INTRODUCTION
Methanol (MeOH) (carbinol, wood alcohol) is a clear, colorless, volatile, flammable liquid with weak odor slightly sweeter than ethanol. Other details are shown in figure 1. It is miscible in water and occurs naturally in humans, animals and plants. It is a natural constituent in blood, urine, saliva and expired air. A mean urinary MeOH level of 0.73 mg/liter in unexposed individuals with a range from 0.06 to 0.32 μg per liter in expired air have been reported. Two most important sources that increase the body burdens of MeOH are diet and metabolic processes. MeOH is available in the diet principally from fresh fruits and fruit juices, vegetables, fermented beverages and any kind of foods (WHO, 1997). Furthermore, in the year 2002, Annual Report of the Toxic Exposure Surveillance System (TESS) by the American Association of Poison Control Centers (AAPCC), reported that about 2,610 people were exposed to MeOH every year from natural emission sources of MeOH that includes volcanic gasses, vegetation, microbes and insects (Owens et al., 1969, Holzer et al., 1977, Graedel et al., 1986).

**MeOH usage**

It is used in the industrial production of many synthetic organic compounds and as an important constituent for a large number of commercially available solvents. It is widely used as solvent in agricultural, pharmacological and cosmetic industry as well as in plastic and varnish production. It is used as a denaturant for ethyl alcohol and in the manufacture of formaldehyde, methacrylates, methyl amines, methyl halides and ethylene glycol. Other uses include de-icing agent, dehydrating agent for pipelines, fungicide, duplicating fluid, stove fuel, motor fuel, an intermediate in the manufacture of methyl tertiary
Figure 1. **Methanol (CH$_3$OH)**

\[
\begin{align*}
\text{H} \\
\downarrow \\
\text{H} - \text{C} - \text{OH} \\
\downarrow \\
\text{H}
\end{align*}
\]

**Structure**

<table>
<thead>
<tr>
<th>Description</th>
<th>Colorless liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>CH$_3$OH</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>32.04 g/mol</td>
</tr>
<tr>
<td>Boiling point</td>
<td>64.6°C</td>
</tr>
<tr>
<td>Melting point</td>
<td>-97.6°C</td>
</tr>
<tr>
<td>Vapor pressure</td>
<td>92 torr at 20°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>Methanol is miscible with water, ethanol, ether and many other organic solvents</td>
</tr>
</tbody>
</table>
butyl ether as an octane booster or directly as an octane booster for gasoline. It is used as fuel in gasoline blend. If MeOH, either 100% or in gasoline blends, becomes a major automotive fuel, emissions of MeOH may arise as uncombusted fuel in exhaust or from evaporation during refueling (Medinsky and Dorman, 1995). For instance, in a public garage, if 100% of vehicles were fueled with MeOH, then methanol concentrations in air will be projected to be 150 ppm. In most cases, exposure of the general public would be brief but repeated over time (Gold and Moulf, 1988). Since MeOH is considered to replace gasoline, possibility of exposure becomes more

Aspartame

Aspartame (L-asparty-L-phenylalanine methyl ester), a new sweetener marketed under the trade name NutraSweet. Aspartame is composed of MeOH and two naturally occurring amino acids aspartic acid and phenylalanine. Because aspartame breaks down into amino acids within the body, it behaves like a protein, providing an energy value of 4 kcal/g. In fact, this energy value is the same as sugar, but since aspartame is used only in very small quantities, food and beverage manufacturers can advertise their products as “calorie-free” releases into the human bloodstream one molecule of MeOH for each molecule of aspartame consumed. This new MeOH source is being added to foods as presumed to reduce the caloric content and, thus, may be consumed in large amounts. Generally, none of these foods could be considered dietary MeOH sources prior to addition of aspartame. When diet sodas and soft drinks, sweetened with aspartame, are used to replace fluid loss during exercise and physical exertion in hot climates, the intake of MeOH can exceed 250 mg/day or 32 times the Environmental Protection Agency’s recommended limit of consumption for this cumulative toxin (Cleland et al., 1977). Even if one avoids aspartame-bearing products, normal dietary exposure to MeOH may still occur from consumption of certain fruits, vegetables
and juices with high pectin content i.e., direct ingestion; (Butchko and Kotsonis, 1991; Frenkel et al., 1998). Furthermore, though some of the pectin content is converted directly to MeOH in these food products, unprocessed pectin can itself be converted in the gut to MeOH by the actions of local bacteria i.e., another indirect form of ingestion; (Siragusa et al., 1988; Lindenger et al., 1997) again resulting in substantive daily increases in blood MeOH content. The question asked whether uncontrolled consumption of this new sweetener might increase the MeOH intake of certain individuals to a point beyond which our limited knowledge of acute and chronic human MeOH toxicity can be extrapolated to predict safety.

