CHAPTER I

INTRODUCTION

Recently, male reproductive function in the general population has attracted increasing attention due to reports suggesting that the occurrence of several biological problems affecting the semen quality has risen during the last 50 years (Carlsen et al., 1992; Becker and Berhane, 1997; Swan et al., 1997, 2000; Telisman et al., 2000; Skakkebaek et al., 2006; De Fleurian et al., 2009). A 45% drop in human sperm count, from an average of 113 million per ml of semen in 1940 to 66 million in 1990, potentially jeopardizing male fertility has been reported (Santamarta, 2001; Carlsen et al., 1992; Skakkebaek et al., 2001). Various authors have reaffirmed the possible significant drop in sperm quality and consequently leading to an increase in male infertility rates since 1970s (Nelson and Bunge, 1974; Pasqualotto, et al., 2003). Male infertility is defined as male’s inability to cause pregnancy in a fertile female after at least one year of unprotected intercourse. Male infertility is a multifactorial diseases process with a number of potential contributing causes including environmental pollutants, occupational exposures and lifestyle (Figà-Talamanca et al., 1996; Tielemans et al., 1999; Homan et al., 2007). These causes of infertility in men can be explained by deficiencies in sperm formation, concentration or transportation.

The increase in environmental pollution in industrial countries in recent decades raises questions about the extent of the contribution of environmental and industrial factors to this decline in the quality of sperm (Osser, 1984; Carlsen et al., 1992). National Institute for Occupational Safety and Health Registry listed about 104,000 chemical and physical agents existing in workplaces and the toxicity of most of these materials is not known or has been partially studied (Gold et al., 1994). The male reproductive system is vulnerable to the effects of these physical and chemical agents. These chemical agents
could adversely affect male reproductive system by either disrupting the gonadal endocrine axis (Skakkebæk et al., 2001; Sharpe and Irvine, 2004) or the spermatogenesis process which may results in poor sperm quality (Assennato et al., 1987; Robins et al., 1997; Wyrobek et al., 1997). It is undeniable that good sperm quality is an essential for reproductive success.

Occupational activities involving exposure to specific chemicals or expositions to toxicants may impair male reproductive health and cause infertility in humans which may directly influence couples who are planning to pregnancy. Furthermore, in recent decades, the industrial world has become inundated with an ever-increasing number of chemical and physical agents whose toxicity on male reproductive system is very little known (Oliva et al., 2001; Sheiner et al., 2003; Kumar, 2004). So it is necessary that the effect of environmental and occupational agents on male reproductive health need to be studied in great detail. Diethanolamine (DEA) is one of these occupational chemical agents.

**DIETHANOLAMINE**

Diethanolamine is an alkanolamine which is reactive and bifunctional, combining both the properties of alcohols and amines. Diethanolamine is a colourless, viscous liquid above its melting point of 28°C (82°F) and a white solid below this temperature (Table 1.1). It is produced by reacting 2 moles of ethylene oxide with 1 mole of ammonia (Dow Chemical Company, 1988). In U.S.A. the estimated annual production of DEA was 106,000 tons in 1995 (TPMC, 2002). Worldwide capacity for ethanolamines production was 1.7 million metric tones (3.7 billion pounds) in 2007 (Greiner, 2009). In 1999 information indicated that DEA was manufactured by seven companies in the United States, three companies each in China and Germany, two companies each in France and India, and one company each in Belgium, Brazil, Canada, Iran, Japan, Mexico, Netherlands, the Russian Federation, Spain, Sweden and the United Kingdom (Chemical
<table>
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<th>Characteristics of diethanolamine</th>
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<td><strong>Structural formula</strong></td>
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<td><strong>Molecular formula</strong></td>
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<td>$\text{C}<em>4\text{H}</em>{11}\text{NO}_2$</td>
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<td><strong>Synonyms</strong></td>
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<td>Bis(hydroxyethyl)amine; bis(hydroxyethyl)amine; $N,N'$-bis(2-hydroxyethyl)amine; DEA; $N,N'$-diethanolamine; 2,2$'$-dihydroxydiethyamine; di-(β-hydroxyethyl)amine; di(2-hydroxyethyl)amine; diolamine; 2-(2-hydroxyethylamino)ethanol; iminodiethanol; $N,N'$-iminodiethanol; 2,2$'$-iminodi-1-ethanol</td>
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<tr>
<td><strong>IUPAC Name</strong></td>
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<tr>
<td>2,2$'$-iminobis[ethanol]</td>
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<tr>
<td><strong>Molecular weight</strong></td>
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<td>105.14</td>
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<td><strong>Appearance and odor</strong></td>
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<tr>
<td>Colourless, viscous liquid with a mild ammonia odour</td>
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<td><strong>Melting point</strong></td>
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<td>28 °C</td>
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<td><strong>Boiling point</strong></td>
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<td>268.8 °C</td>
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<td><strong>Solubility in water</strong></td>
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<td><strong>Density</strong></td>
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<td>1.0966 (at 20 °C)</td>
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<td><strong>Partition coefficient</strong></td>
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<td><strong>Vapour pressure</strong></td>
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<td>&lt;0.01 mm Hg (1.33 Pa) at 20 °C</td>
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<td><strong>Conversion factor</strong></td>
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<td>1 ppm = 4.370 mg/m$^3$</td>
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Information Services, 1999). Dow chemical company (2010) reported that DEA is commercially available with a minimum purity of 99.0%, containing 0.5% maximum ethanolamine and 0.5% maximum triethanolamine.

