CHAPTER I
INTRODUCTION

The growing population and expanding industrialization are responsible for an increasing and complex range of health hazards in developed and developing countries. In the present world, people are exposed to a great variety of natural and man-made substances. Under certain conditions such exposures cause adverse health effects, ranging from subtle biologic changes to even death. The ever increasing quest of society to identify and present these ill-effects has led to the dramatic evolution of toxicology.

An escalation in the concentration of toxic pollutant in the biosphere and their ultimate entry into the biological system will pose serious problems on human and natural resources and also on the ecological balance. Indiscriminate use of metals in various industrial and agricultural processes also leads to various health hazards in the environment.

The unplanned and uncontrolled extraction of ground eater has disturbed the hydrological balance resulting in rapid depletion of water table as well as deterioration of water quality. The high concentration of fluoride and nitrate in potable water makes it unfit for human consumption. The fluoride and nitrate in groundwater depends on the geological, chemical and physical characteristics of the soils and rocks, temperature and the action of other chemicals.

The sources of fluoride are drinking eater, air, food, industrial exposure, drugs, cosmetics, dental products etc. The high fluoride in different food products were
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reported in tea (4.97 ppm), canned fish (4.57 ppm), shellfish (3.36 ppm) and cooked wheat cereal (1.02 ppm) (ATSDR, 2001). The fluoride pollution sources of greatest magnitude are those associated with industrial sites, including manufacturers of bricks, iron, fertilizers, glass, cial-fired power stations and particularly aluminum smelters (Polomski et al., 1982a; Pickering, 1985; Haidouti, 1991; Gritsan et al., 1985). More widespread source of fluoride pollution in agricultural soils is the long-term application of phosphate fertilizers (McLaughlin et al., 1996), whereas water extractable fluoride tends to increase with depth (Polomski et al., 1982b; Haidouti, 1991).

In India, fluoride is the major inorganic pollutant of natural origin found in groundwater. The fluoride research in the past decades suggests that concentration below 1 ppm are beneficial in the prevention of dental caries of tooth decay, but above 1.5 ppm increase the severity of the incurable disease fluorosis. The latest estimation suggests that around 200 million people, from among 25 nations the world over, are under the dreadful fate of fluorosis. Fluorosis is the most widespread geochemical disease in India, affecting more than 66 million people including 6 million children less than 14 years age. Though fluoride has spread its tentacles in 3,988 habitations (DDWS, 2004) and the number of people falling prey to fluoride poisoning have been steadily increasing, an exact exposure-health relationship is yet to be properly elucidated. There is an essential relation between poverty and fluorosis as malnutrition is found to play an aggressive role in its severity.

Naturally, occurring fluorides in groundwater are a result of the dissolution of fluoride containing rock minerals by water while artificially high soil fluoride levels
eruptions. Fluorine cannot be destroyed in the environment; it can only change its form. Fluorides released into the atmosphere from volcanoes, power plants, other high temperature processes usually hydrogen fluoride gas or attached to very small particles. Fluorides contained in windblown soil are generally found in larger particles. These particles settle to the ground or are washed out by rain. Fluorides that are attached to very small particles may stay in the air for many days. Fluorides associate with various elements present in the water, mainly with aluminum in freshwater and calcium and magnesium in seawater, and settle into the sediment where they are strongly attached to sediment particles. When deposited on land, fluorides are strongly retained by soil, forming strong associations with soil components. Leaching removes only a small amount of fluorides from soils. Fluorides may be taken up from soil and accumulate in plants, or they may be deposited on the upper parts of the plants. Animals that eat fluoride-containing plants may accumulate fluoride. However, the fluoride accumulates primarily in the bones or shell rather than in edible meat.

**LETHAL DOSE \textsubscript{50} OF FLUORIDE IN ANIMALS**

In rats, LD\textsubscript{50} values for sodium fluoride administered by oral gavages ranging from 31 to 126.3 mg fluoride/kg (Whitford et al., 1990). Differences in rat strains, variations in weight (and presumably differences in ages), and gender differences may account for the reported differences in LD\textsubscript{50} values. LD\textsubscript{50} values were higher in younger female rats (52–54 mg fluoride/kg) than in older female rats (31 mg fluoride/kg) (DeLopez et al., 1976). LD\textsubscript{50} values (84.3–146.3 mg fluoride/kg) were also
estimated in rats administered monofluorophosphate (Whitford et al., 1990). These 
LD50 values were similar to the LD50 values for sodium fluoride (85.5-126.3 mg 
fluoride/kg) measured in the same study. The LD50 value for mice is 54.41 mg F/ kg 
body weight while the females have LD50 value of 51.6 mg F/kg body weight (Pillai et 
al., 1987; 1988) and for male and female rats are 250 mg and 180 mg F/kg body 
weight respectively (Chinoy, 1991a).

HALF LIFE

The toxicokinetic studies revealed that the absorbed fluoride is distributed 
between blood, soft tissues and the skeleton. The half life of fluoride in blood and of 
tissues has been reported to be few hours, while in skeleton, it has a longer half life 
of about 8 years (WHO, 1984).

WORLDWIDE OCCURRENCE OF FLUORIDE

Fluoride is the most electro negative and reactive of all elements and thus, in 
nature, is rarely found in its elemental state. The fluorosis endemic countries are 
Argentina, United States of America, Morocco, Algeria, Libya, Jordan, Egypt, Syria, 
Turkey, Iraq, Iran, Pakistan, Kenya, Tanzania, South Africa, China, Australia, New 
Zealand, Japan and Thailand (Connett, 2000; Wang et al., 2007).

At present, fluorosis is prevalent in 20 states of India. The endemic states are 
Andhra Pradesh, Gujarat, Bihar, Madhya Pradesh, Rajasthan, Assam, Tamil Nadu, 
Uttar Pradesh, Punjab, Haryana, Maharashtra, Kerala, Jammu and Kashmir and Delhi, 
around 20 million people are severely affected by fluorosis and around 40 million are
exposed to its risk in India (Chinoy, 1991a). Fluoride has both notable chemical qualities and physiological properties, which are of great interest and significant to human health.

**SOURCES OF FLUORIDE**

Fluoride is found in man's natural environment and under normal conditions. It is present in our food, water, soil, air and vegetation. The extensive distribution of fluoride in the nature is a direct source for human population resulting in adverse health hazards.

**FLUORIDE IN AIR**

Gaseous and particulate both forms of fluoride emitted into the air. Traces of fluoride in the air of rural communities and cities arise from both natural sources and human activities. The natural dispersal of fluoride into the air has long been recognizing in regions of volcanic activity and in vicinity of industries (US EPA, 1980). Other natural sources of fluoride in the air are the dust from soils, and sea-water droplets, carried up into the atmosphere by winds.

The burning of fluoride containing fuels (coal, wood, oil and peat) and due to pollution from industrial sources were increases air borne fluoride with increasing urbanization.


**Introduction**

**FLUORIDE IN THE LITHOSPHERE**

Fluoride has an atomic weight of 19.0 and an atomic number of 9. Fluorides account for about 0.032% of the earth’s crust. The mean fluoride content of rocks lies between 0.1 to 1.0 g/kg. The main primary fluoride containing minerals are fluorspar (CaF₂), fluorapatite (Ca₁₀[PO₄]₆F₂), Cryolite (Na₃AlF₆), and apatite Ca₅(PO₄)₃(F,Cl,OH), but in most soils it is associated with micas and other clay minerals. It is 17th in the order of abundance of elements in the earth’s crust. Sodium fluoride and magnesium fluoride is also found as natural minerals.