**Routes of MeOH exposure**

The primary routes of MeOH exposure are inhalation and ingestion, whereas the dermal exposure is currently considered with much less importance in terms of total daily intake for both the general and occupational populations. Inhalation of MeOH is the most common route of entry as it is unavoidable in an occupational setting. Experiences in occupational health and volunteer studies showed that MeOH is rapidly absorbed after inhalation (Liesivuori and Savolainen, 1987; Kawai et al., 1991; d’Alessandro et al., 1994). Around 60-85% of inhaled MeOH is absorbed in the lung of humans (Sedivec et al., 1981). Although ingestion of MeOH historically has been shown to be the most frequent route of poisoning, percutaneous absorption of MeOH liquids or inhalation of its vapor is as effective as the oral route in producing MeOH acute toxic syndrome in adult and pediatric poisonings (Buller and Wood, 1904; Wood and Buller, 1904; Giminez et al., 1968; Kahn and Blum, 1979; Dutkiewicz et al., 1980; Becker, 1983). Sufficient quantities of MeOH can be absorbed through the skin and can lead to systemic intoxication (Niosh, 1977; Downie et al., 1992).
Cluster of infant deaths was reported due to topical application of MeOH after a vaccination programme in Egypt (Darwish et al., 2002, Gimenez et al., 1968) also reported that about 48 children were intoxicated with percutaneously applied MeOH. Thus, all exposure routes are presumed to be toxicologically equivalent (Tephly and McMartin, 1984). No differences exist between the capabilities for absorption of MeOH among various animal species, and blood levels are entirely predictable based on the concept that regardless of the exposure route, MeOH distributes uniformly to the body water content.

**Lethal dose**

The average intake of MeOH from natural sources varies but limited data suggests an average intake of considerably less than 10 mg/day (Cleland et al., 1977). Alcoholics may average much more, with a potential range of between 0 and 600 mg/day, depending on the source and in some cases the quality of their beverages. The lethal dose of MeOH for humans is not known for certain. The minimum lethal dose of MeOH in the absence of medical treatment is between 0.3 and 1 g/kg (WHO, 1997). Death has also been reported with ingestion of as little as 15 ml of 40% solution and survival has been reported after ingestion of 500 to 600 ml of a solution of the same concentration (Rumauk, 1989). The lowest dose reported to be associated with toxicity is 4 ml of absolute MeOH (Kulig, 1984). Two important determinants of human susceptibility to MeOH toxicity appear to be (1) concurrent ingestion of ethanol, which slows the entrance of MeOH into the metabolic pathway, and (2) hepatic folate status, which governs the rate of formate detoxification.

The LD$_{50}$ value for single intra-peritoneal administration for male Wistar rats was 7.59 gm/kg (Tichy et al., 1985). Based on the range of oral LD$_{50}$'s, 0.4 to 14.2 g/kg, for monkeys, rats, mice, and rabbits (Rowe and Mc Collister, 1981) and
Rowe and Mc Collister (1982) reported that the $L_D_{50}$ value for rat as 7.4 to 13 g kg.

**Excretion**

Following uptake and distribution, MeOH clears from the body. MeOH is either excreted unchanged in the urine or through breath. However, this route of elimination is not faster than the metabolic pathway. The time course for the disappearance of MeOH from the circulation is dependent upon the combined action of both direct excretion and metabolism. The elimination of MeOH from the blood appears to be very slow in all species, especially when compared to ethanol (Tephly and McMartin, 1984). Excretion of MeOH in urine is initially high and decreases with time following exposure. Following uptake and distribution, MeOH is either excreted unchanged (direct excretion) in urine or exhaled through breath, or it enters a metabolic pathway in the liver, whose ultimate product is carbon dioxide. The primary route of MeOH elimination from the body is via oxidation to formaldehyde and then to formic acid, which may be excreted in the urine or further oxidized to carbon-dioxide.