Uses

Diethanolamine is widely used as an industrial chemicals (Wagner, 2006), in agricultural chemicals, metal working fluids and personal care products like cosmetics, shampoos and hair conditioners (CIR, 1983, 1986). In cosmetics, DEA is used as a pH adjuster (Gottschalck and Bailey, 2010). The concentration of diethanolamine in cosmetic formulations may range from 1 to 25% (NTP, 1999). Aqueous DEA solutions are used as solvents for numerous drugs that are administered intravenously (NTP, 1999; Cavender, 2001). It is used in pharmaceutical industries as buffer and stabilizer for certain drugs and also used as raw materials in the production of antihistamines, antimalarials, antibiotics, local anesthetics, antidepressants, and muscle relaxants drugs (Soreat, 1973). Diethanolamine is widely used in preparation of DEA salts of long chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners (Wagner, 2006). Other applications of DEA are in adhesives, antistatic agents, cement and concrete work, coatings, electroplating, an epoxy hardener, a fuel-gelling agent, printing inks, metal cleaning and lubricating, mining, natural gas treatment, paint and pigments, paper, petroleum and coal production, a pharmaceutical intermediate and an ointment emulsifier, polymers and polymer production, rubber processing, soldering flux, textile finishing and polyurethane production (Hammer et al., 1987; Bollmeier, 1992; Knaak et al., 1997; Dow Chemical Company, 1998). Food and Drug Administration (FDA) in 2011 indicated that dithanolamine is used in 22 formulations, 10 are leave-on and 12 are rinse-off formulation.
Exposure

Diethanolamine production and its wide use in industrial and consumer products may result in its release to the environment (Yordy and Alexander, 1981; Beyer et al., 1983; Environment Canada, 1995; Mathews et al., 1995; Knaak et al., 1997). According to the Environmental Protection Agency Toxics Release Inventory, air emissions of DEA was about 149 200 kg in the United States from 358 industrial facilities in 1994 (Environmental Protection Agency, 1996). National Pollutant Release Inventory of Canada reported that release of diethanolamine to air was about 40 000 kg from 74 facilities (Environment Canada, 1995). Because of industrial and consumer uses of DEA and its miscibility with water, large amount of chemical can be discharged into waste water and sewage in an unaltered form (Yordy and Alexander, 1981; Mathews et al., 1995).

General populations are exposed to DEA via dermal exposure through consumer products such as soaps, shampoos and cosmetics. Occupational exposure to DEA may occur by inhalation of vapors and aerosols and by skin contact during the use of DEA in many industries (Knaak et al., 1997; Blum and Lischka, 1997). Diethanolamine is reported to be present in hair sprays. 95% to 99% of the aerosols released from cosmetic sprays have aerodynamic equivalent diameters in 10 to 110 µm range (Johnsen, 2004; Rothe, 2011). So the most of aerosols incidentally inhaled from these sprays are deposited in the nasopharyngeal region and are not respirable (Rothe et al., 2011; Bremmer, 2006). At workplace DEA exposure could occur in a manufacturing facility or a facility which makes products using DEA as a raw material. The potential exposure may occur during process sampling, filter changes and material loading. Diethanolamine has been detected in workplace air in the metal manufacturing industry. Diethanolamine was present in bulk cutting fluids at concentrations ranging from 4 to 5% (Kenyon et al., 1993). National Occupational Exposure Survey estimated that about 800 000 metal workers and others in
United States were potentially exposed to DEA (NOES, 1999). General populations may also be exposed to DEA through cigarette smoking (Hoffman et al., 1982).