The mean fluoride content of mineral soils is 0.2-0.3 g/kg, whereas, that of organic soils is usually lower. However, in soils which have developed from fluoride containing minerals it may range from 7 to 38 g/kg (WHO, 1984). The fluoride content of top soil may be increased by the addition of fluoride containing phosphate fertilizers, pesticides, irrigation water, or by deposition of gaseous and particulate emissions.

**FLUORIDE IN WATER**

Fluoride is present in both surface and ground water. So, some fluoride compounds in the earth's upper crust are fairly soluble in water. The natural concentration of fluoride in ground water depends on factors such as the geological, chemical, and physical characteristics of the water-supplying area, the consistency of the soil, the porosity of rocks, the pH and temperature, the complexing action of other elements, and the depth of wells. Owing to these factors, fluoride concentrations in ground water fluctuate within wide limits e.g., from <1 to 25 or
more mg per liter. In some areas of the world e.g., India, Kenya, and South Africa, the levels can be much higher than 25 mg/liter. In surface fresh waters, less influenced by fluoride containing rocks, the fluoride content is usually low, 0.01-0.3 mg/liter than in sea (WHO, 1984). Air borne fluoride is returned by the way of snow and rainfall, when occurring over land, it eventually reaches the oceans via rivers (WHO, 1984).

**FLUORIDE IN FOOD AND BEVERAGES**

An assortment of values for fluoride concentrations in vegetables have reported. The fluoride content of vegetables cooked in fluoridated water is higher than the content of vegetables cooked in water containing negligible amounts of fluoride. Spinach, cabbage, lettuce, and parsley have higher values than other vegetables. The fluoride content of water used in industrial food production and home cooking affects the fluoride content of ready-to-eat products ranging from 0.60 to 1.0 mg/kg (US EPA, 1980). Mineral waters may contain fluoride levels higher than 1 mg/litre. Substitutes for human milk like Infant formulae, infant gruel, syrups, and juices prepared with fluoridated water contain 0.9-1.3 mg fluoride/liter. About 40-90% of the fluoride in tea leaves is eluted by brewing. Tea leaves are usually very rich in fluoride, and contain levels ranging from 3.2-400 mg/kg dry weight.

**FLUORIDE INTAKE IN HUMAN BEING**

The human intake can be estimated by the fluoride content present in water, air and food. Estimations on those persons, living near industrial sources of fluoride
could inhale 0.06 mg fluoride during a day of maximal pollution. Occupational exposure may add considerably to the total intake of fluoride viz., the mining and processing of fluorspar, cryolite and apatite (WHO, 1984). In communities, where the water is fluoridated, people would consume a mean of 2.7 mg fluoride/day as compared to 0.9 mg/day, where the water is not fluoridated. Accidental intake of sodium fluoride tablets has occasionally resulted in fluoride intoxication in children (Duxbury et al., 1982).

Fluoride also finds its way into the body through fluoridated toothpastes (WHO, 1984). In the past 30 years, toothpaste has become a far greater source of fluoride in the world than fluoridated water. Using fluoride is the most effective and economical method of protecting the tooth against decay. When tested for cost effectiveness, it has been calculated that it costs 100 times more to treat an individual decayed tooth than to prevent one through the use of fluoride. In India, around 50% of the populations are known to use toothpastes.

**ABSORPTION OF FLUORIDE**

Probably by simple diffusion, fluoride salts are rapidly and almost completely absorbed from gastrointestinal tract. Fluoride from insoluble substance or sparingly soluble substance such as calcium fluoride and cryolite is less efficiently absorbed. However, some fluoride may be more easily dissolve in the stomach because of the low pH, and hydrogen fluoride will then be formed. This chemical may easily penetrate biological membrane, and its chemical reactivity is the probable cause of resulting gastrointestinal symptoms when large amount have been ingested (WHO,
Approximately 75-90% of the fluoride ingested each day is absorbed from the alimentary tract. The half-time for absorption is approximately 30 minutes, so peak plasma concentrations usually occur within 30-60 minutes. Absorption across the oral mucosa is limited and probably accounts for less than 1% of the daily intake. Absorption from the stomach occurs readily and is inversely related to the pH of the gastric contents (Whitford and Pashley, 1984).

Within the stomach, low pH gastric acid favors the formation of the hydrogen fluoride complex, which comprises over 90% of the total fluoride at pH 2 (Doull et al., 2006). Hydrogen fluoride is readily absorbed from both the stomach and small intestine by a process of simple diffusion, and once it enters the less acidic mucosa, it dissociates to release fluoride (Whitford, 1996). About half of the absorbed fluoride is quickly incorporated into developing bone and teeth, where nearly all of the body's fluoride is found, and the remainder is excreted in the urine (Cerklewski, 1987). The uptake of fluoride by the skeleton is most efficient in children and decreases with age (Whitford et al., 1999), but this process can continue up to age 55 (Rao, 2003). Once incorporated into hard tissues, fluoride is retrievable, but this entails an extremely slow process of osteoclastic resorption that occurs over many years (Doull et al., 2006). Because the absorption of soluble inorganic fluoride is largely controlled by acidity in the stomach, systemic fluoride absorption from drinking water does not vary with overall water quality (Maguire et al., 2005). However, the absorption of less soluble inorganic and organic fluorides is more complicated, and a variety of dietary factors can either increase or decrease the amount that is absorbed (Cerklewski, 1987).
FLUORIDE AND BIOLOGICAL FUNCTIONS

Fluoride may have a role interrelated with absorption or utilization of some dietary nutrients. There is evidence that fluoride could enhance the intestinal absorption of iron.

FLUORIDE EFFECTS

ACUTE EFFECTS

Acute exposures are now rare, but over exposures causing toxic signs and symptoms. The clinical course of systemic toxicity from ingested fluoride begins with gastric signs and symptoms, and can develop with alarming rapidity. Treatment involves minimizing absorption by administering solution containing calcium, monitoring and managing plasma calcium and potassium concentrations, acid-base status, and supporting vital functions (Whitford, 2011). Enzymes involved in vital processes are inhibited, and severe hyperglycemia has been noted in some cases. Fluoride is a fairly effective inhibitor of cholinesterase, and this characteristic, with the decrease in plasma calcium concentration that has been observed, may be responsible for effects on the nervous system. The decrease in calcium concentrations have also been postulated to affect blood clotting and membrane permeability as well as an increase in skeletal muscle excitability, hyperactive reflexes and painful spasm (WHO, 1984). Cell damage and necrosis produce massive impairment in the function of vital organs, and particularly when fluoride is given orally, there are severe local effects on the gastric and intestinal mucosa. The symptoms of acute fluoride poisoning usually include nausea, vomiting, excessive
Smedley, 2005). The mechanism(s) that leads to skeletal fluorosis are poorly understood; however, the stages of development are well-documented (Susheela, 2003). The chemical structure of the bone is adversely affected causing osteomalacia, osteoporosis and osteosclerosis. In patients suffering from endemic genu valgum (knock knee), besides osteoporosis, cystic expansion of short and long bones occur. Skeletal fluorosis affects young and old alike. Fluoride ingested by a mother can accumulate in the skeleton of the growing fetus. In general, elevated dietary fluoride results in an acceleration of bone mineralization. However, the increase in mineralization is accompanied by a decrease in bone strength. An X-ray examination of the bones reveals thickening and high density of bones. In some patients with calcium deficiency osteomalacia type changes are seen. Constriction of vertebral canal and intervertebral foramen - pressure on nerves leads to paralysis.

Investigations have shown that fluoride affects not only bones and the skeleton, but also the muscles (Vani and Reddy, 2000), gastro-intestinal systems (Susheela et al., 1992), erythrocytes (Yur et al., 2003; Bouaziz et al., 2006), endocrine glands (Balabalkin et al., 1995; Gupta et al., 2001) and vital organs (Dote et al., 2000; Xiong et al., 2006; Agrawal and Sharma, 2008).

