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Impact of Glycerol, Mannitol, Neurotol and Neurotol Plus Administration in Alcohol Induced Ischemic Rat Model

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Abstract: The aim of the present study was to evaluate the effect of various treatment of glycerol, mannitol, neurotol and neurotol plus on xanthine oxidase, adenylate kinase activities and MDA levels in alcohol induced ischemic rat model. Twenty Wistar rats (weighing 100- 200 g) were divided into four groups, glycerol treated group (20%), mannitol treated group (10%), fixed dose combination of glycerol (10%)+ mannitol (10%) (Neurotol) treated group and neurotol plus treated group (glycerol (10%)+mannitol (10%)). A significant decrease in xanthine oxidase activity, adenylate kinase activity and MDA levels were observed on treatments but decrease was maximum in neurotol plus treated groups suggesting that it has better free radical scavenging activity than glycerol, mannitol and neurotol.

Key words: Xanthine oxidase, adenylate kinase, stroke, neurotol plus

INTRODUCTION

Brain injury is one of the major causes of increased intracranial pressure (ICP), secondary deterioration and death in patients after stroke (Hacke *et al.*, 1996; Muizelaar *et al.*, 1991). Oxidative stress in the brain occurs when the generation of ROS overrides the ability of the endogenous antioxidant system to remove excess ROS subsequently leading to cellular damage. Several cellular features of the brain suggest that it is highly sensitive to oxidative stress. For example, brain is known to possess the highest oxygen metabolic rate of any organ in the body (Maiese, 2002). It consumes approximately 20% of the total amount of oxygen in the body (Shulman *et al.*, 2004). This enhanced metabolic rate leads to an increased probability that excessive levels of ROS will be produced. It has been proposed that toxic metabolites from xanthine oxidase (XO) contribute to the development of injury seen during reperfusion of a variety of ischemic tissues (Patt *et al.*, 1988). Adenylate kinase is also a marker enzyme for brain tissue injury.

Various new therapeutic strategies have been developed to reduce ICP that preserve the integrity of nerve cells and also prevent the accumulation of toxic metabolites. Substances such as glycerol and mannitol have been shown to decrease edema formation in the brain (Kaufmann and Cardoso, 1999; Kofke, 1993; Sheikh *et al.*, 1996). Combination of mannitol and glycerol comprises of two sugars with better osmotic diuretic properties (Freshman *et al.*, 1993; Manno *et al.*, 1999; Takagi *et al.*, 1984). Either glycerol or mannitol can be administered individually, however the addition of glycerol to mannitol avoids rebound edema likely to be observed with the intravenous administration of only mannitol

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(Berger *et al.*, 1995; Soustiel *et al.*, 2006). This provides a strong rationale for combining glycerol and mannitol in the management of cerebral edema and raised intracranial tension or pressure (Soustiel *et al.*, 2006). This combination strategy is able to enhance the diffusion of water from cerebrospinal fluid back into plasma by elevating the osmolality of the plasma (Yilmaz *et al.*, 2007). It rapidly enters cerebrospinal fluid and brain compartments and favorably affects the recovery from stroke in two ways, one is by redistribution of cerebral blood flow with increase in regional cerebral blood flow and regional cerebral blood volume in ischemic brain and other is by reducing focal cerebral edema (Sacco *et al.*, 2004).

In the present study, we evaluated effect of various treatment of glycerol, mannitol, neurotol and neurotol plus on xanthine oxidase, adenylate kinase activities and MDA levels in alcohol induced ischemic rats.

MATERIALS AND METHODS

Study Conduct

The study was carried out from 10th April 2009 to 25th July 2009 in pre-clinical unit of Venus Medicine Research Centre, Venus Remedies Ltd. Baddi (India).

Chemicals

All the biochemicals used in the present study were procured from Sigma, St. Louis, MO, USA. Other chemicals, purchased locally, were of analytical grade.