**Pharmacokinetics**

The disposition of DEA varies across species, is dose-dependent and characterized by a relatively longer elimination time (Mathews et al., 1995; Mendrala et al., 2001). After application of DEA to the skin of rats (2 to 28 mg/kg bw), DEA was bound to be slowly absorbed (3 to 16% after 48 hours of exposure) (Mathews et al., 1997). After application to mouse skin (8 to 80 mg/kg bw), 25 to 60% was absorbed after 48 hours, with the percent of the applied dose absorbed increasing with dose. The dermal penetration is much higher in mice than that in rats (Sun et al., 1996). Diethanolamine is metabolized by biosynthetic routes common to endogenous alkanolamines (ethanolamine and choline) and incorporated into phospholipids in liver, kidney, spleen and brain of mice and rats (Mathews et al., 1995). It interacts with lipid metabolism in vivo probably by the enzyme-catalyzed transphosphatidylation of phosphatidylcholine by phospholipase D (Dippe et al., 2008). Diethanolamine can be incorporated into phospholipids and inhibit the in vitro and in vivo synthesis of phospholipid derivatives of choline and ethanolamine (Barbee and Hartung, 1979a). Matthews et al. (1997) administered carbon$^{14}$ (C$^{14}$) labeled DEA to rats via the oral, intravenous and dermal routes of exposure to determine how this chemical is taken up and excreted. Mice were also exposed via the dermal route (Mathews et al., 1997). Oral administration of DEA (7 mg/kg bw) once to examine the metabolic profile after a single dose and then daily for up to eight weeks to look at DEA’s potential for bioaccumulation (Mathews et al., 1997). Oral doses of DEA were well absorbed but excreted very slowly. Accumulation of DEA is high in certain tissues, particularly liver and kidney. Diethanolamine is excreted predominantly unchanged with a half-life of approximately one week in urine (IARC, 2000). Only small portion is found as its mono
and dimethylated derivatives. As a result of progressive methylation after repeated oral administration (eight weeks), the relative amounts of N-methyldeethanolamine and N,N-dimethyl-2-oxomorpholinium appearing together with unchanged DEA in urine was found to increase markedly (Mathews et al., 1995, 1997). If a source of nitrite is available (for example, from a nitrite-preserved food), it may combine with DEA in vivo to form a nitrosoamine. N-nitrosodiethanolamine was excreted in the urine of male Sprague-Dawley rats by receiving diethanolamine via the skin and sodium nitrite in the drinking water (Preussaman et al., 1981).

**Toxicological evaluation**

National Cancer Institute nominated DEA for study because of its large annual production, known human exposure, potential for conversion to a known carcinogen in the presence of nitrite N-nitrosodiethanolamine (NDEA), and because there was little adequate toxicity and carcinogenicity data on this chemical (NTP, 1992). The toxicology of DEA has been reviewed by Knaak et al. in 1997. Diethanolamine is an irritant to skin and eyes and cause systemic toxicity mainly in liver, kidney, red blood cells and the nervous system following oral and/or dermal exposure in laboratory animals (Anon., 1983; BIBRA, 1990, 1993). Diethanolamine caused intoxication include increased blood pressure, diuresis, salivation and papillary dilation (Beard and Noe, 1981). Diethanolamine was applied dermally to B6C3F1 mice and F344/N rats with different doses which caused ulceration, irritation and crusting at the application site. Microscopically, ulceration with acanthosis and inflammation was also observed in these animals and ulcerative necrosis extended into the underlying dermis (NTP, 1992). New Zealand White rabbits showed irritation of eye and skin after application of pure DEA (98%). Irritation of skin was moderate, whereas irritation in eye was severe after 72 hours (Dutertre-Catella et al., 1982). In a 13 weeks study of dermal application of DEA
significantly decreased final mean body weight of B6C3F1 mice (Melnick et al., 1994a) and F344/N rats (Melnick et al., 1994b). National Toxicology Program also reported that DEA decreased body weight gain in mice and rats for 2 weeks and 13 weeks of their oral and dermal studies (NTP, 1992). Exposure to 400 mg/m³ DEA resulted in decreased body weight of Wistar rats by inhalation (Gamer et al. 2008).