The primary symptoms of gastrointestinal disorders are nausea, vomiting and abdominal pain. The lining of the stomach and duodenum were severely damaged by the toxic effects of fluoride, which results in stomach and abdominal pain of abrasion with loss of microvilli (Sharma et al., 2009).
TOXIC EFFECTS OF FLUORIDE ON EXPERIMENTAL ANIMALS AND LIVESTOCK

Fluoride enters in plants through water and soil in endemic areas as well as from air in the vicinity of industries. Once fluoride enters to plant, it moves to animals. Animals such grazing on vegetation has been found to be affected adversely. There have been reports concerning fluorosis in cattle reared in a polluted area, where the animals were fed on vegetation contaminated by fluoride (Chinoy, 1995). The animals exhibited a nonspecific and typical lameness or stiffness associated with calcification of periarticular structures and tendon insertions, thickening of bones and mineralization of the tendons. Thus, lameness is often found to be transitory in nature and limits feeding or grazing time, thereby impairing performance of the animal (US EPA, 1980).

In some research, rapid absorption of fluoride by ruminants was demonstrated by using radioactive fluoride. The symptoms in teeth were chalkiness and mottling. Field studies have found poor reproduction, diarrhoea and overgrowth of the hoofs due to high consumption of fluoride in cattle.

EFFECTS OF FLUORIDE ON GENERAL BODY METABOLISM

The toxicity of fluoride in aggravated mainly through it adverse effect on general body and tissue metabolism. Use fluoridated toothpaste that we must avoid (Balan, 2012), as fluoride is associated with food stuffs we eat.
fluoride at a phosphate fertilizer factory in North China was significantly higher than that of controls (Zhang and Meng, 1999).

The incidence of Down's syndrome with increasing concentrations of fluoride has been reported in human population residing in endemic areas in Sweden (Berglund et al., 1980). Takahashi (1998) has also reported fluoride related incidence of Down's syndrome births in young mothers in five countries of metropolitan Atlanta, Georgia and in several regions of USA with fluoridated water. Populations between areas with low (<0.2 mg/litre) and high (0.8-2.6 mg/litre) fluoride levels, did not find the difference in the occurrence of Down's syndrome.

The above information clearly demonstrates that at present there is no established opinion regarding the genotoxic effects of fluoride and its potential as a mutagenic agent. It is apparent that further investigations are necessary in order to clarify this important issue. But efforts in this direction are underway at present in our laboratory, it was recently reported that simultaneous exposure to both xenobiotics increased the induction of genotoxic effects in in vitro and in vivo system fluoride and arsenic induces genotoxicity (Chinoy et al., 1996; Pant and Rao, 2010; Tiwari and Rao, 2010; Barbier et al., 2010).

TERATOGENIC EFFECTS OF FLUORIDE

Embryo and fetal toxicity from high doses of fluoride have been reported in experimental animals. High doses of fluoride (3 to 12 mg/kg body weight/day) have been found to cause abortions, necrosis of placenta and affect fetal growth in rats. Studies carried out by Glenn et al. (1982) suggest that fluoride may also exert effects
formation of hydrofluoric acid in the gut were noticed. Fluoride affects cellular protein synthesis in the gastrointestinal organs (Shashi et al., 1987). Scanning electron microscopic studies carried out by Susheela et al. (1992) revealed widespread damage to the stomach mucosa viz., loss of microvilli and desquamated epithelium due to fluoride intake. The corrosive nature of hydrogen fluoride possibly leads to inflammation, ulceration and other mucosal abnormalities in the stomach and proximal small intestine.

**INTESTINE**

The intestinal cell lining plays an important role in digestion and absorption. It automatically becomes the most exposed site of contact to fluoride following ingestion. Study has shown significant alterations in the formation of lipid peroxides in rat intestine following oral administration of fluoride (Shayiq et al., 1986). Rastogi et al. (1987) observed that higher fluoride concentrations cause substantial damage to the intestinal brush border membrane.

**LIVER**

Direct affect of any toxic substance can be seen on liver in the body. Zonal necrosis is the most common symptom in liver of NaF treated rats, mice and mudskippers (Chinoy, 1991a,b; 1992). The hepatic lobules were hyalinized with loss of cells and vacuolization of cytoplasm. The shape of hepatocyte nuclei was irregular and they were pycnotic. The arrangement of hepatic cord was also disturbed (Kour et al., 1981; Chinoy, 1991a,b). Adachi et al. (2007) observed severe hepato-cellular
species of animals, pulmonary damage due to exposure to reactive gases of fluoride was evident (Morris and Smith, 1982). In acute toxicity, respiratory depression, and coagulation, necrosis and congestion in lung were reported. In the reports of industrial vicinity, pneumonia, carcinoma and lung abscess besides the common respiratory obstacles in inhabitants. The delicate tissues of the lung got intensely and fatally damaged in industrial workers and bronchial asthma was evident by fluoride. Thus, exposure to fluoride compounds is harmful and damage respiratory tract.

**EFFECTS ON CARDIOVASCULAR SYSTEM**

There is limited information available on the role of fluoride on cardiovascular functions. Intravenous infusion of fluoride caused a depression of blood pressure, heart and respiratory rate. Caruso et al. (1970) observed a direct vasodilatory effect by fluoride. Vascular changes, characterized by micro-vascular injury, perivascular disintegration of tissue cells, and vascular proliferation were predominated by fluoride ingestion. It is believed that calcification of arteries is an integral feature of skeletal fluorosis.

**HEART**

Fluoride causes the chronic myocarditis and dystrophic changes in heart muscle fibres of rats. Fluoride is reported to decrease the blood pressure and heart beat. High doses of fluoride have been reported to cause severe heart damage leading to cardiac irregularities and irregular electrocardiogram in humans (Zhiliang et al., 1987). The aorta is known to accumulate the highest amount of fluoride as
PANCREAS

NaF treatment brought about no alterations in the histology of pancreas as compared to control except that the Islet cells appeared more pyknotic as compared to normal (Chinoy, 1991a,b). Clinical study on the effect of high fluoride intake revealed that the β-cells of pancreatic islets were damaged (Xie et al., 1999). Hence insulin production may be affected.

THE PINEAL GLAND AND FLUORIDE

Pineal gland is known to be the primary target organ for the accumulation of fluoride in all soft tissues and its impact is on melatonin synthesis and regulation in animals including man. Luke (1997; 2001) conducted experiments in animals and found its maximum accumulation in pineal gland and also found the increasing amount of fluoride correlated with concomitant lower levels of circulating melatonin as reflected by reduced levels of melatonin metabolite in animal’s urine. In conclusion, the human pineal gland accumulates the highest concentration of fluoride in the body. It is also associated with depressed pineal melatonin synthesis by prepubertal gerbils followed by an accelerated onset of sexual maturation in the female gerbil (Luke, 1997).

HYPOTHALOMO – HYPOPHYSEAL SYSTEM

Fluoride also affects hypothalamo-hypophyseal axis in mammals. Morphological changes in hypothalmo – hypophyscal neurosecretary systems attributed to accumulation of fluoride in rats and guinea pigs (Zhavoronkov and
SPERMATOZOA

Recent studies on fluoride treated rats and mice have revealed inhibition of sperm acrosomal enzymes, namely, hyaluronidase and acrosin (Narayana and Chinoy, 1994b; Chinoy and Sharma, 2000). Schoff and Lardy (1987) reported that fluoride is a strong inhibitor of glycolysis and respiration process in spermatozoa. The sperm of NaF treated rabbits when stained with silver nitrate (specific for acrosomal integrity) exhibited head to head agglutination, deflagellation and loss of acrosome (Chinoy et al., 1991a). These alterations in sperm structure and metabolism are the result of the hostile internal milieu of epididymis affecting sperm maturation which ultimately led to a decline in sperm count, motility (Kumar et al., 2012) and their fertilizability and subsequently to a significant reduction in fertility after NaF treatment (Chinoy, 1991a; Chinoy and Sequeira, 1992; Rao and Bhatt, 2012). Human spermatozoa lost their motility in vitro in the presence of 250 mM NaF within 20 minutes incubation (Chinoy and Narayana, 1994).

