Analysis Of Chronic Nicotine Administration On Carbohydrate Metabolism In The Brain Tissue Of Male Albino Rat With Reference To Aging

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Abstract: The present study is aimed at investigating the effect of nicotine administration on carbohydrate metabolic enzymes in the brain tissue of male albino rat. In vitro studies showed that Red grape (Vitis vinifera) has significant antioxidant activity, antimicrobial, anti-inflammatory action. Nicotine induces oxidative stress both in vivo and in vitro that causes a peroxidant/antioxidant imbalance in tissues. Pathogen free, Wistar strain male albino rats were used in the present study, 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 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 brain 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 brain 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.

Keywords: Nicotine, Red Grape (Vitis vinifera) Extract, Total Carbohydrates, Glycogen, Total free amino acids, Brain tissue and Male albino rats.

I. INTRODUCTION

Grape is non-climatic fruit that grows on the perennial and deciduous woody vines of the genus Vitis. Grapes can be eaten raw or used for making jam, juice, jelly, vinegar, wine, grape seed extracts and grape seed oil. The fruit of the grape is one of the most palatably edible foods, having many established nutritional and medicinal properties for consumers. Grape fruit contains various nutrient elements, (Table.1) such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Polyphenols are the most important phytochemicals in grape because they possess many biological activities and health-promoting benefits (Shrikhande, 2000; Wada et al., 2007). Anthocyanins and other pigment chemicals of the larger family of polyphenols in red grapes are responsible for the varying shades of purple in red wines. (Waterhouse et al., 2002; Brouillard et al., 2003). 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.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>(82%)</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>(12–18%)</td>
</tr>
<tr>
<td>Proteins</td>
<td>(0.5–0.6%)</td>
</tr>
<tr>
<td>Fat</td>
<td>0.3–0.4%</td>
</tr>
<tr>
<td>Potassium</td>
<td>(0.1–0.2%)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>(0.01–0.02%)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>(0.001–0.0015%)</td>
</tr>
<tr>
<td>Calcium</td>
<td>(0.01–0.02%)</td>
</tr>
</tbody>
</table>

Source: Sivasankar R, 2014

Table 1: Red Grape- Nutrients

Consumption of grape flavonoids has been shown to confer antioxidant protection, inhibit platelet activity, reduce thrombus formation and lead to the concentration of inflammatory biomarkers (Castilla, et al. 2006; O’Byrne et al., 2002). The antioxidant activity of grape seed phenolic compounds is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders (Yilmaz, Y. and Toledo, R. T. 2004). Flavonoids are polyphenolic antioxidants, occur naturally in vegetables and fruits (Soong, Y.Y and Barlow, P.J. 2004). Flavonoids possess several physiological properties: antioxidant, antibacterial, antiviral, antiinflammatory, antimutagenic and antitumoral activity, as well as the activation or inactivation of certain enzymes (Rice, L. 1998). A number of studies have demonstrated that the polyphenolic (flavonoid) compounds derived from grape products can improve endothelial function and increase endothelial nitric oxide (NO) production (Folts et al., 2002). Dietary resveratrol (a polyphenol antioxidant) has been shown to modulate the metabolism of lipids and to inhibit oxidation of low-density lipoproteins and aggregation of platelets. (Chan, and Delucchi, 2000). Resveratrol is found in wide amounts among grape varieties, primarily in their skins and seeds which, in muscadine grapes. (Le Blanc, 2005). These flavonoids exerts many-health-promoting effects including the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals. There are many references in the literature to the composition and antioxidant properties of grape polyphenols.

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. 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 (Pessone et al., 2001) and contributes to progression of HBV-related cirrhosis (Yu et al., 1997). Like other organs of the body, liver structure and functions are also altered with age advances. (Lee et al., 1999).

Nicotine binds to brain tissues with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers (Benwell et al., 1988; Breese et al., 1997; Perry et al., 1999). The increase in the binding is caused by a higher number of nicotinic cholinergic receptors in the brain of the smokers. The time course of nicotine in the brain and in other body organs and resultant pharmacologic effects are highly dependent on the route and rate of dosing. Smoking a cigarette delivers nicotine rapidly to the pulmonary venous circulation, from which it moves quickly to the left ventricle of the heart and to the systemic arterial circulation and to the brain. The lag time between a puff of a cigarette and nicotine reaching the brain is 10 to 20 s. Although the delivery of nicotine to the brain is rapid, there is nevertheless significant pulmonary uptake and some delayed release of nicotine as evidenced by pulmonary positron emission tomography data and the slow decrease in the arterial concentrations of nicotine between puffs (Lunell et al., 1996; Rose et al., 1999).

