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RESEARCH ARTICLE

ALCOHOL INDUCED OXIDATIVE STRESS IN TESTES OF EXPERIMENTAL ANIMALS

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Abstract

A study was conducted to evaluate the alcohol induced oxidative Stress (OS) in the testes of experimental animals. Albino rats of Wister stain weighing 200-220 g were used for the study. The experimental groups received 1.6 g of ethanol/kg of body weight/day for 12 weeks. It was observed that alcohol used for a long period caused significant OS in the testes as a result of the generation of Super Oxide (SO) radical due to the alcohol metabolism. A significant reduction in glutathione and increased in barbituric acid reactive substances were observed. SO dismutase activity and glutathione reductase activity decreased significantly in the experimental group.

Introduction:-

Alcohol is capable of generating oxygen radicals inhibiting glutathione synthesis, inducing reduced glutathione levels in the tissue, increasing malondialdehyde (MDA) levels and impairing antioxidant defense mechanisms in humans and experimental animals. In addition, acetaldehyde, the metabolite of ethanol, is a very reactive molecule with a high affinity for sulf hydryl groups, enabling binding to a variety of intracellular proteins. It can impair enzyme functions by forming Schiff bases with sulf hydryl groups at the active sites of several enzymes. Acetaldehyde also enhances lipid peroxidation and interferes with protective responses in the cell, including interference with glutathione synthesis and reduction in the activity of free radical scavenging enzymes (Leiber 1998).

Oxidation stress (OS) occurs as a consequence of an imbalance between the production of Reactive Oxygen Species (ROS) and available antioxidant defense against them (Sikka et al., 1995, Sharma et al., 1996). Ethanol oxidation eventually generates reactive free radicals leading to OS, a condition that is further reinforced by the depletion of the antioxidants glutathione and vitamin E as observed in chronic alcoholics (Bjorneboe et al., 1998;). OS is also involved in the peroxidation of membrane and lipoprotein lipids, thus facilitating cellular necrosis and apoptosis (Ziol et al.,2001 ; Natori et al., 2001).

Many processes and factors are involved in causing alcohol induced OS. Alcohol metabolism results in the formation of NADPH, or NADH (either by alcohol dehydrogenase pathway or by cytochrome P450 pathway) which enhances activity of the respiratory chain, increasing heightened O_2 use and Reactive Oxygen Species (ROS) formation. Out of the byproducts of alcohol metabolism, acetaldehyde interacts with protein and lipids to form ROS. Peroxidation of membrane lipids leads to mitochondrial leakages, which may cause decreased ATP production. Alcohol also induces hypoxia that results in tissue damage.

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Research has contributed substantially to our understanding of the relation of drinking to specific disorders, and has shown that the relation between alcohol consumption and health outcomes is complex and multidimensional (Das et al., 2006). Ethanol increases the rate of generation of free radicals, decreases the antioxidant levels, and potentiates oxidative stress (Das et al., 2005). Finally, Alcohol reduces the levels of antioxidants that can eliminate ROS. Following ethanol intoxication, the balance between pro oxidants and antioxidants is disturbed to such an extent that it results in the oxidative damage of bio molecules, such as fats, proteins, or DNA, and finally leading to cell injury (Das and Vasudevan, 2007).

An ethanol induced oxidative stress is not restricted to the liver, where ethanol is actively oxidized, but can affect various extra hepatic tissues as shown by experimental data obtained in the rat during acute or chronic ethanol intoxication. Chronic ethanol administration elicits in the tests an enhancement in mitochondrial lipid per oxidation and a decrease in the glutathione level, which appear to be correlated to the gross testicular atrophy observed (Nordmann et al., 1990).

Chronic ethanol abuse causes testicular atrophy and male infertility in alcoholic men. It is well known that ethanol exposure disrupts the hypothalamic-pituitary-gonadal axis, adversely affects the secretory function of sertoli cells, and produces oxidative stress within the tests. Studies showed that ethanol enhances testicular germ cell apoptosis (Zhu et al., 2000). Ethanol is a testicular toxin and it causes fertility abnormalities with low sperm count and impaired sperm motility in men. A study by Maneesh et al (2006) investigated plasma testosterone level and hypothalamic pituitary gonadal (HPG) axis function in alcoholic men and also effect of ethanol on systemic oxidative stress. Antioxidants, to a large extent, inhibit oxidative stress and apoptosis induced by ethanol (Guan et al., 2004).

Aim and Objectives:-

The aim and objectives of the present study was to demonstrate the role of oxidative stress in long term ethanol induced testicular damage and to evaluate the effects of graded dose of ethanol on various oxidative stress related parameters.

Materials and Methods:-

Animal Selection:

16 – 18 week-old male albino rats of wistar strain weighing 200-220g were used. Food and water were given ad libitum (Das and Vasudevan, 2006).The animals were weighed daily and its general condition was recorded including their daily intake of liquid. The animal Ethics Committee of the Institution approved the procedures in accordance with the **CPCSEA** guideline.

The rats were divided in to the following 2 groups of 6 each:

Group 1 : Control rats were fed normal diet and water.

Group 11: Ethanol treated rats (1.6 g ethanol/Kg body weight/day for 12 weeks)

Ethanol was diluted with distilled water to get desired concentration and fed orally.

At the end of the experimental period, the animals were sacrificed after overnight fast, by applying intra – peritoneal thiopentone (thiosol / Na+) (euthensia). The testes was dissected out and cleaned with ice- cold saline, blotted dry, and immediately transferred to the ice chamber. The following investigations were carried out.