VAS DEFERENS

The histoarchitecture of the deferens of fluoride treated mice indicated nuclear pyknosis in the epithelial region, clumping of stereocilia, increase in thickness of lamina propria and muscle coat as well as absence of sperm in the lumen (Chinoy and Sharma, 1999b; Rao and Bhatt, 2012). In rabbits treated with NaF (10 mg/kg body weight) for 18 to 29 months, loss of stereocilia on the epithelial cell lining the lumen of the vas deferens with abundant mucus droplets (Susheela and
Kumar, 1991) was observed. Alterations in histology of other sex accessory organs were also reported (Chinoy and Sequeira, 1989b; Chinoy et al., 1991a).

**MALE ACCESSORY GLANDS**

NaF (10mg/kg) treated mice showed decrease in acid phosphatase, a marker enzyme for prostate function, suggests changes in prostate metabolism (Chinoy et al., 1994c; 2005b). Sharma et al. (2008a) carried out fluoride toxicity in rats, revealed significant reduction in seminal vesicle weight. Sodium fluoride treatment resulted in lowered fructose concentration in the seminal vesicles. Similarly, the acid phosphatase and protein levels were also affected by NaF which were to a great extent responsible for low sperm motility resulting in reduction of fertility (Chinoy and Sharma, 1998).

**FEMALE REPRODUCTION**

**OVARY**

The follicular cells surrounding the primary and secondary follicles were found to be pyknolic (Chinoy and Patel, 1998b). Atrophy and confluence of endometrial glands was observed with nuclear pyknosis (Chinoy and Patel, 1998b). The fluoride water induced reduction in weighs of ovary, uterus, vagina, kidney and adrenal gland. The tissue and serum biochemistry were altered and increased cholesterol concentration of ovary and adrenal gland (Sharma et al., 2008b). Fluoride induced free radical toxicity in the mouse ovary observed by Jhala et al. (2008).
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histology of ovary of mice after 30 days of NaF treatment showed vacuolisation of the stromal region and corpora lutea (Chawla and Rao, 2012).

UTERUS

Jhala et al. (2004) exposed sodium fluoride (5 mg/ kg body weight) and arsenic trioxide (AS$_2$O$_3$) to adult female mice for 30 days for their effects on ovarian histology and steroidogenesis in female mice. Sharma et al. (2006a) investigated that fluoride water (5.8 ppm) for 15 and 30 days to female rats caused irregular estrus cycle, reduced fertility rate, weight of ovary, uterus and vagina. NaF treatment for 60 days brought about a decrease in the thickness of serosa and myometrium of uterus. Vacuolisation was observed in the serosa with dense pyknosis in the endometrium (Solanki et al., 2007).

OTHER ACCESSORY GLANDS

Epidemiological study of gynecological problems in female workers in a superphosphate manufacturing plant offers a direct evidence of fluoride effects in human pregnancies. The female workers were found to suffer more menstrual irregularities, vaginal and uterine inflammation, and more frequent toxicosis during pregnancy with hypotension and threatened abortions, a higher percentage of untimely discharge of the amniotic fluid and weakness of labor. In male patients with fluorosis, Tokar and Savechenko (1977) found reduced amount of testosterone but enhanced concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH).
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Fluoride in concentrations over 60 ppm in the feed of dairy cows were interfered with breeding efficiency. A dose of 150 to 300 mg fluoride per kg body weight over 0-72 hour interval blocked gonadotropin stimulation of the rabbit ovary. Similarly, fluoride at a dose of 10 mg/kg in a 21 days period blocked follicular development in guinea pigs. Studies on histopathological changes in rabbit ovary, during experimental fluorosis revealed atrophy of follicles along with oocyte disintegration and marked necrosis of cells (Shashi, 1990).

The histopathology of ovary revealed loss of follicular maturation and other atrophic conditions as its protein levels were reduced, resulting in weight loss by the organ (Chawla and Rao, 2012). However, high fluoride level also affects reproduction of both sexes in animals and human.

FLUORIDE TOXICITY AND ITS AMELIORATION

Various antioxidants i.e. Vitamin A, C, D, E; mineral elements like calcium, zinc and compounds like amino acids, proteins, selenium, melatonin, black tea, tamarind, amla etc., alone or in combination of different doses and durations have been used to mitigate the fluoride-induced toxicity in animals including man. The economically weaker sections of the society having low nutritional status are affected more. Poor nutrition also plays an important role in aggravating endemic fluorosis. The studies conducted by Gupta et al. (1995), revealed that people living in fluoride endemic areas have taken low dietary protein, calcium and vitamin C than the required amounts, which aggravated the fluorosis condition.
Induced fluorosis in monkey and demonstrated clinical improvement following vitamin C administration. Ascorbic acid is a biologically active and found metabolic significance in animal, human tissue and biological fluids to activate numerous hydroxylating enzymes, participates in metabolic processes as a supplementary source of energy in several tissues including sperms (Chinoy et al., 1982). Its involvement during steridogenesis is via the formation of free radical, monodehydroascorbic acid that if coupled with steroids, viz., pregnenolone and testosterone might produce progesterone or their active metabolites in the rat corpora lutea or testis.

Vitamin C prevents free radical damage in the lungs and may help to protect the central nervous system from such damage (Kronhausen and Kronhausen, 1989). Vitamin C helps the immune system to fight off foreign invaders and tumor cells (Gaby and Singh, 1991). It also supports the cardiovascular system by facilitating fat metabolism and protecting tissues from free radical neurotransmitters.

Vitamin C is of "anti-stress" factor. It is needed for healthy adrenal function; helps expel heavy metals and other toxic substances from the body. This is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat to cellular organelles called mitochondria, for conversion to energy (Carr and Frei, 1999). Ascorbic acid is involved in the metabolism of cholesterol to bile acids, which may have implication for blood cholesterol levels and the incidence of gallstones (Simon and Hudes, 2000). Vitamin C, a water-soluble glucose derivative, has considerable antioxidant activity, in part because of its ease of oxidation and
because the semi-dehydroascorbate radical derived from it is of low reactivity. Vitamin C is an essential cofactor on its antioxidant effects (Halliwell, 2001). The antioxidant ascorbic acid plays an important role in various physiological processes in the body including detoxification of different toxic compounds (Salem et al., 2001).

Vitamin C supplements use and bone mineral density in post menopausal women were studied by Morton et al. (2001). They also revealed that Vitamin C supplement use appears to have a beneficial effect on levels of bone, mineral density, especially among postmenopausal women using concurrent estrogen therapy and calcium supplements. It could further antagonize the inhibitory effect of higher concentration of fluoride on proliferation and differentiation of osteoblasts (Zhang et al., 2003b). The altered biochemical parameters in male mice reproductive organs were partially restored by withdrawal of NaF, whereas vitamin C supplementation showed complete recovery (Chinoy et al., 2005c).

Jhala et al. (2008) observed sodium fluoride produced free radicals toxicity with an increased lipid peroxides in the ovary, which were balanced by vitamin C, calcium or vitamin E alone and in combined treatment. Vitamin C is an antioxidant helping in maintaining normal body physiology. Further, it has been reported to prevent dyslipidaemia and oxidative stress caused during the aging process (Yokozawa et al., 2007).