Eastman (1989) reported that DEA decreased hemoglobin and hematocrit with an increased white blood cell (WBC) count in male albino rats which received feed containing 0.01, 0.1 or 1% DEA. Poorly regenerative, normochromic, microcytic anemia in male and female rats as indicated by dose-dependent decreases in erythrocyte and reticulocyte counts, mean corpuscular volume (MCV), hemoglobin concentration and hematocrit were reported by NTP in their oral and dermal studies (NTP, 1992). Depletion of femoral bone marrow cells was seen in high DEA treated male rats (NTP, 1992). Diethanolamine is also reported as an immunotoxic agent (NTP, 2002). Mendrala et al. (2001) also observed anemia, as an accumulation of radiolabelled DEA in erythrocytes. Grice et al. (1971) reported that DEA produced cytoplasmic vacuolization, basophilia and mitochondrial swelling in hepatocytes and necrosis and cytoplasmic vacuolization in renal tubular epithelium at 4 and 24 hours after doses of 100 or 500 mg/kg DEA. Repeated dermal administration of DEA significantly increased liver weight and caused cell proliferation and apoptosis in liver of mice (Mellert, 2004). Melnick et al. (1994a) reported that DEA increased absolute and relative liver weights, associated with hepatocellular changes and hepatocellular necrosis in male mice dosed with 320-1250 mg/kg DEA by dermal application. Melnick et al. (1994b) also found changes in kidney weight including renal lesions and nephropathy in rats by dermal application of DEA. Similarly liver and kidney toxicity was observed in mice and rats by oral studies (Melnick et al., 1994a,b). Gamer et al. (2008) reported DEA-induced systemic toxicity in liver and
kidney at or above 150 mg/m³ by repeated exposure of DEA. Diethanolamine also increased activity of the serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in mice (Blum et al., 1972). In mice DEA also increased total glycogen, alkaline phosphatase (ALP) and decreased total lipids (Annau et al., 1950; Annau and Manginelli, 1950). Barbee and Hartung (1979b) reported that DEA caused alterations in hepatic mitochondria structure and function. Diethanolamine inhibited the in vitro synthesis of both phosphatidylcholine and phosphatidylethanolamine in liver tissue. (Barbee and Hartung, 1979a). It also inhibits phosphatidylcholine synthesis in rat liver tissue in vivo (Browning and Snyder., 1987)

National Toxicology Program reported that DEA may induce tumors by disrupting phospholipid metabolism, which may perturb cell and organelle membrane function and the synthesis of fatty acid second messengers. Long-term toxicity (NTP, 1999) studies suggest that DEA could have carcinogenic potential in experimental animals. Recently, Kamendulis and Klaunig (2005) have reported that DEA increased liver cell proliferation in in vitro. Hayashi et al. (2003) reported the localization of β-catenin protein in hepatocellular neoplasms and hepatoblastomas in B6C3F1 mice exposed dermally to 0-160 mg/kg bw DEA for 2 years which also caused genetic alterations in the Catnb and H-ras genes. Diethanolamine altered choline homeostasis and caused choline deficiency (Stott et al., 2000; Leung et al., 2005). Dietary choline deficiency is known to promote spontaneous hepatocarcinogenesis in rodents (Newberne et al., 1982; DeCarmargo et al., 1985; Rogers et al., 1987). It was found that DEA treatment caused biochemical changes consistent with choline deficiency in mice (Lehman-McKeeman et al., 2002). Diethanolamine treatment also perturbed choline metabolite concentrations in rodents liver (Lehman-McKeeman et al., 2002). Diethanolamine inhibits choline uptake into cultured Syrian hamster embryo (SHE) and Chinese hamster ovary cells (CHO) and to inhibit the
synthesis of phosphatidylcholine in *in vitro* systems in a concentration-dependent, competitive and reversible manner (Lahman-McKeeman and Gamsky, 1999, 2000). Diethanolamine with phenobarbital altered DNA methylation in GC-rich region resulting in choline deficiency in mouse hepatocytes (Bachman *et al.*, 2006). An altered DNA methylation has been implicated in aberrant expression of genes and has been suggested as an epigenetic mechanism of carcinogenesis (Goodman and Watson, 2002; Eden *et al.*, 2003).

Melnick *et al.* (1994a) reported that DEA increased heart weight and cardiac myocyte degeneration in high dose male and female mice. Demyelination of medulla oblongata of brain and spinal cord was seen by dermal and oral application of DEA (NTP, 1992; Melnick *et al.*, 1994a,b). Diethanolamine also has degenerative effect on the nerve cells of the anterior horn of the spinal cord (El-Mehallawi *et al.*, 2007a). Diethanolamine also caused mild gastric ulceration, hemorrhage or inflammation and lymphoid depletion of the spleen and thymus in rats (NTP, 1992). Microscopic changes in the submandibular salivary gland, diagnosed as cytologic alterations were observed in high dose DEA-treated male and female mice (NTP, 1992). Gamer *et al.* (2008) reported that DEA caused concentration-dependent increase in laryngeal squamous hyperplasia as well as incidence and severity of local inflammation of larynx and trachea.