In vivo Studies

Twenty Wistar rats (weighing 100-200 g) were used in the experiment. The rats were fed standard pelleted diet and sterile water *ad libitum*. The test room was air conditioned with temperature $23\pm 2^{\circ}\text{C}$, humidity $65\pm 5\%$ and with artificial fluorescent light (10 and 14 h of light and dark, respectively). Experimental rats were intubated intragastrically with single dose of 25% alcohol. The rats were divided into four groups of six animals each as given below:

- Glycerol treated group (20%)
- Mannitol treated group (10%)
- Neurotol treated group (FDC of glycerol (10%)+mannitol (10%))
- Neurotol plus treated group (FDC of glycerol (10%)+mannitol (20%))

The respective drugs were administered intravenously ($20\ \mu\text{L g}^{-1}$ body weight). Blood samples were collected at 30 min and 2 h intervals before and after respective treatment by the retro-orbital plexus into heparinized vials under the light anesthesia.

Plasma Preparation

Citrate containing blood samples were centrifuged at 7600 rpm for 15 min at $0-4^{\circ}\text{C}$ and plasma was separated out. It was used for the analysis.

Xanthine Oxidase Activity

Assay of xanthine oxidase was carried out essentially according to the method described by Roussos (1967). The assay mixture, in final volume of 3.0 mL, consisted of 0.30 mL Tris-HCl buffer, 50 mM pH 7.4; 0.30 mL CuSO_4 , 10 mM; 0.05 mL Xanthine, 2.58 mM per mL in 0.05 M glycine buffer, pH 7.4; 0.1 mL of diluted blood and water to make up the volume. Change in absorbance was recorded at 290 nm at 15 sec interval for 1 min. Suitable control was run simultaneously. One unit of activity has been defined as change in absorbance at 290 nm in 1 min by 1 mL enzyme preparation.

Adenylate Kinase Assay

Adenylate kinase assay was performed by method of Lamprecht and Trautschold with minor modification (Lamprecht, 1963). The reaction mixture 3.0 mL consisted of ADP 0.30 mL (4.0 mM), 0.55 mL of glucose (10 mM), 0.55 mL of $MnCl_2$ (10 mM) 0.30 mL of NADP (0.2 mM), 0.58 mL of Tris buffer (50 mM, pH 7.4), 10 μ g of hexokinase (10 unit), 10 μ g of glucose 6 phosphate dehydrogenase (1 unit) and added water to make up the 3.0 mL. The reaction was started by adding 20 μ L of sample. Change in absorbance was recorded at 340 nm at 15 sec interval for 1 min. Suitable control was run simultaneously. One unit of AK activity in the forward direction was defined as 1 μ mole of ADP removed/min at 37°C under experimental condition.

Estimation of Malonaldehyde Level

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldehyde (MDA) formed, essentially according to Ohkawa *et al.* (1979). It was determined by thio barbituric acid reaction. The reaction mixture consisted of 100 μ L of blood suspension, 0.20 mL of 8.1% Sodium Dodecyl Sulphate (SDS), 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% thio barbituric acid (TBA) and water to make up the volume to 4.0 mL. The tubes were boiled in water bath at 95°C for 1 h, immediately cooled thereafter under running tap water and 1.0 mL of water and 5.0 mL of mixture of n-butanol and pyridine (15:1 v/v) was added and vortexed. The tubes were centrifuged at 3500x g for 30 min. The upper layer was aspirated out and absorbance measured at 532 nm. The reference used was 1, 1, 3, 3 tetra ethoxy propane. The study was approved by the institutional animal ethical committee.

RESULTS

Xanthine Oxidase Activity

A significant increase in xanthine oxidase activity was observed 2 h after the intoxication with alcohol as compared to 30 min after intoxication. Its activity was significantly reduced on treatment with glycerol, mannitol, neurotol and neurotol plus as compared to its activity after 30 min of intoxication and 2 h of intoxication, respectively. A significant decrease in xanthine oxidase activity was observed after 30 min and 2 h of the treatment with the study drugs as compared to just before the initiation of the treatment i.e., 2 h after the alcohol intoxication. The percentage decrease in xanthine oxidase activity after 30 min of treatment was 65.8, 61.8, 68.5 and 73.6% in glycerol, mannitol, neurotol and neurotol plus treated groups respectively as compared to respective control value before the initiation of the treatment. The percent decrease after 2 h of the treatment was 74, 70, 73.1 and 85.4% in glycerol, mannitol, neurotol and neurotol plus treated groups, respectively as compared to respective control value before the initiation of the treatment (Fig. 1).