Vitamin D acts as a regulator of calcium and phosphate metabolisms. It is used in treating conditions such as reduced renal functions, calcium malabsorption and osteoporosis. The rodent data (Chinoy and Sharma, 1998; Chinoy and Patel, 1998b) revealed that ingestion of vitamin E and/or vitamin D to fluorotic mice
manifested a significant recovery in all NaF induced effects in the tissues studies. Sherlin and Verma (2000) concluded that administration of vitamin C, vitamin D, vitamin E and a combination (vitamins C+D+E) along with NaF, caused amelioration in serum calcium and serum phosphatase in fluoride treated rats. Ekambaram and Paul (2003) reported that administration of vitamin D along with NaF prevented hypocalcaemia. The co-administration of calcium and vitamin E with fluoride resulted in a significant recovery from testicular disorders and oxidative stress in the testis and male accessory sex organs (Das et al., 2006).

Helal and Dakdoky (2006) observed less fetal growth retardation in the rat with exposure of fluoride + antioxidants than only fluoride treated group. They used sodium fluoride at the dose level of 40 mg/kg body weight and antioxidants at 25 mg/kg body weight (Vitamins A, C and D, and selenium) from the 8th to 19th day of gestation to pregnant rats. Guney et al. (2007) reported that combined uses of vitamin E and C would effectively protect endometrial damage in the uterus via its antioxidant and anti-inflammatory effects on fluoride-induced damage.

Vitamins C, D and calcium showed a significant improvement in skeletal, clinical fluorosis and biochemical parameters in children consuming water containing 4.5 ppm of fluoride (Gupta et al., 1996). Vitamin A, C, and E and selenium combination (25 mg/kg b.wt.) was found to be protective against fluoride (40 mg/kg/b.wt.) induced toxicity in pregnant rats and their foetuses (Helal and Dakdoky, 2006).

Patel and Chinoy (1998) investigated that sodium fluoride (5 mg/kg b.wt.) caused irregular estrus cycle, altered nucleic acid and protein metabolism in ovary
and uterus of female mice. These changes were ameliorated through exposure of amino acids, glycine and glutamine.

Calcium plays a crucial role in bone development. Calcium works in conjunction with various parts of the body, helping to control the pace of heart. It allows important nutrients to be able to move in and out of the cells in the body and play a crucial role in nerve function. It is even known to lower cholesterol levels and blood pressure. Calcium helps in blood clot. It's found most often in dairy products like cheese, milk, yogurt, beans, and dark green vegetables. It is also responsible for construction, formation and maintenance of bone and teeth. This function helped to reduce the occurrence of osteoporosis (Sizer and Whitney, 1997).

The effects of NaF are transient and reversible with the administration of ascorbic acid and calcium (Chinoy and Sharma, 2000). Therefore, ascorbic acid and calcium were proposed therapeutic agents for populations residing in endemic areas for the amelioration of fluoride effects on reproductive functions. Calcium chloride administered simultaneously with sodium fluoride reduces the bioavailability of fluoride poisoning in mice (Heard et al., 2001).

Chinoy et al. (2004) administered ascorbic acid, calcium and vitamin E alone or in combination to sodium fluoride (NaF, 5 mg/kg b.w.t.) and/or arsenic trioxide (0.5 mg/ gm b.w.t.) treated mice for 30 days observed significant recovery in all altered parameters studied. According to Yan et al. (2007), Zhou et al. (2007) and Wang et al. (2008) supplementation with protein and calcium was found to play a protective role against high fluoride damage.
Supplementation with protein and calcium was found to remove fluoride-induced metabolic and biochemical changes in bones and nonspecific immune function (Zhou et al., 2007, Wang et al., 2008, He et al., 2008). Trivedi et al. (2007) reported amelioration of sodium fluoride (6 and 12 mg/kg b.wt.) toxicity in male mice with 2% black tea for 30 days.

**MELATONIN**

Melatonin, also known chemically as N-acetyl-5-methoxytryptamine, is a naturally occurring compound found in animals, plants, and microbes (Caniato et al., 2003; Paredes et al., 2009). Melatonin was discovered in 1958 and named for its skin-bleaching effect upon melanin (Skin pigment) (Brainard et al., 2001). In animals, circulating levels of the hormone melatonin vary in a daily cycle, thereby allowing the entrainment of the circadian rhythms of several biological functions (Altun and Ugur-Altun, 2007). Many biological effects of melatonin are produced through activation of melatonin receptors (Boutin et al., 2005), while others are due to its role as a powerful antioxidant (Hardeland, 2005), with a particular role in the protection of nuclear and mitochondrial DNA (Reiter et al., 2001). In mammals, melatonin is secreted into the blood by the pineal gland in the brain, Known as the "hormone of darkness", it is secreted in darkness in both day-active (diurnal) and night-active (nocturnal) animals (Challet, 2007). Melatonin-rich plant feed, such as rice, ingested by chicks has been shown to reach and bind to melatonin receptors in their brains (Hattori et al., 1995).
SYSTEMIC (IUPAC) NAME:
N-[2-{5-methoxy-1 H-indol-3-yl} ethyl] ethanamide

COLOUR: Pale yellow leaflets

MELTING POINT: 116-118°C

FORMULA: C13H16N2O2

MOLECULAR WEIGHT: 232.278 g/mol

PHASE: Solid (at STP)

DENSITY: 1.272 g/cm³

SOLUBILITY: Insoluble in water

HALF LIFE: 30 to 50 minutes

EXCRETION: Urine

IN ANIMALS

Many animals use the variation in duration of melatonin production each day as a seasonal clock (Lincoln et al., 2003). In animals including humans (Arendt, 2005)
the profile of melatonin synthesis and secretion is affected by the variable duration of night in summer as compared to winter. The change in duration of secretion thus serves as a biological signal for the organisation of daylength-dependent (photoperiodic) seasonal functions such as reproduction, behaviour, coat growth and camouflage colouring in seasonal animals (Arendt and Skene, 2005). In seasonal breeders which do not have long gestation periods and which mate during longer daylight hours, the melatonin signal controls the seasonal variation in their sexual physiology, and similar physiological effects can be induced by exogenous melatonin in animals including mynah birds (Chaturvedi, 1984) and hamsters (Chen, 1981).

**IN MAMMALS**

Melatonin produced in the pineal gland, which is outside of the blood-brain barrier, acts as an endocrine hormone since it is released into the blood. By contrast, melatonin produced by the retina and the gastrointestinal (GI) tract acts as a paracrine hormone. Melatonin can suppress libido by inhibiting secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland, especially in mammals that have a breeding season when daylight hours are long. The reproduction of long-day breeders is repressed by melatonin and the reproduction of short-day breeders is stimulated by melatonin. During the night, melatonin regulates leptin, lowering the levels. Light/dark information reaches the suprachiasmatic nuclei (SCN) via retinal photosensitive ganglion cells, intrinsically photosensitive photoreceptor cells, distinct from those involved in image forming (that is, these light sensitive cells are a third type in the retina, in addition to rods.
and cones). These cells represent approximately 2% of the retinal ganglion cells in humans and express the photopigment melanopsin (Nayak et al., 2007). The sensitivity of melanopsin is consistent with that of a vitamin A-based photopigment with a peak sensitivity at 484 nm (blue light) (Roberts, 2005). This photoperiod cue entrains the circadian rhythm, and the resultant production of specific "dark"- and "light"-induced neural and endocrine signals which regulate behavioral and physiological circadian rhythms. Melatonin is secreted in darkness in both day-active (diurnal) and night-active (nocturnal) animals (Challet, 2007).