A reproductive and developmental toxicity was also reviewed by Knaak *et al.*, (1997) for DEA. Dermal application of DEA (0,20,80 or 320 mg/kg) on C57BL/6 mice caused significant decrease in sperm motility and epididymis weight in parental male mice treated with 80 mg/kg group at postnatal day and reductions in weights of reproductive organs of high dose male pups (Lee *et al.*, 2007). Melnick *et al.* (1994a) reported that DEA decreased testis, epididymis weights at doses of 1200 ppm in drinking water in male Fischer 344/N rats. Inhalation exposure of DEA aerosols for 6 hours per day on days 6-15...
of gestation caused an increased incidence of cervical ribs in the fetuses (Game et al., 1993; Marty et al., 1999). Maternal exposure to DEA (80 mg/kg/day) during pregnancy caused diminished proliferation and increased apoptosis of neural progenitor cells in the fetal hippocampus (Craciunescu et al., 2006). Diethanolamine in vitro treatment decreased choline uptake, resulting in diminished choline and phosphocholine in mouse neural precursor cells (Niculescu et al., 2007). It has been previously reported that dermal application of DEA caused adverse effect on testis and sperms in rats (El-Mehallavi et al., 2007b). National Toxicology Program also observed alterations in testis and epididymis of rats by DEA in their oral studies (NTP, 1992).

Diethanolamine creates choline deficiency which has been discussed earlier (Stott et al., 2000; Leung et al., 2005). Other effects associated with choline deficiency include increased generation of free radicals and increased susceptibility to oxidative damage which may induce DNA damage and alter gene expression, increased cell death and increased cellular proliferation (Ghoshal et al., 1983; Rushmore et al., 1984; Counts et al., 1996). It has been previously reported that choline deficient mitochondria leak large amount of reactive oxygen species (ROS) in rats (Banni et al., 1989; Vrablic et al., 2001). So the over production of ROS is also due to abnormal mitochondrial function. Diethanolamine also caused alterations of mitochondrial structure and function which is also discussed earlier (Barbee and Hartung, 1979b). Several studies reported that excess ROS triggers apoptosis in many tissues of both rodent and human (Albright et al., 1996; Albright and Zeisel, 1997; da Costa et al., 2006). Oxidative stress can leads to various disorders including infertility (Cheeseman and Slater, 1993).

About 70-80% of the world population mainly in developing countries used herbal medicines primarily for treating mild and chronic ailments because of the general belief that herbal medicines have no side-effects besides being cheap and locally available
(Gupta and Raina, 1998; Kamboj, 2000). According to the World Health Organization (WHO), the use of herbal medicines throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994). WHO has also listed over 21000 plant species used around the world for medicinal purposes. About 2500 plant species belonging to more than 100 genera are being used in indigenous systems of medicine in India (Yadav et al., 2006). These natural products and especially those derived from higher plants have historically played a pivotal role in the discovery of new pharmaceuticals (Mukherjee, 2002).

**CURCUMIN**

Curcumin is a major chemical component isolated as yellow pigment of turmeric powder produced from the rhizome of the plant *Curcuma longa* (Fig. 1.1) which is commonly used as dietary spice and colouring agent in food (Buescher and Yang, 2000). The plant *Curcuma longa* is of Indian origin and has been used in India for thousands of years and is a major part of Siddha medicine (Chattopadhyay, 2004). Curcumin commonly called as diferuloyl methane, is a low molecular weight hydrophobic polyphenol, first characterized in 1910, which is regarded as the most active constituent and comprises 2-8% of most turmeric preparations (Milobedzka et al., 1910; Heath et al., 2004).

**Chemical properties of Curcumin**

Curcumin was first isolated in 1815 by Vogel and Pelletier, obtained in crystalline form in 1870 and its chemical structure was determined by Rougley and Whiting (Vogel and Pelletier, 1818; Daube, 1870; Roughley and Whiting, 1973). Curcumin has a molecular weight (MW) of 368.37, molecular formula of C_{21}H_{20}O_{8} and melting point of 183°C.

