RGEt rats in the liver of both age groups when compared to control rats. Red Grape literature is not available regarding glycogen in this matter. However other evidence indicates that Shibib et al., (1993) reported *Momordica charantia* (Bitter Milon, Family of Cucurbitaceae ) fruit juice increase the hepatic glycogen synthesis and decreases the hepatic gluconeogenesis. So that in our studies glycogen content was increased may be due to up regulation of glycogen metabolism by the Red Grape Extract treatment rats.

From the present investigation it was observed that the liver glycogen levels decreased due to aging (Table; 2). The decrease in the glycogen content with advancement of age may be due to augmented glycogen degradation, through glycolysis or due to decreased in the synthesis of glycogen during aging. Takahashi et al., (1970) reported reduction in glycogen levels with advancement of age. The decrease in glycogen (Prahota and Gutmann, 1963), ATP (Frubel Osipova, 1969), ATP/ADP ratio (Ermini et al.,1971) and Creatine phosphate (Ermini, 1970) levels with advancement of age. The decreased mitochondrial oxidation revealed by decreased activity of ICDH, SDH and MDH clearly indicates the prevalence of hypoxic conditions in the tissues, which normally increases glycogen utilization. In the present investigation elevated glycogen content levels were observed in the liver of both age groups in the combination treatment (Nt+RGEt), suggesting Red Grape Extract treatment may beneficial for the nicotine subject to improve the glycogen content under induced nicotine conditions.
Total Free Amino acids:
In the present study in total free amino acids content was significantly decreased in both age groups (young and old) of nicotine treated rats (young by -52.08 %; old by -36.42 %) when compared to control rats. In Red Grape Extract treated rats of both age groups (young and old) significantly an increase was observed when compared to the control rats (young by 16.73 %; old by 29.04 %). In the combination treatment (Nt+RGEt) non significantly increase was observed when compared to the control rats of both age groups. (Table. 3).

In the present investigation more amount of free amino acids (FAA) were found in the liver of young age group compared to old age group. Obled and Arnal, (1991) suggested that, with advancement of age protein synthesis was decreased and FAA concentration was increased. In general, we can conclude that the age by including tissue proteolysis, elevated free amino acids with a decline in protein synhesis in rats. In this present study it is reported that total free amino acids content was decreased due to nicotine treatment in both age groups. This decrease may be due to the effect of nicotine products on the FAA content in the plasma. However, contradictory reports are also available regarding the influence of nicotine on total free aminoacid pool. Besides these, the enhanced level of FAA may be due to ammonia intoxication (Krishna Mohan Reddy, 1986).

The total FAA content was increased in the Red Grape Extract treatment rats of both the age groups in the liver tissue. Amino acids are added to the pool through the synthesis of non-essential amino acids and precursors within the tissue and through release of amino acids from the breakdown of dietary and cellular proteins in the tissue. The increased amino acid content in the Red Grape Extract treatment liver may be due to augmented activity of acidic, alkaline and neutral proteases. This elevation in amino acid level may also be attributed to the enhanced proteolysis as well as decreased amino acid utilization for protein synthesis (Bylund-
Fellenius et al., (1984). The low levels of FAA in the liver tissue due to nicotine treatment may also be due to high utilization of these to carbohydrate sources via gluconeogenesis pathway to meet the energy demand under the influence of nicotine intoxication. In the present study we observed an elevation of FAA pool in the liver tissue due to combination treatment (Nt + RGEt), suggests that Red Grape extract treatment enhances the supply of FAA content to counter the nicotine toxicity.

CONCLUSION

To conclude, the present findings suggest that 2 months Red Grape extract treatment with the selected mgs (50 mg/kg body weight) that was adapted may be beneficial in countering the age associated and nicotine induced alterations in carbohydrate metabolic profiles. This investigation draws a conclusion stating that Red Grape extract treatment to the old age as well as young age male subjects may be beneficial especially for the nicotine subject.

ACKNOWLEDGEMENTS

I am very much grateful to the UGC-MRP Fellow, New Delhi for providing the financial support (UGC-MRP Letter No.39-612/2010 (SR), dated 10-01-2011) sanctioned by Dept. of Zoology. S. V. University, Tirupati., and my humble thanks to my Research supervisor, Dr. K. Chennaiah. Assistant, professor, Department of Zoology S.V.University, Tirupati.

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