IN HUMAN CIRCADIAN RHYTHM

In humans, melatonin is produced by the pineal gland, a gland about the size of a pea, located in the center of the brain but outside the blood-brain barrier. The melatonin signal forms part of the system that regulates the sleep-wake cycle by chemically causing drowsiness and lowering the body temperature, but it is the central nervous system (more specifically, the SCN) that controls the daily cycle in most components of the paracrine and endocrine systems (Richardson, 2005; Perreau-Lenz et al., 2004) rather than the melatonin signal (as was once postulated). In infants' melatonin levels become regular in about the third month after birth, with the highest levels measured between midnight and 08:00 AM (Ardura, 2002). In humans, 90% of melatonin is cleared in a single passage through the liver, a small amount is excreted in urine, and a small amount is found in saliva.
LIGHT DEPENDENCE

Production of melatonin by the pineal gland is inhibited by light and permitted by darkness. For this reason melatonin has been called "the hormone of darkness". Its onset each evening is called the Dim-Light Melatonin Onset (DLMO). Secretion of melatonin as well as its level in the blood, peaks in the middle of the night, and gradually falls during the second half of the night, with normal variations in timing according to an individual’s chronotype. It is principally blue light, around 480nm that suppresses melatonin (Brainard et al., 2001) increasingly with increased light intensity and length of exposure. Until recent history, humans in temperate climates were exposed to few hours of (blue) daylight in the winter; thereafter fires gave predominantly yellow light. Wearing glasses that block blue light in the hours before bedtime may avoid melatonin loss. Kayumov et al. (2005) showed that light containing only wavelengths greater than 530 nm does not suppress melatonin in bright-light conditions. Use of blue-blocking goggles the last hours before bedtime has also been advised for people who need to adjust to an earlier bedtime, as melatonin promotes sleepiness.

BIOSYNTHESIS OF MELATONIN

Melatonin is produced by pinealocytes in the pineal gland (located in the brain) and also by the retina, lens and GI tract in higher animals. Tryptophan is the precursor of melatonin, which is metabolized consistently into 5-hydroxy-tryptophan (by tryptophan-hydroxylase), 5-HT (by aromatic amino acid decarboxylase), N-acetyltransferase, AA-NAT) and then into melatonin (by hydroxyindole-O-
methytransferase, HIOMT). It has been established that AA-NAT and HIOMT are the key enzymes of this pathway (Axelrod and Weissbach, 1960; Fig. 1). Production of melatonin by the pineal gland is under the influence of suprachiasmatic nucleus (SCN) of the hypothalamus which receives information from the retina about the dully pattern of light and darkness. Both SCN rhythm city and melatonin production are affected by non-visual light information traveling not through the optic nerve, but through the recently-identified hypothalamic tract. Melatonin synthesis is mainly observed at night and correlates with the peak of AA NAT activity. Recent reports indicate that the main factor regulating rhythmic and light-induced changes in AA-NAT activity is the steady-state level of AA-NAT protein, which in turn reflects the balance of its synthesis and degradation (Falcon et al., 2001). Both of these processes can be regulated by distinct mechanisms and the relative importance of each of them is species dependent. In humans, nocturnal production of melatonin in the pineal gland is mainly regulated by the central circadian clock, situated in the hypothalamic suprachiasmatic nucleus (Barinaga, 2002). The circadian clock stimulates norepinephrine release from dense pineal synthetic fibers. Norepinephrine elevates the intracellular cAMP concentration via β-adrenergic receptors and activates the cAMP-dependent protein kinase A - the crucial pathway for the regulation of AA-NAT synthesis and its activity in some mammals, cAMP dependent Protein kinase A protects the enzyme from degradation (Schomerus and Korf, 2005). Thus, in primates pinealocytes constantly synthesize AA-NAT from continually available AA-NAT mRNA. During the day, in the absence of noradrenergic stimulation, this protein immediately undergoes proteasomal proteolysis, while the
nocturnal elevation in the cAMP level causes phosphorylation of AA-NAT by protein kinase A and protects the enzyme from degradation. Consequent increments in the intracellular concentration AA-NAT are paralleled by increases in enzyme activity. In rodents, the cAMP/protein kinase A pathway induces transcriptional activation of the AA-NAT gene, primary mechanism initiating melatonin biosynthesis (Khavinson et al., 2012).

Figure 1. Biosynthesis of melatonin
STRUCTURAL PROPERTIES OF MELATONIN

Melatonin is an indoleamine. It contains an indole heterocycle and two side chains, namely, a 5-methoxy group and 3-amide group. The chemical structure of melatonin is illustrated figure 2:

![Chemical Structure of Melatonin](image)

It is very well documented that the core structure for melatonin required to scavenge free radicals is the indole heterocycle. The electron-rich indole moiety with high resonance stability and electrophoresitivity determines melatonin's potent free radical scavenging capacity (Poeggeler et al., 1993). If the indole moiety is replaced by structurally similar moieties such as benzofuran and naphthalene, the antioxidant activity of these agents decreases substantially when compared with melatonin (Gozzo et al., 1999). Methoxy and aminoacetyl side groups are connected at the C5 and C3 positions, respectively, of the indole moiety in the melatonin molecule. These side chains appear to contribute significantly to the free radical scavenging capacity and they limit pro-oxidative actions of melatonin. Initially, Tan et al. (1993) reported the influence of the side chains on the •OH scavenging ability by comparing melatonin with several analogs. It was found that the methoxy group as
well the acetyl group of the amide was essential for melatonin to display potent 
-OH scavenging activity. The -OH scavenging capacity of 5-methoxy tryptamine, which 
is devoid of a acetyl group, was about 50% that of melatonin. Moreover, a compound 
lacking both the methoxy and acetyl groups was a pro-oxidant rather than an 
antioxidant (Tan et al., 2002).

Tan et al. (1998) elucidated the pathway of melatonin's interaction with •OH 
and with the formation of cyclic 3-hydroxy melatonin; the function of the N-acetyl 
group became apparent. The formation of cyclic 3-hydroxymelatonin requires 
melatonin to scavenge two •OH and this reaction also requires the acetyl group to 
be intact on the side chain. When melatonin interacts with the first •OH it forms the 
cyclic 3-hydroxy melatoninyl radical. The unpaired electron captured from the •OH 
shifts from the newly formed heterocycle moiety and localizes at the carbonyl 
structure of the acetyl group. The highly localized unpaired will easily interact with 
the second •OH to yield the stable final product (Tan et al., 2002). If a melatonin 
analog lacks this nitrogen connected carbonyl structure or related structures 
such as 5-methoxytryptamine (one acetyl group less than melatonin), it may also 
lack the ability to capture the second •OH. This would explain stoichiometrically 
while the •OH scavenging capacity of 5-methoxy tryptamine is about half that of 
melatonin, i.e., melatonin scavenges two •OH and 5-methoxy tryptamine scavenges 
one •OH. Several investigators (Poeggeler et al., 2002) have confirmed the lack of 
pro-oxidative actions for melatonin, if the methoxy group is replaced by a hydroxyl 
group (as in serotonin and other hydroxyindoles) the dual behavior (pro-oxidation 
and antioxidation) is observed (Ng et al., 2000; Poeggeler et al., 2002). This unshield
hydroxyl group may form O-centered radical intermediates (Perez-Reyes and Mason, 1981) and induce peroxidative reactions.

The methoxy and acetyl side chains are not only important chemically but also physically. The physical property of being both lipophilic and hydrophilic (Costa et al., 1995) enables the molecule to cross the membranes with ease but also to distribute in sufficiently high portions in the lipid and the aqueous phases of the cell. Thus, melatonin effectively protects molecules in various compartments of the cell including the membrane, cytosol, mitochondrion and nucleus against oxidative insults. Modifications of the side chains, such as hydroxylation in C5, influence both the chemical and the physical properties of melatonin, thus altering its antioxidant efficacy in in vivo situations.