Curcumin is insoluble in water, but dissolves in acetone, dimethylsulphoxide and ethanol. It is a hydrophobic natural product which contain two phenolic rings, each
Figure 1.1: *Curcuma longa* (A) Whole plant, (B) Rhizome and turmeric powder
substituted with a methoxy ether functionality in the ortho-position (Fig. 1.2). These two phenolic rings are connected via an aliphatic unsaturated heptene linker in the para-position that also contains an α, β diketonic functionality on carbon-3 and -5. Several studies have indicated that the diketone functionality can undergo reversible tautomerization between enolic- and ketonic-forms (Payton et al., 2007). These tautomerization of curcumin is pH-dependent. The bis-keto form predominates in acidic and neutral solutions, and the enol-form in alkaline solutions (Sharma et al., 2005). In the bis-keto form, carbon-4 of the heptene linker can function as an extremely powerful proton donor, while in the enol form it functions mainly as an electron donor, chemical activity that bestows upon curcumin is its antioxidant properties (Sharma et al., 2005). At pH 3–7, curcumin acts as an extraordinarily potent H-atom donor (Jovanovic et al., 1999). The antioxidant properties of curcumin are several times more potent than those exhibited by vitamin E (Zhao et al., 1989) Curcumin functions as a pro-oxidant under certain conditions (Aggarwal et al., 2003) most likely as a result of electron transfer to molecular oxygen to generate ROS (Choi et al., 2006). Turmeric contains curcumin along with other chemical constituents are called as curcuminoids. (Srinivasan, 1952). Commercially produced curcumin is composed of three major curcuminoids: curcumin I (described above), demethoxycurcumin (curcumin II; lacks one methoxy functionality, MW-338, Typically 10-20%), and bisdemethoxycurcumin (curcumin III; lacks both methoxy functionalities, MW-308, Typically less than 5%) (Aggarwal et al., 2003; Sharma et al., 2005) (Fig. 1.2).

**Biological and medicinal properties of curcumin**

Curcumin possesses wide variety of desirable preventive or putative biological and medicinal properties (Fig. 1.3). The most important feature of curcumin is that it has no side effect and therapeutic agent with multiple beneficial functions (Kamboj, 2000). Many
Figure 1.2: Chemical structures of the curcuminoids.
Figure 1.3: Biological and medicinal properties of curcumin
scientists have reported that curcumin possessed various promising biological activities such as anti-inflammatory (Ramsewak, 2000), anti-platelet (Srivastava et al., 1986), antioxidant (Venkatesan and Rao, 2000; Rukkumani et al., 2003), cancer chemoprotective (Mariadason et al. 2000), antimutagenic (Nagabhushan et al., 1987), anticancer (Aggrawal et al., 2003), anti- HIV (Jordan and Drew, 1996), anti infection (Duvoix, 2005) and antibacterial (Bhavani Shankar and Sreenivasa Murthy, 1979). Other uses of curcumin are in treatment of Alzheimer’s diseases (Lim et al., 2001; Frautschy et al., 2001), cataract prevention (Awasthi et al., 1996), high cholesterol prevention (Patil and Srinivasan, 1971; Asai and Miyazawa, 2001), liver protection (Morikawa et al., 2002) and as therapeutic agent for multiple sclerosis (Natarajan and Bright, 2002), osteoarthritis (Kulkarni et al., 1991), rheumatoid arthritis (Funk et al., 2006), ulcerative colitis (Ukil et al., 2003) to suppress thrombosis (Srivastava et al., 1986) myocardial infarction (Nirmala and Puvanakrishnan, 1996a,b), diabetes (El-Azab et al., 2011), protection from pulmonary toxicity and fibrosis (Venkatesan, 2000; Punithavathi et al., 2000), nephrotoxicity (Venkatesan et al., 2000) and lung injury (Venkatesan and Chandrakasan, 1995).

Bhavani Shankar et al. (1980) reported that curcumin did not show pathological, behavioural abnormalities or lethality in wistar rats, guinea pigs and monkeys of both sexes at a dose of 300 mg/kg body weight. Similarly, Sambaiah et al. (1982) did not observe any adverse effect on both growth and the level of erythrocytes, leucocytes, blood constituents such as haemoglobin, total serum protein, alkaline phosphatase etc. Curcumin did not show toxicity on human when administered at doses of 1-8 gm/day (Chainani-Wu, 2003) and 10 gm/day (Aggarwal et al., 2003).