In the non-human primate (cyanomolgus monkey), exhalation is the primary route of MeOH excretion (41-72%) both during and after exposure. The clearance half time at high dose (1 g kg$^{-1}$) is approximately 1 day while a clearance of only about 3 hrs was observed at lower dose (0.1 g kg$^{-1}$) (Tephly and McMartin, 1984). Blood MeOH concentrations were non-detectable after cessation of exposure within 4-6 hrs in the Macaca fascicularis monkey (Burbacher et al., 2004) or 8-10 hrs in the cyanomolgus monkey (Dorman et al., 1993). MeOH was oxidized at a constant rate of 24 mg/kg/h during the first 28 hrs following intraperitoneal administration of a 10% $^{14}$C-MeOH solution to male albino rats.
Reports on MeOH poisoning

MeOH has been recognized as a human toxic agent since the end of the 19th century. From 20th century onwards, many hundreds of cases of MeOH intoxication have been reported as single cases and as groups in many countries. MeOH is one of the major adulterants of illicit liquor causing blindness and death (Kumar et al., 2003, Mittal et al., 1991, Ravichandran et al., 1984). It should be noted that the highest morbidity and mortality have been associated with deliberate or accidental oral ingestion of MeOH containing mixtures (WHO, 1997). The preponderance of MeOH poisonings have resulted from the consumption of adulterated alcoholic beverages, e.g., “moonshine”, or “bootleg whiskey”, wood alcohol and spirits mixed with whiskey. Buller and Wood (1904) and Wood and Buller (1904) reported 235 cases of blindness and death primarily connected with drinking adulterated beverages or wood alcohol products, but these also included 10 deaths involving inhalation or absorption of MeOH through skin. Bennett et al., 1953) described a case that occurred in Atlanta, Georgia, USA, in 1951, when within a 5-days period, 323 people consumed bootlegged whiskey contaminated with 35-40% MeOH and 41 of them died.

Kane et al. (1968) reported the poisoning of 18 individuals, out of whom 8 died, when a diluted paint thinner containing approximately 37% MeOH was used as an alcoholic beverage in Lexington, Kentucky, USA. An epidemic in the State Prison of Southern Michigan in 1979 in which MeOH diluents used in photocopying machines was used as “home-made” spirits (containing approximately 3% MeOH) resulted in 46 definite cases of MeOH intoxication and 3 deaths were reported (Swartz et al., 1981).

An outbreak of acute MeOH intoxication involving 28 young men in Papua New Guinea in 1977, each of whom consumed an equivalent of 60-600 ml pure
MeOH resulted in all becoming hospitalized within 8-36 hours due to acute metabolic acidosis, severe visual impairment and acute pancreatitis. Four died within 72 hrs after hospitalization and out of the 24 who recovered, 16 showed no residual complications, 6 had bilateral visual impairment and 2 had difficulty in speech as well as visual impairment (Dethlefs and Naraqi, 1978; Naraqi et al., 1979).

MeOH is present in all alcoholic beverages (Jones, 1987) and has been proved to contribute to some of toxic manifestations of ethanol intake, such as ‘hangover’ (Youssef et al., 2006). From the above, it is clear that MeOH remains to be a major public and environmental health hazard as MeOH is well absorbed from the gastrointestinal tract mucosa as well as through the skin and lungs.

**Signs and symptoms**

The symptoms and signs of MeOH poisoning, which may not appear until after an asymptomatic period of about 12 to 24 hrs, include visual disturbances, nausea, abdominal and muscle pain, dizziness, weakness and disturbances of consciousness ranging from coma to clonic seizures. The concept that the MeOH metabolite is the causative agent for toxicity helps to explain the delayed onset of symptoms (Tephly, 1991). MeOH poisoning is generally manifested by effects on the eye. Common signs of overexposure to MeOH include hyperemia of the optic disc and adjacent retina, peri-papillary edema, central scotoma, diminished pupillary reaction to light, diminished visual acuity, atrophy of the optic nerve heads and complete loss of vision (Dethlefs et al., 1978). Workers exposed to MeOH levels that average 200 ppm for 8-h work shift reported dimmed vision and nasal irritation as the most frequent symptoms (Kawai et al., 1991). Medical examination revealed severe gastric irritability, marked hyperesthesia in both arms and hands, incomplete paralysis of the extensor muscles with wrist drop, mild
ptosis of eyelids and restricted partial amblyopia. Over the ensuing 4 months, there was reported to be a gradual recovery with residual blurring of vision (Niosh, 1976). Optic atrophy is a late finding and permanent blindness may result, although some cases of blindness appear to have resolved (HSDB, 1992).