Lipid Peroxidation – Method of Sinnhuber et al 1958

The tissue Malone dialdehyde (MDA) was allowed to react with thiobarbituric acid (TBA).The MDA – TBA adduct formed during the reaction in acidic medium was extracted to the organic layer and the absorbance was measured at 535 nm.

Glutathione – Method of Ellman 1959

The acid soluable hydryl groups forms an yellow colored complex with dithionitrobenzene (DTNB). The absorbance of measured at 412nm .

Catalase Activity – Method of Beers and Sizer 1952

Catalase catalyses the decomposition of H_2O_2. In the ultra violet range H_2O_2 shows a continual increase in absorption with decreasing wave length. The decomposition of H_2O_2 can be followed directly by the decrease in extinction at 240 nM.

$$2H_2O_2 \longrightarrow 2H_2O + O2$$

Glutathione Reductase (GR) Activity -Method of Goldberg & Spooner 1983

Activities of holo and apo forms of glutathione reductase in tissue are measured, with and without addition of FAD, by spectrophotometric determination of NADP formed. The reactions are given below.

Apo. Enzyme + FAD $\longrightarrow$ Holo enzyme

G – S – S – G + NADPH + H $\longrightarrow$ 2GSH + NADP

Glutathione S – transferase (GST) Activity – Method of Habig et al, 1974

The GST activity was assayed spectrophotometrically at 340 nm with standard substrate (1 – chloro -2, 4 – dinitrobenzoic acid , CDNB) and co- substrate (reduced glutathione , GSH) as described by (Habig et al ., 1974) and expressed in U / ml/min. The total glutathione estimation was done in testes.

Superoxide Dismutase (SOD) Activity – Method of Marklund and Marklund, 1974

This is a spectrophotometric assay for superoxide dismutase (SOD). SOD's are metallo enzymes that catalyze the dismutation of the superoxide ion into hydrogen peroxide and oxygen. Three classes of SOD's have been described and they vary only in the catalytic metal that is in the active site. They are C/Zn, Mn and Fe-SOD- mediated increase in the rate of auto oxidation of 5, 6, 6a, 11b-tetrahydro-3, 9, 10-trihydroxy-benzo[c] fluorine.

Statistical analysis

All data were analyzed using the statistical package SPSS (version 11.0, SPSS lnc., Chicago, IL). Results were expressed as mean ± SD (Standard Deviation)

Results:-

Effects of graded dose of ethanol on various oxidative stress related Parameters was studied. Reduced glutathione (GSH) concentration significantly decreased on ethanol exposure, where as thiobarbituric acid reactive substances (TBARS) significantly increased. No significant change in catalase activity was observed, while superoxide dismutase activity decreased significantly on ethanol exposure (1.6 ethanol/kg body wt/ day for 12 weeks) compared to the control group. Ethanol exposure caused significant decrease in glutathione reductase activity and increase in glutathione –S- transferase activity in testes homogenate .

Effects of different doses (per kg body weight per day) of ethanol on reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) level, glutathione reductase activity, catalase activity, superoxide dismutase activity and glutathione –S- transferase activity in testes homogenate are given in table.1

Table 1:- Oxidative Stress and Anti Oxidant Status in Test and Control Animals:

Parameter	**Control**	**Test**
GSH (nmol /mg protein)	26.7±2.53	15.17±1.03*
TBARS(µmole of MDA /min/100mg tissue)	0.348±0.013	0.589± 0.044*
Glutathione reductase (nmol NADPH break down/ min / mg of protein)	26.4 ±1.4	16.8± 0.8*
Catalase (µmol H_2O_2 decomposed /min/mg of protein)	3.87 ±0.36	3.26 ±0.31*
SOD (U /mg of protein)	6.13 ±0.31	4.07 ±0.2*
GST (µmol of CDNB conjugate formed / min / mg of protein	3.84 ±0.10	6.26 ±0.31*

All values are Mean ± SD * P<0.05

Discussion:-

The thiobarbituric acid reactive substances (TBARS) level in testes was increased due to ethanol exposure. One of the proposed mechanisms of chronic ethanol induced toxicity is the membrane damage due to the direct effect of lipid peroxidation products (Das and Vasudevan, 2005),i.e., TBARS, which was found to be increased in the ethanol treated rats in the present study.

Catalase activity was unchanged in the present study. The amount of SOD is organ specific. Three types of SOD have been purified; Cu Zn – SOD, Mn – SOD (McCord, 1979) and extra cellular SOD (ECSOD) (Marklund, 1984). Cu Zn-SOD consists of two protein subunits each unit has an active site containing one Cu ion and one Zn ion (McCord, 1979). Cu ion serves as an active redox site and Zn ions maintain the protein structure (Fridovich, 1999).

Ethanol exposure caused significant decrease in glutathione reductase (GR) activity and increase in glutathione S-tranferase (GST) activity. A significant decrease in hepatic GR activity and glutathione level after ethanol treatment is indicative of impaired reduction of oxidized glutathione to reduced form (Dinu and Zamfir, 1991). Glutathione- S – transferase (GST) plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. Increased Glutathione S Transferase activity and decreased glutathione reductase activity, followed by thiol depletion, are important factors sustaining a pathogenic role for oxidative stress (Aniya and Daido, 1994; Das and Vasudevan, 2005).

Conclusion:-

Our study revealed that higher doses of ethanol (1.6 g/ kg body weight/ day) for a long period cause significant oxidative stress in the testes as a result of the generation of superoxide radicals by ethanol metabolism. Intermediates of oxygen reduction may in fact be associated with the development of ethanol induced organ damage.

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