**MELATONIN AS A POWERFUL ANTIOXIDANT**

Besides its function as synchronizer of the biological clock, melatonin also exerts a powerful antioxidant activity. The discovery of melatonin as an antioxidant was made (Tan et al, 1993). In many less complex life forms, this is its only known purpose (Tan et al, 2007). Melatonin is an antioxidant that can easily cross cell membranes and the blood-brain barrier (Hardeland, 2005). Melatonin is a direct scavenger of OH, O$_2^-$, and NO (Poeggeler et al., 1994). Unlike other antioxidants, melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation repeatedly. Redox cycling may allow other antioxidants (such as vitamin C) to act as pro-oxidants, promoting free radical formation. Melatonin, on the other hand, once oxidized, cannot be reduced to its former state.
Introduction

because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan et al., 2000).

Recent research indicates that the first metabolite of melatonin in the melatonin antioxidant pathway may be N(1)-acetyl-N(2)-formyl-5-methoxykynuramine (or AFMK) rather than the common, excreted 6-hydroxymelatonin sulfate. AFMK alone is detectable in unicellular organisms and metazoans. A single AFMK molecule can neutralize up to 10 ROS/RNS (reactive oxygen species/reactive nitrogen species) since many of the products of the reaction/derivatives (including melatonin) are themselves antioxidants. This capacity to absorb free radicals extends at least to the quaternary metabolites of melatonin, a process referred to as "the free radical scavenging cascade". This is not true to other, conventional antioxidants (Tan et al., 2007).

In animal models, melatonin has been demonstrated to prevent the damage to DNA by some carcinogens, stopping the mechanism by which they cause cancer (Karbownik et al., 2001). It also has been found to be effective in protecting against brain injury caused by ROS release in experimental hypoxic brain damage in newborn rats (Tutunculer et al., 2005; Rao et al., 2010). Melatonin's antioxidant activity may reduce damage caused by some types of Parkinson's disease, may play a role in preventing cardiac arrhythmia and may increase longevity; it has been shown to increase the average life span of mice by 20% in some studies (Oaknin et al., 1995; Anisimov et al., 2003).
IMMUNE SYSTEM

Melatonin interacts with the immune system (Carrillo-Vico et al., 2005; Arushanian and Beier, 2002), the details of those interactions are unclear. There have been few trials designed to judge the effectiveness of melatonin in disease treatment. Most existing data are based on small, incomplete clinical trials. Any positive immunological effect is thought to result from melatonin acting on high affinity receptors (MT1 and MT2) expressed in immunocompetent cells. In preclinical studies, melatonin may enhance cytokine production (Carrillo-Vico et al., 2006), and by doing this counteract acquired immunodeficiencies. Some studies also suggest that melatonin might be useful fighting infectious disease (Maestroni, 2001) including viral, such as HIV, and bacterial infections, and potentially in the treatment of cancer (Maestroni, 1999). Gagnier (2001) noted that the therapeutic potential of melatonin in migraines and other headache types.

AUTISM

Individuals with autism spectrum disorders (ASD) may have lower than normal levels of melatonin. A study carried out in 2008 has been found that unaffected parents of individuals with ASD also have lower melatonin levels, and that the deficits were associated with low activity of the ASMT gene, which encodes the last enzyme of melatonin synthesis (Melke et al., 2008).
TOXICOLOGY

Melatonin has a very low toxicity in rats. Rat maternal toxicity, the no observable adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) were 100 and 200 mg/kg/day, respectively, and the developmental toxicity NOAEL was ≥ 200 mg/kg/day (Jahnke et al., 1999).

CANCER

A systematic review of unblinded clinical trials involving a total of 643 cancer patients using melatonin found a reduced incidence of death (Navara and Nelson, 2007). Another clinical trial is due to be completed in 2012 (Schernhammer et al., 2004). Melatonin levels at night are reduced to 50% by exposure to a low-level incandescent bulb for only 39 minutes, and it has been shown that women with the brightest bedrooms have an increased risk for breast cancer (Koppisetti et al., 2008). Reduced melatonin production has been proposed as a likely factor in the significantly higher cancer rates in night workers (Tan et al., 1999).

FERTILITY

A research team in Italy has found that melatonin supplementation in the evening in perimenopausal women produces an improvement in thyroid function and gonadotropin levels, as well as restoring fertility and menstruation and preventing the depression associated with the menopause (Bellipanni et al., 2005). However, at the same time, some resources warn women trying to conceive not to take a melatonin supplement. One study reported that 3 mg of melatonin taken in
the evening raised prolactin levels in six out of seven women (Terzolo et al., 1993). Melatonin also lowers FSH levels. It is believed that these hormonal changes in some women impair fertility.

**AMLA (EMBLICA OFFICINALIS)**

The world craves new ideas and looks to the Far East and Asia for inspiration and innovation. One Indian plant stands out as being exceptional for its ethnic, ethnobotanical and ethnopharmaceutical use. There is a wealth of technical data to support the safe use of this plant and in this review a monograph will be produced that justifies the use of this plant in a wide range of personal care applications. Amla is one of the most celebrated herbs in the Indian traditional medicine system, Ayurveda. Amla's traditional uses include as a laxative, eye wash, appetite stimulant, restorative tonic, and to treat anorexia, indigestion, diarrhea, anemia, and jaundice.

Amla is becoming increasingly well known for its unusually high levels of Vitamin C, which is resistant to storage and heat damage due to cooking.

*Emblica officinalis* (EO) enjoys a hallowed position in Ayurveda an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in the universe. It belongs to family Euphorbiaceae. It is also named as Amla, *Phyllanthus Emblica* or Indian gooseberry. The other vernacular names of EO have been listed below.

In Sanskrit- Dhatriphala, Amla, Amaliki, Amalakan, Sripalham, Vayasth; Hindi: Amla; English: Emblica myroblan; Italian: Mirabolano emblico; German: Amla;
The species is native to India and also grows in tropical and subtropical regions. Tibetan: Skyu-ru-ra including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. The fruits of EO are widely used in the Aryuveda and are believed to increase defense against diseases. It has its beneficial role in cancer, diabetis, liver treatment, heart trouble, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is also helpful in neutralizing snake venom and as an antimicrobial. It is often used in the form of Triphla which is an herbal formulation containing fruits of EO, *Terminalia chebula* and *Terminalia bellerica* in equal proportions. A general description about EO has been summarized below.

**USED PARTS:** Dried fruits, Fresh fruit, seed, leaves, rootbark, flowers

**FRUITS:** Ripen from November to February nearly sphenical or globular, wider than long and with a small and slight conic depression on both apexes. Fruit is 18-25mm wide and 15-20mm long. Surface is smooth with 6 obscure vertical pointed furrow. Mesocarp is yellow and endocarp is yellowish brown in ripened condition. In fresh fruit mesocarp is acidulous and in dried fruit it is acidulous astringent.
LEAVES: Leaf is 8-10 mm or more long and 2-3 m broad, hairless light green outside, palegreen or often pubescent beneath. It contains gallic acid, ellagic acid, chebulic acid, chebulinic acid, chebulagic acid, a gallantonic called amlic acid, alkaloids phyllantidine and phyllantine.

SEEDS: Four-Six, smooth, dark brown a fixed oil, phosphatides and a small quantity of essential oil. The fixed oil (yield 16% and has the following physical and chemical characteristics: acid value 12.7; saponification value 185; iodine value 139.5; acetyl value 2.03; unsaponifiable matter 3.81%; sterol 2.70% ; saturated fatty acid 7%. Contains linolenic acid (8.78 %), linoleic (44%). oleic (28.40%), steric (2.15%), palmitic (2.99%) and miristic acid (0.95%).

BARKS: Thick to 12 mm, shining grayish brown or grayish green. Leukodelphinidin, tannin and proanthocyanidin.