Assay of Adenylate Kinase

A significant increase in adenylate kinase activity was observed 2 h after the intoxication with alcohol as compared to 30 min after intoxication. Its activity was significantly reduced on treatment with glycerol, mannitol, neurotol and neurotol plus as compared to its activity after 30 min of intoxication and 2 h of intoxication, respectively. A significant decrease in adenylate kinase activity was observed after 30 min and 2 h after the treatment with the study drugs as compared to just before the initiation of the treatment i.e., 2 h after the alcohol

intoxication. The percentage decrease in adenylate kinase activity after 30 min of the treatments was 12.1, 24.4, 31.6 and 25.6% in glycerol, mannitol, neurotol and neurotol plus treated groups, respectively as compared to respective control value before the initiation of the treatment. The % decrease in its activity after 2 h of the treatments was 21.37, 19.66, 32.5 and 45.2% in glycerol, mannitol, neurotol and neurotol plus treated groups respectively as compared to respective control value before the initiation of the treatment. In glycerol and mannitol treated groups no significant change in adenylate kinase activity was observed between 30 min after the treatment and 2 h after the treatment, where as decrease was significant in neurotol and neurotol plus treated groups (Fig. 2).

Estimation of Malonaldehyde Level in Blood

A significant increase in MDA levels were observed 2 h after the intoxication with alcohol as compared to 30 min after intoxication. A significant decrease in MDA levels were