Nicotine is primarily metabolized to cotinine in the liver, but also in brain and lung (Turner et al., 1975; Miksys et al., 2000) via CYP2A6 in humans and CYP2B1/2 in the rat, members of the cytochrome P-450 enzyme family (Hammond et al., 1991; Messina et al., 1997), and aldehyde oxidase. Cotinine is subsequently metabolized to 3-hydroxycotinine via CYP2A6 (CYP2B1/2). Though nicotine pharmacokinetic studies (Table 2) have largely excluded adolescent rats, the main metabolizing enzymes of nicotine, i.e., CYP2B1/2, are present and functioning in neonates (Shimada et al., 1994). Further to this, in vitro preparations of adolescent (PD40) and adult (PD100) rat hepatic microsomes exhibit similar rates of C and N-oxidation of nicotine in Wistar and Long Evans rats, indicating that metabolism of nicotine may be mature during the adolescent period (Kyerematen et al., 1988). Specific age differences in nicotine pharmacokinetics have been observed and may have implications in terms of the reinforcing properties of nicotine. For example, the propensity for nicotine to accumulate in brain increases, peaking at PD12 in mice (Ilback and Stalhandske, 2003), whereas nicotine-induced toxicity increases and metabolism decreases with advanced age in rats (>24 months, Okamoto et al., 1994).

<table>
<thead>
<tr>
<th>Half-life</th>
<th>Nicotine</th>
<th>Cotinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 min</td>
<td>18 hr</td>
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<table>
<thead>
<tr>
<th>Volume of distribution</th>
<th>180 L</th>
<th>88 L</th>
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</thead>
<tbody>
<tr>
<td>Total clearance</td>
<td>1,300 mL/min</td>
<td>72 mL/min</td>
</tr>
<tr>
<td>Renal clearance</td>
<td>200 mL/min</td>
<td>12 mL/min</td>
</tr>
<tr>
<td>Non renal clearance</td>
<td>1,100 mL/min</td>
<td>60 mL/min</td>
</tr>
</tbody>
</table>

Source: Average values based on data from Benowitz, Jacob et al., (1982) and Table 2: Human pharmacokinetics of Nicotine and Cotinine Benowitz, Kuyt et al., (1983).
II. 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/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 (Hindustan Lever Ltd, Mumbai) and water ad libitum.

SELECTION AND MODE OF NICOTINE TREATMENT

Nicotine was first distilled from tobacco sap in 1809. Nineteen years later, the main base of tobacco was isolated and separated in pure form from fermented tobacco by Posselt and Reimann (Pailer, 1964). They called it nicotine and characterized it as a water clear liquid, boiling under atmospheric pressure at 246 °C, miscible with water, alcohol and ether. Historically nicotine had been recommended for treatment of numerous symptoms.

PHYSICAL AND CHEMICAL PROPERTIES OF NICOTINE

✓ Nicotine Scientific name: Nicotiana tobaccomm
✓ Nicotine Family: Solanaceae
✓ Chemical formula: C₁₀H₁₄N₂
✓ Molecular Weight: 162.23
✓ IUPAC Name: 3-[2-(N-methylpyrrolidinyl)]pyridine
✓ Appearance: Oily, colourless hygroscopic liquid
✓ Characteristic odour: Turns brown on exposure to air
✓ Boiling point (decomposes): 246 °C
✓ Density: 1.01 g cm⁻³
✓ Solubility in water: Miscible

DOSEAGE 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 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, 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 hrs after the last treatment session by cervical dislocation and the brain tissue were be isolated at 4°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.

III. BIOCHEMICAL ANALYSIS

A. TOTAL CARBOHYDRATES

The total carbohydrate content was estimated by the method of Carroll et al., (1956). The Brain tissue was homogenized in 10% Trichloro acetic acid (TCA) to prepare 1% (w/v) homogenates. The proteins precipitated were removed by centrifuging the homogenates for 15 minutes at 3000g at 4°C. The clear supernatant was taken for the estimation of total carbohydrates. To 0.5 ml of supernatant, 5 ml of anthrone reagent was added and kept in a boiling water bath for 15 minutes. Then, the contents were cooled and read at 620 nm against the reagent blank. The total carbohydrate content was expressed as mg of glucose/gm wet weight of the tissue.