**Causes of death**

Breathlessness and tachypnea coincide with the onset of acidosis but is rapid and shallow rather than deep and labored. Death in coma is usually attributed to respiratory failure rather than circulatory collapse and complications of metabolic acidosis including coma, convulsions, cardiovascular collapse, cerebral edema and pulmonary edema. Death may be rapid or occur several hours after the onset of coma (Winchester, 1998).

**MeOH and oxidative stress**

MeOH intoxication is also associated with mitochondrial damage and increased microsomal proliferation resulting in increased production of oxygen radicals (Liesivuori and Savolainen 1991; Tephly, 1991). MeOH intoxication may result in oxidative stress results in impaired immune function (Skrzydlewska and Farbiszewski 1997; Dhabhar and McEwen, 1997). MeOH induced oxidative stress disturbs the HPA-axis function by altering the level of corticosterone, which leads to varied non-specific and specific immune responses in experimental rats were also reported (Parthasarathy et al., 2006).

**Treatment for MeOH toxicity**

In mammalian species, MeOH is metabolized to formaldehyde in the liver and by subsequent oxidative steps, formic acid and carbon dioxide are formed (Makar and Tephly, 1977; Jyrki and Savolainen, 1991). Specifically, formic acid
is the toxic metabolite responsible for the metabolic acidosis (Bells, 1991, Murray et al., 1991, Lee et al., 1994a) Carbicarb was chosen to treat the metabolic acidosis (Shapiro et al., 1989, Kucera et al., 1989, Filley and Kindig, 1984, Forsythe and Schmidt, 2000, Bersin and Arieff, 1988) Elimination of MeOH and lactate was tried successfully with enzyme loaded carrier erythrocytes (Jyrkä et al., 1991, Mauro, 1993)

Existing treatment guidelines for MeOH toxicity

The treatment protocol for MeOH poisoning as reported by Barceloux et al. (2002), includes

1. Correction of metabolic acidosis by bicarbonate infusion
2. Administration of folinic acid (active form of folic acid) to facilitate formate metabolism,
3. Administration of alcohol dehydrogenase inhibitor, Fomepizole (alcohol dehydrogenase inhibitor) to prevent further breakdown of MeOH. It is a selective hemodialysis to eliminate MeOH and formate

Focus needed

MeOH exposure could be added to the available MeOH load in the body. In MeOH poisoning, the attention is focused when there is acute exposure leading to clinical symptoms and even then, the focus is only to the visual system. Not only MeOH metabolites but MeOH itself is reported to be toxic to brain (Jeganathan and Namasivayam, 1989) and the brain biogenic amines. Moreover, MeOH is known to induce free radical generation as well as toxic metabolites. In sub lethal exposures, the changes have not been monitored or reported so far. Based on such lacuna the current study is focused to understand the sub lethal
exposure effects of methanol on free radical scavenging enzyme natural free radical scavenging antioxidants

Selection of DL-α-Lipoic acid (LA)

Normally, oxidation process induced by the free radicals could be prevented by using antioxidant LA was chosen to understand because it is a strong antioxidant (Packer et al., 1995) and with increasing interest as an antioxidant and is widely used for the treatment of diabetic poly neuropathy in humans (Kleemann et al., 1989) It is noteworthy that during MeOH poisoning the salient feature observed is the retinal toxicity The retinal pigment epithelium (RPE) undergoes oxidative stress because it is metabolically very active (Snodderly 1995, Miceli et al., 1994, Kennedy et al., 1995) because there are large oxygen fluxes across its boundaries (Wangsa-Wirawan and Linsenmeier, 2003) and because it experiences exposure to sunlight (Cruckshanks et al., 2001; Beatty et al., 2000) LA involves multiple pathways and that LA could be effective against oxidative stress and mitochondrial dysfunction in RPE cells (Ludmila A, 2005) LA also interacts with reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, hypochloric acid, peroxyl radicals, and singlet oxygen (Biewenga et al., 1997) Under these criteria to reduce the MeOH toxicity, LA was selected