Thanqapazham et al. (2007) reported beneficial effects of curcumin and the potential of this compound to be developed as a potent nontoxic agent for treating skin diseases. Topical application of curcumin markedly inhibited TPA- and arachidonic acid-
induced epidermal inflammation (ear edema) in mice (Huang et al., 1991). Curcumin at a level of 0.1% in the diet lowered serum and cholesterol in rats fed with 1% cholesterol-containing diets for 7 weeks (Rao et al., 1970). Soudamini et al. (1992) also reported that oral administration of curcumin lowered serum cholesterol level and lipid peroxidation (LPO) in liver, lung, kidney and brain of mice treated with carbon tetrachloride, paraquat and cyclophosphamide. Curcumin and turmeric decrease blood sugar level in alloxan-induced diabetes in rats (Arun and Nalini, 2002). Sajitlal et al. (1998) reported that curcumin decreased advanced glycation end products induced complications in diabetes mellitus. In vivo study revealed that curcumin treatment for 16 weeks significantly reduced the induction of diabetic retinopathy in rats (Gupta et al., 2011). A recent Egyptian study investigated that curcumin treatment significantly reversed streptozotocin-induced hyperglycemia, glucose intolerance, hypoinsulinemia, and pancreatic islet damage; attenuated pancreatic lipid peroxidation; upregulated antioxidant enzyme activity; and suppressed serum levels of tumor necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1β) (EI-Azab et al., 2011). Curcumin also shows antimicrobial effect against Plasmodium falciparum and Leishmania major organisms (Rasmussen et al., 2000).

Curcumin is effective against carrageenin-induced oedema in rat (Brouet and Ohshima, 1995) and mice (Srimul and Dhawal, 1985). Curcumin also functions as a potent anti-inflammatory agent in periodontal disease (Guimaraes et al., 2011). Deodhar et al. (1980) also investigated antirheumatic activity of curcumin in patients who showed significant improvement in symptoms after administration of curcumin. Venkatesan (1998) showed protective effect of curcumin on acute Adriamycin (ADR) myocardial toxicity in rats. Srivastava and his colleagues (1986) reported that curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation in vitro as well as in vivo in rat thoracic aorta. A Chinese study reported the potential
effect of curcumin as a therapeutic agent in Alzheimer’s disease in vitro (Zhang et al., 2011). Administration of curcumin exerted a strong protective effect in rat suffered from spinal cord injury via its antioxidant potential (Sahin et al., 2011). A recent study reported for the usefulness of curcumin as an agent for the treatment of gastrointestinal disorders such as diarrhea, abdominal cramps and irritable bowel syndrome (Kumar et al., 2010). Curcumin and its analogues shows protective activity in cultured rat hepatocytes against carbon tetrachloride, D-galactosamine, peroxide and ionophore-induced toxicity (Hikino, 1985; Song et al., 2001; Kang et al., 2002). Curcumin showed protective effect against diethylnitrosamine and 2-acetylaminofluorine-induced altered hepatic foci development (Shukla and Arora, 2003). Curcumin also protect the liver damage induced by biliary obstruction in rats (Erenoglu met et al., 2011). Gukovsky et al. (2003) reported that curcumin ameliorates pancreatitis in two rat models. Curcumin also increases the activity of pancreatic lipase, amylase, trypsin and chymotrypsin (Platel and Srinivasan, 2000).

Venkatesan and Chandrakasan (1995) investigated an increase antioxidant defense mechanism in rats fed with curcumin for 7 days prior to being treated with cyclophosphamide to induce lung injury. Curcumin exerted protective effect against nicotine-induced lung toxicity by modulating the biochemical marker enzymes, LPO and augmenting antioxidants status in bronchoalveolar lavage fluid (BALF) and bronchoalveolar lavage (BAL) of nicotine-treated Wistar rats (Kalapana and Menon, 2004). Curcumin also exerted a potent protective effect on the lungs following a cardiopulmonary bypass (Liu et al., 2012). Venkatesan et al. (2000) reported that curcumin prevents Adriamycin (ADR)-induced nephrotoxicity in rats. Curcumin prevented ADR-induced proteinuria, albuminuria, hypoalbuminemia and hyperlipidemia. An Egyptian study reported that pretreatment with curcumin exerted protective effects in rats subjected to acute renal injury (Awasd and EI-sharif, 2011). Renoprotective effect of
Curcumin by a remarkable improvement of renal function in gentamicin injected rats was also reported (El-Zawahry and Abu El-Kheir, 2007). Curcumin also showed protective effect against cadmium chloride-induced nephrotoxicity in Swiss albino mice and rat (Singh et al., 2010; Tarasub et al., 2011).