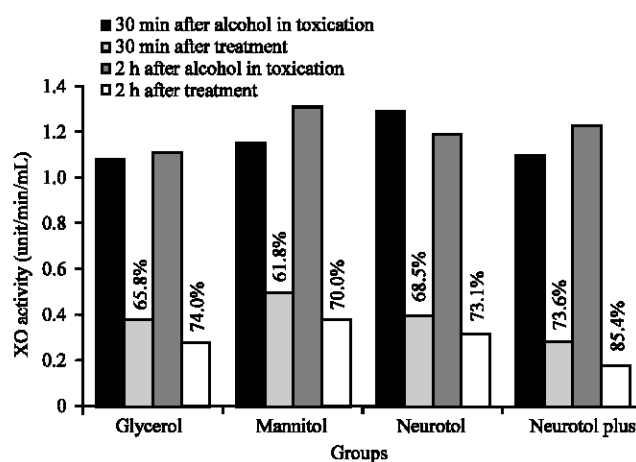


Fig. 1: Effect of various treatments on xanthine oxidase activity

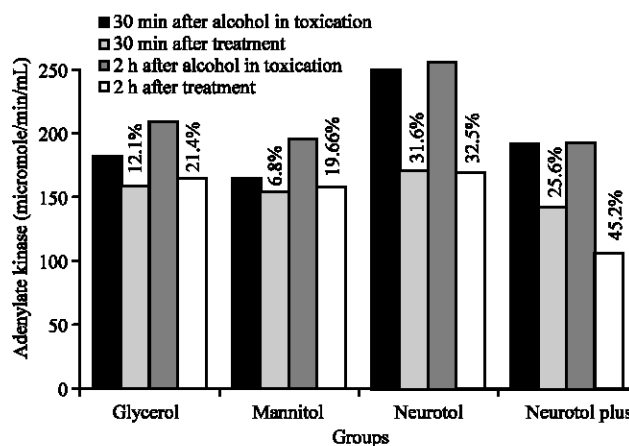


Fig. 2: Effect of various treatments on adenylate kinase activity

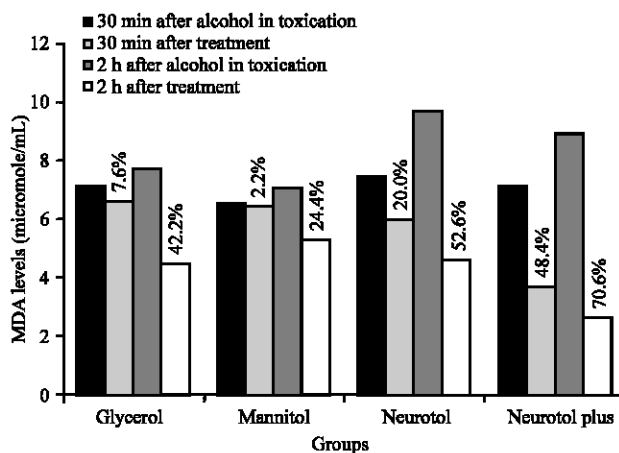


Fig. 3: Effect of various treatments on MDA levels

observed after 30 min and 2 h after the treatment with the study drugs as compared to just before the initiation of the treatment i.e., 2 h after the alcohol intoxication. The percentage decrease in MDA levels observed were 7.6, 2.2, 20.0 and 48.4% in glycerol, mannitol, neurotol and neurotol plus treated groups, respectively as compared to respective control value before the initiation of the treatment. The percentage decrease after 2 h of the treatment was 42.2, 24.4, 52.6 and 70.6% in glycerol, mannitol, neurotol and neurotol plus treated groups, respectively as compared to respective control value before the initiation of the treatment. In glycerol and mannitol treated groups no significant change in a MDA levels was observed between 30 min after the treatment and 2 h after the treatment, where as decrease was significant in neurotol and neurotol plus treated groups (Fig. 3).

DISCUSSION

Acute ischemic stroke is a major cause of mortality (Ikeda and Long, 1990). Stroke generally refers to a local interruption of blood flow to the brain and is the leading cause of long-term disability, third leading cause of death (Young *et al.*, 2007). Approximately 12% of strokes are hemorrhagic (rupture of a cerebral blood vessel), whereas the remaining 88% are ischemic and result from occlusion of a cerebral artery (either thrombotic or embolic). Blockage of a cerebral artery results in interruption of the blood flow and supply of nutrients, glucose and oxygen to the brain. Collins *et al.* (1998) has been established alcohol intoxication to cause ionic imbalances leading to increase in ICP and brain edema, which is further known to be associated with oxidative stress, lipid peroxidation and neurodegeneration.

Oxidative stress is the one of the cause of neuronal injury during brain ischemia/reperfusion (Ozkul *et al.*, 2007; Reynolds *et al.*, 2007; Taylor and Crack, 2004). It was accompanied by increase in free radicals, including superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), along with simultaneous decrease in endogenous antioxidant system, including antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase, or antioxidants, glutathione (GSH), Vitamin (Vit) C and Vit E (α -tocopherol). The highly reactive $^{\bullet}OH$ is formed from H_2O_2 in the presence of divalent metal ions, especially Fe^{2+} and Cu^{2+} via the Fenton reaction. In addition, during ischemia,

xanthine dehydrogenase undergoes irreversible proteolytic conversion to XO, producing $O_2^{\bullet-}$ and H_2O_2 in the presence of oxygen (Ozkul *et al.*, 2007). $O_2^{\bullet-}$ does not directly induce LPO but can react with $^{\bullet}NO$ to form cytotoxic peroxynitrite ($ONOO^-$) (Bromont *et al.*, 1989; Collins *et al.*, 1998).

In the present study, a significant decrease in xanthine oxidase activity was observed on treatments but decrease was maximum in neurotol plus treated groups suggesting that it has better free radical scavenging activity than glycerol, mannitol and neurotol. Steinberg *et al.* (1996) reported that adenylate kinase is a marker enzyme for ischemic brain injury and its activity has been reported to increase on injury. Similarly, a significant decrease in adenylate kinase activity was observed in all treated groups but maximum decrease was found that in neurotol plus treated group as compared to all other treated groups.

Tien *et al.* (1981) reported that mannitol produced more than 95% inhibition of lipid peroxidation initiated by Fenton reaction generated hydroxyl radical. Glycerol has also been reported to reduce lipid peroxidation induced by lipid polysaccharides. There other reports suggesting glycerol as oxygen radical scavenger. In the present study, a significant decrease in MDA levels were observed in all the treated groups as compared to respective controls and decrease in MDA levels were much more in neurotol plus treated group as compared to other treated groups clearly indicating that neurotol plus has been most effective in treatment of alcohol induced ischemic brain injury.

In conclusion, these results establish that neurotol plus was more effective and fast free radical scavenger than glycerol, mannitol and neurotol in the treatment of ischemic brain injury.

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