B. GLYCOGEN

The Glycogen was estimated by the method of Kemp and Van Heijnigen (1954). The Brain tissues were homogenized in 80% (w/v) methanol to prepare 5% (w/v) homogenates. The suspension was centrifuged at 3000g for 15 minutes at 4°C the supernatant containing glucose was decanted. (The glycogen content present in the Brain tissue homogenates was estimated after extraction of the glucose with 80% methanol). Now the
tissue residue was suspended in 5 ml of deproteinizing solutions (5% TCA containing 0.1% silver sulphate) and the fluid level was marked on centrifuge tube and the tube was covered with a glass cap and placed in a boiling water bath for 15 minutes. Then the tube was cooled in running tap water and deproteinizing solution was added up to the mark to compensate the loss due to evaporation. The contents were centrifuged at 5000g for 15 minutes at 4°C, 1 ml of clear supernatant was added to 3 ml of concentrated sulphuric acid in a wide mouthed test tube mixed by vigorous shaking. The mixture was heated in a boiling water bath for exactly 6.5 minutes and subsequently cooled under running tap water. The intensity of the pink colour developed was read against the blank at 520 nm in a spectrophotometer. The glycogen content was expressed in mg of glucose/gram wet weight of the tissue.

C. TOTAL FREE AMINO ACIDS

The total free amino acids were estimated by the method of Moore and Stein (1954). 5% (w/v) homogenates of Brain tissues were prepared in 10% (w/v) Trichloro acetic acid (TCA) and centrifuged the contents at 2000g for 15 min at 4°C. To 0.5 ml of supernatant, 2.0 ml of Ninhydrin reagent was added and the contents were exactly boiled for 6½ minutes in a boiling water bath. The contents were cooled to laboratory temperature. The samples were made up to 10 ml with distilled water and the colour intensity was read at 570 nm in a spectrophotometer against the reagent blank. The total free amino acid content was expressed in mg of free amino acids per gram wet weight of the tissue.

D. 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.

IV. RESULTS AND DISCUSSION

TOTAL CARBOHYDRATES

A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen, usually with a hydrogen oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula Cn(H2O)m. (Some exceptions exist; for example, deoxyribose, a component of DNA, has the empirical formula CnH2nO2n.) Carbohydrates are one of the three major food groups needed for proper nutrition, (proteins – 20-25%, carbohydrates – 50-60%. Fat – 20-30%). Carbohydrates in food are important and immediate source of energy for the body. Starch refers to carbohydrates found in plants (grains). Vegetables and fruits are a source of starch and are broken down to sugar or glucose. Carbohydrates are present in at least small quantities in most food, but the chief sources are the sugars and the starches. Carbohydrates may be stored in the body as glycogen for future use. If they are eaten in excessive amounts, however, the body changes them into fats and stores them in that form. If carbohydrates are not properly broken down before they are absorbed, then adverse health consequences may occur. To some extent every cell depends on glucose. The cells of the nervous system and the brain almost exclusively use glucose for energy. Fibers are different than starches in that they cannot be broken down by the digestive system, and therefore they provide little or no energy for the body. Fiber has been shown to protect against heart disease and diabetes by lowering cholesterol and glucose levels.

The U.S. Surgeon General C.Everett Koop recommends in his 1988 report increasing consumption of complex carbohydrates as the best alternative to eating fats and cholesterol. The report implies that Americans already eat enough protein and should not increase their intake of this nutrient. Building glycogen reserves may improve endurance in some activities, such as running marathons. However, Sharon Vitousek, M.D., a physician and an athlete adds that glycogen-loading diets for sports may be less effective than originally thought. Diabetics also may benefit from a diet high in complex carbohydrates and low in fat and sugar, according to the American Dietetic Association. Some researchers believe that dietary fiber improves the ability of diabetics to process blood sugar. Carbohydrate metabolism focuses on the synthesis and usage of glucose, a major fuel for most organisms. In vertebrates, glucose is transported throughout the body in the blood. If cellular energy reserves are low, glucose is degraded by the glycolytic pathway. Glucose molecules not required for immediate energy production are stored as glycogen in liver and muscle. The energy requirements of many tissues (e.g., brain, red blood cells, and exercising skeletal muscle cells) depend on an uninterrupted flow of glucose. Depending on a cell’s metabolic requirements, glucose can also be used to synthesize, for example, other monosaccharides, fatty acids, and certain amino acids. So, the carbohydrates play several crucial roles in the metabolic processes of living organisms. They serve as energy sources and as structural elements in living cells.