Curcumin is considered to be an effective antioxidant against oxidative damage. Curcumin acts as a scavenger of oxygen free radicals (Ruby et al., 1995; Subramanian, 1994). Curcumin can significantly inhibit the generation of (ROS) like superoxide anions, hydrogen peroxide ($H_2O_2$) and nitrite radical generation by activated macrophages in vitro, which play an important role in inflammation (Joe and Lokesh, 1994). Curcumin also lowers the production of ROS in vivo (Joe and Lokesh, 1994). Curcumin shows powerful inhibitory effect against $H_2O_2$-induced damage in human keratinocytes and fibroblasts (Phan et al., 2001) and in NG 108-15 cells (Mahakunakorn et al., 2003). Curcumin also reduced oxidative damage and amyloid pathology in an Alzheimer transgenic mouse model (Lim et al., 2001). Pulla and Lokesh (1994) reported that curcumin decreased lipid peroxidation in rat liver microsomes, erythrocytes membranes and brain homogenates. The antioxidant mechanism of curcumin is due to its unique conjugated structure, which includes two methoxylated phenols and an enol form of β-diketone, this structure which shows typical radical-trapping ability and as a chain-breaking antioxidant (Sreejayan and Rao, 1994; Masuda et al., 2001).

Several studies indicate that curcumin plays important role in its anticarcinogenic effects. Curcumin suppressed tumorigenesis of the skin, mammary gland, oral cavity, forestomach, oesophagus, stomach, intestine, colon, lung, and liver (Rao et al., 1984; Kuttan et al., 1985, 1987; Huang et al., 1988; Limtrakul et al., 1997; Deshpande et al., 1998; Piper et al., 1998; Chuang et al., 2000; Ushida et al., 2000; Lee et al., 2005). Inano et al. (1999, 2000) also reported preventive effect of curcumin on diethylstilbestrol (DES)-
dependent promotion in radiation initiated mammary tumorigenesis in rats. Curcumin also induced apoptotic cell death by DNA-damage in human cancer cell lines, TK-10, MCF-7 and UACC-62 by acting as topoisomerase II poison (Martin et al., 2003). Several researchers reported that curcumin inhibits the proliferation of a wide variety of tumor cells, including B-cell and T-cell leukemia (Kuo et al., 1996; Ranjan et al., 1999; Piwocka et al., 1999; Han et al., 1999), colon carcinoma (Chen et al., 1999), epidermoid carcinoma (Korutla and Kumar, 1994), head and neck squamous cell carcinoma (Aggarwal et al., 2004), multiple myeloma cells (MM) (Bharti et al., 2003), and mantle cell lymphoma (Shishodia et al., 2005). Curcumin also suppressed the proliferation of various breast carcinoma cell lines in culture (Mehta et al., 1997; Simon et al., 1998; Ramachandran and You, 1999).

Curcumin has been shown to reduce the number of aberrant cells in cyclophosphamide-induced chromosomal aberration in Wistar rats at 100 and 200 mg/kg body wt doses (Shukla et al., 2002). Antimutagenic potential of curcumin against sodium azide-induced clastogenic damage in Allium cepa was reported by Raganathan and Panneerselvum (2007). Curcumin reduced the lipid peroxidation and genotoxic damage induced by tinidazole in cultured human lymphocytes (Siddique et al., 2010). Singh and Sankhla (2010) also reported protective effect of curcumin on cadmium chloride induced genotoxicity in bone marrow chromosomes of Swiss albino mice.

Diethanolamine is selected for evaluation because of its large scale production and pattern of use indicating the potential for widespread human exposure. Review of literature of DEA indicates studies on the liver and other vital organs. However, very limited studies have been done on reproductive system. Since there is continuous decline in fertility worldwide, we made an attempt to study its effect on male reproductive system in detail.
The present study is an attempt to investigate DEA-induced biochemical and histopathological changes in reproductive organs of male Swiss albino mice. In addition, ameliorative effect of curcumin, on DEA-induced toxicity will also be evaluated.
AIMS AND OBJECTIVES

Occupational chemicals are known to have a negative impact on male reproduction and eventually create male infertility. Male infertility is a major clinical worldwide problem which affects the people medically and psychologically. Hence the main aims and objectives of present study were to evaluate the:

(a) Effect of DEA on human spermatozoa in *in vitro* and its possible amelioration by curcumin

(b) Effect of DEA on biochemical parameters and histopathology of reproductive organs of male Swiss albino mice and its possible amelioration by curcumin
HYPOTHESES TESTED

The study was designed to test the reproductive toxicity of DEA and its possible amelioration by curcumin. Therefore, the following hypotheses were framed and tested in this present work which was made in Null form.

Hypotheses Proposed:

(1) DEA may not be causing significant concentration and time-dependent effect on human spermatozoa.

(2) DEA may not be causing significant dose-dependent alterations in biochemical parameters and histopathology of male reproductive organs of Swiss albino mice.

(3) The therapeutic treatment of curcumin will not be effective against the toxicity of DEA.