RESULTS AND DISCUSSION

In the present study in total carbohydrates content was decreased in both (young and old) nicotine treatment rats (young by -18.29%; old by -32.28%) when compared to control rats. In red grape extract treatment rats of both (young and old) an increased (young by 6.54%; old by 3.78 %) was observed than the, control rats. In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups. (Table.3)

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Control</th>
<th>Young (±6.44)</th>
<th>Old (±6.14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>95.71 (±4.47)</td>
<td>78.20 (±6.52)</td>
<td>101.97 (±5.70)</td>
</tr>
<tr>
<td></td>
<td>107.07 (±6.44)</td>
<td>72.50 (±9.23)</td>
<td>111.1 (±9.22)</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**

| Table 3: 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 Brain of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in mg/gm wet weight of the tissue |

In the present investigation it was observed that the age induced slight elevation in total carbohydrate content in the brain, 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 brain of 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 Kidney (Sailaja, 1997; Gurumurthy, 2001), heart tissue slices (Bilwanath,1996). Enzymes of Kreb's-citrull 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. 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-citrull cycle enzymes which are necessary for the catabolic process of carbohydrates, may lead to increase the total carbohydrate content in the brain of old age rat.

Several authors have been reported decreased total carbohydrate in afferent tissue with reference to different toxic treatments. Subramanyam, (1984) reported decreased total carbohydrate content in different tissues with acetaldehyde toxicity. 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); Linda and Charles, (1983).

We observed in the present study the induced effect of red grape extract treatment (RGEt) increase the total carbohydrates content in both age groups at the same time decrease was observed in the nicotine treatment (Nt) rats when compare to control rats. Interestingly, in the present investigation with combination treatment (Nt+RGEt) an increase in the levels of total carbohydrate content was found in the brain of both age groups of rats. Thus, these results clearly suggest that, the red grape extract treatment (RGEt) was beneficial for nicotine induced toxicity.

**GLYCOGEN**

In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, and functions as the secondary long-term energy storage (with the primary energy stores being fats held in adipose tissue). Glycogen is a multibranch polysaccharide that serves as a form of energy storage in animals (Sadava et al., 2011). The amount of glycogen present in tissues varied widely with diet and physiological status (Nelson and Cox, 2001). Glycogen is the analogue of starch, a glucose polymer in plants, and is sometimes referred to as animal starch, having a similar structure to amylopectin but more extensively branched and compact than starch. Glycogen is found in the form of granules in the cytosol/cytolasm in many cell types, and plays an important role in the glucose cycle. Glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose, but one that is less compact than the energy reserves of triglycerides (lipids). Polysaccharide represents the main storage form of glucose in the body. Found in the liver and muscles, muscle glycogen is converted into glucose by muscle cells, and liver glycogen converts to glucose for use throughout the body including the Central Nervous System.

In the liver hepatocytes, glycogen can compose up to eight percent of the fresh weight (100–120 g in an adult) soon after a meal. (Campbell et al., 2006) Only the glycogen stored in the liver can be made accessible to other organs. In the muscles, glycogen is found in a low concentration (one to two percent of the muscle mass). The amount of glycogen stored in the body especially within the muscles, liver, and red blood cells (Moses et al., 1972; Ingermann and Virgin, 1987; Miwa and Suzuki, 2002). Mostly depends on physical training, basal metabolic rate, and eating habits such as intermittent fasting. Small amounts of glycogen are found in the brain and even smaller amounts in certain glial cells in the brain and white blood cells. The uterus also stores glycogen during pregnancy to nourish the embryo. (Campbell et al., 2006). Glycogen is a branched biopolymer consisting of linear chains of glucose residues with further chains branching off every ten glucose or so. Glucoses are linked together linearly by α (1→4) glycosidic bonds from one glucose to the next. Branches are linked to the chains they are branching off from by α (1→6) glycosidic bonds between the

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first glucose of the new branch and a glucose on the stem chain. (Berg et al., 2012). Due to the way that glycogen is synthesised, every glycogen granule has at its core a glycogenin protein. (Berg et al., 2012).

RESULTS AND DISCUSSION

In the present study the glycogen content was decreased in both (young and old) nicotine treatment rats (young by -25.65%; old by -20.83%) 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 9.32%; old by 10.05%). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table 4).

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Young</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
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<tr>
<td>Nt</td>
<td>90.78**</td>
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<tr>
<td>RGEt</td>
<td>133.48*</td>
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<tr>
<td>Nt+RGEt</td>
<td>123.20*</td>
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</table>
| Values in parentheses denote per cent change over respective control. ** Values are significant at P < 0.01 * Values are non significant.

Table 4: 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 Brain 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.

All the values are ± SD of six individual observations.

From the present investigation it was observed that the brain tissue glycogen levels were decreased in the nicotine treatment (Nt) rats 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, kidney, and muscle, under guanidine toxicity. Hariprasad, (1996) observed decreased Glycogen content in fish with ammonium toxicity. Decrement in tissue glycogen levels has been reported during ammonia stress (Santhi 1991; Nadamuni Cherry, 1992 and Obula Reddy, 1994). The decreased glycogen content in the brain 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).

In our present findings, it is observed that the glycogen content was increased in RGEt rats in the brain both age groups when compared to control rats. Red grape extract 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 upregulation of glycogen metabolism by the RGEt rats.

From the present investigation it was observed that the brain glycogen levels significantly decreased due to aging (Table; 4). 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, 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 Isocitrate dehydrogenase (ICDH), Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (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 brain of both age groups in the combination treatment (Nt+RGEt), suggesting RGEt may beneficial for the nicotine subject to improve the glycogen content under induced nicotine conditions.

**TOTAL FREE AMINOACIDS**

Amino acids are the building blocks of proteins, have a pivotal role to play in cellular metabolism. Biological value of proteins is considered on the basis of tissue amino acid composition. The diverse physical, chemical and biological properties of the proteins are dependent on the nature and arrangement of their constituent’s namely free amino acids. A hydrolysis of dietary proteins as well as the breakdown of endogenous proteins result in the of a large amino acid pool in the body conversely amino acids are e precursors for the synthesis of various cellular proteins. In addition, they also precursors for gluconeogenesis, glycogen synthesis as well as ketoacids and hence they are of greater significance. A pool of free-amino acids which form the precursors for protein synthesis and gluconeogenesis will be present in every tissue (Nelson and Cox, 2001; Murray et al., 2000). All types of physiological processes relating to sports energy, recovery, muscle/strength gains and fat loss, as well as mood and brain function are intimately and critically linked to amino acids. It's no wonder aminoacids have become major players in athletes' supplementation, especially among bodybuilders. For all practical purposes, free amino acids are the currency through which protein metabolism operates.

There will be a constant flux of amino acids from plasma to tissue and *vice versa*. Because of the heavy traffic of these vital molecules which contribute to various metabolic pathways as well as to the structural machinery, of cells, sensitive control mechanisms are warranted to keep the amino acid pools in each tissue at equilibrium. The physiological state of the cell also depends upon the free amino acid reserve (Adibi, 1980). The breakdown of proteins, the transport of amino acids across the cell membranes and the rate of incorporation of amino acids into cellular proteins and their oxidation towards other metabolic pathways are the factors that govern the size of amino acid pools. Free amino acids (FAA) also play an important role in the maintenance of osmotic pressure in the cells. The quality and quantity of free
amino acid pool can be considered as a best diagnostic tool to decide the physiological state of the cell.

In view of the extensive traffic passing through the free amino acids (FAA) pool, one may expect sensitive control mechanisms to maintain the size of the pool constantly. Since the FAA represent intermediates protein metabolism, consequently the size of the amino acid pool is the resultant of a balance between input and removal. Any abnormality in the protein or amino acid metabolism of brain will have its own consequences not only in the host tissue but also in other tissues due to heavy traffic of these protein catabolic products. In view of this, the levels of total FAA in the brain of control and experimental rats were studied to gain an insight into the pattern of amino acid turnover.

RESULTS AND DISCUSSION

In the present study in total free amino acids content was decreased in both (young and old) nicotine treatment rats (young by -52.43%; old by -35.21%) 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 13.19%; old by 17.91%). In the combination treatment (Nt+RGEt) slightly increased was observed when compared to control rats of both age groups (Table 5).

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Control</th>
<th>Young</th>
<th>Old</th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>67.89</td>
<td>66.35</td>
<td>62.39</td>
<td>70.44</td>
<td>66.93</td>
</tr>
<tr>
<td></td>
<td>±8.35</td>
<td>±9.58</td>
<td>±7.92</td>
<td>±10.34</td>
<td>±9.47</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 5: Changes in Total free amino acids 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 Brain 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.

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). Total Carbohydrates, Glycogen, Total free amino acids, Brain tissue and Male albino rats.

In the present investigation more amount of free amino acids (FAA) were found in the brain of young age group compared to old age group. Obled and Arnal, (1991) suggested that, with advancement of age in 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 synthesis in rats. In the present study 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 brain. 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 RGEt rats; in both the both the age groups of brain 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 RGEt brain 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 brain 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 brain tissue due to combination (Nt + RGEt), suggests that RGEt enhances the supply of FAA content to counter the nicotine toxicity.

CONCLUSION

To be defined, the findings of the present investigation 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. Chronic nicotine administration produces molecular and metabolic changes in brain. In addition, our present findings suggest that the disturbance in carbohydrate metabolic balance play a part in rendering brain tissue and more vulnerable to free radical-induced injuries. 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.

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