ROOTS: Ellagic acid and lupeol

HABITAT

Found in India, Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. Mainly it is found in the Deccan, the sea-coast districts and Kashmir (Nadkarni and Nadkarni, 1999). It is common all over tropical and sub-tropical India and also found in Burma, it is abundant in deciduous forests of Madhya Pradesh (Thakur et al., 1989). It is also grows in tropical and subtropical parts of Ceylon,
Malay Peninsula and China. In Ceylon, it is very common in exposed places on patana land in the moist regions up to 4000 feet altitude.

**THE AYURVEDIC DESCRIPTION OF AMLA**

The fruit has these properties using the Ayurvedic classifications:

**RASA (TASTE):** Sour and astringent are the most dominant, but the fruit has five tastes, including sweet, bitter, and pungent.

**VEERYA (NATURE):** Cooling.

**VIPAKA (TASTE DEVELOPED THROUGH DIGESTION):** Sweet.

**GUNA (QUALITIES):** Light, dry.

**DOSHAS (EFFECT ON HUMORS):** Quietens all three doshas: *vata, kapha, pitta*, and is especially effective for *pitta*.

Because of its cooling nature, amla is a common ingredient in treatments for a burning sensation anywhere in the body and for many types of inflammation and fever; these are manifestations of *pitta* (fire) agitation (Williamson, 2002).

Amla has been considered the best of the Ayurvedic rejuvenative herbs, because it is *tridosaghna*. Uniquely, it has a natural balance of tastes (sweet, sour, pungent, bitter and astringent) all in one fruit, it stimulates the brain to rebalance the three main
components of all physiological functions, the water, fire, and air elements within the body.

IDENTIFICATION AND CHEMICAL CONSTITUENTS OF *EMBLICA OFFICINALIS*

Identification of correct genotype of medicinal plant material remained challenging to botanical drug industries. Limitations of chemical and morphological approaches for authentication have created need for newer methods in quality control of botanicals. DNA based marker for identification of EO were developed. Random Amplified Polymorphic DNA (RAPD) technique was used to identify a putative marker (1.1 kb) specific for EO. RAPD amplicon was used to generate Sequence Characterized Amplified Region (SCAR) marker. The SCAR marker was found beneficial for identification of EO in its commercial samples (Dnyaneshwar et al., 2006).

EO primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C (478.56 mg/100 mL). The fruit when blended with other fruits, boosted their nutritional quality in terms of vitamin C content (Jain and Khurdiya, 2004). Compounds isolated from EO were gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O-galloyl beta D glucose, 3 ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid) and isostrictiniiin (Zhang et al., 2003a). Phyllanthus emblica also contains flavonoids, kaempferol 3 O alpha L (6" methyl) rhamnopyranoside and kaempferol 3 O alpha L (6"ethyl) rhamnopyranoside (Habib-ur-Rehman et al., 2007). A new acylated apigenin glucoside (apigenin 7 O (6"
4.12% and moisture 3.83%. Amla fruit ash contains chromium, 2.5 ppm; zinc 4 ppm; and copper, 3 ppm.

**EMBLICANIN**

The low molecular weight hydrolyzable tannins (<1,000), namely Emblicanin A and Emblicanin B, along with pedunculagin and punigluconin are the key ingredients in Emblica (Chaudhuri et al., 2003). Figure 3 shows structure of pedunculagin, one of the ellagitannins of emblica. Each of the ring structures is a phenol, gallic acid.

**USES OF AMLA**

Amla has been used as a valuable ingredient of various medicines in India and Middle East. The extract of amla also has antimicrobial properties. Amla is used for
all Pitta diseases, all obstinate urinary conditions, anemia, biliousness, bleeding, colitis, constipation, convalescence from fever, cough, diabetes, gastritis, gout, hepatitis, hemorrhoids, liver weakness, to relieve stress, osteoporosis, palpitation, spleen weakness, tissue deficiency, vertigo rebuilds blood, bones, cells, and tissues. It increases red blood cell count and regulates blood sugar; heart tonic, cleanses mouth, stops gum bleeding, stops stomach and colon inflammation; cleans intestines, strengthens teeth, aids eyesight, worms, acidity, eye and lung inflammations, ulcerations, G.I. disorders, painful urination, and internal bleeding.

APPLICATIONS OF *EMBILICA OFFICINALIS* IN CANCER:

Triphala has been reported to exhibit chemopreventive potential as it containing EO as one of components. The presence of Triphala in diet had significantly lowered the benzo(a)pyrene (B(a)P) induced forestomach papillomagenesis in mice. It was more effective in reducing tumor incidences compared to its individual constituents. Triphala also significantly increased the antioxidant status of animals which might have contributed to the chemoprevention (Deep et al., 2005). The breast cancer is one of the most common cancers in women. Lipid-metabolizing enzymes, lipids and lipoproteins have been associated with the risk of breast cancer. Kalpaamruthaa (KA) is a modified Siddha preparation containing EO, *Semecarpus anacardium* (SA and honey). The elevated levels of free cholesterol, total cholesterol, triglycerides, phospholipids and free fatty acids and decreased levels of ester cholesterol in plasma, kidney and liver found in cancer
suffering animals were reverted back to near normal levels on treatment with KA and SA (Veena et al., 2006).

Chemoprevention with food phytochemicals is presently considered as one of the most important strategies to control cancer. EO is valued for its unique tannins and flavanoids, which exhibit very powerful antioxidant properties. The inhibition of tumor incidences by fruit extract of this plant has been evaluated on two-stage process of skin carcinogenesis in Swiss albino mice. Chemopreventive potential of EO fruit extract on 7,12-dimethylbenz(a)anthracene (DMBA) induced skin tumorigenesis in Swiss albino mice have been found (Sancheti et al., 2005). The cytotoxic effects of aqueous extract of Triphala were investigated on a transplantable mouse thymic lymphoma (barcl-95) and human breast cancer cell line (MCF-7). The differential response of normal cells and tumor cells to Triphala in vitro and the substantial regression of transplanted tumor in mice fed with Triphala indicate to its potential use as an anticancer drug for clinical treatment (Sandhya et al., 2006a). The suppression of the growth of cancer cells due to the gallic acid—a major polyphenol as observed in "Triphala" have been reported (Kaur et al., 2005).

Ethanolic extract of EO was experimentally evaluated for protection against genotoxicity induced by DMBA. EO fruit administered orally at different concentrations (100, 250, 500 mg/kg body weight) for seven consecutive days in Swiss albino mice prior to a single intraperitoneal injection of DMBA decreased the frequency of bone marrow micronuclei. The protection provided by EO may be due to its antioxidant capacity and through its modulatory effect on hepatic activation and detoxifying enzymes (Banu et al., 2004). Phenolic compounds derived from plant
exhibit a number of beneficial effects and can potentially inhibit several stages of carcinogenesis. Efficacy of EO polyphenols fraction (EOP) on the induction of apoptosis in mouse and human carcinoma cell lines and its modulatory effect on N-nitrosodiethylamine (NDEA) induced liver tumors in rats was also investigated. EOP treatment could induce apoptosis in Dalton's Lymphoma Ascites (DLA) and CeHa cell lines. EOP also inhibited DNA topoisomerase in Saccharomyces cervisiae, mutant cell cultures and the activity of cdc25 tyrosine phosphatase (Rajeshkumar et al., 2003). In vitro antiproliferative activity of extracts from medicinal plants toward human tumor cell lines, including human erythromyeloid K562, T-lymphoid Jurkat, B-lymphoid Raji, erythroleukemic HEL cell lines were compared. Extracts from EO were the most active in inhibiting in vitro cell proliferation have been found (Khan et al., 2002).

Cyclophosphamide is one of the most famous alkylating anticancer drugs in spite of its toxic side effects including hematotoxicity, immunotoxicity and mutagenicity. EO or its medicinal preparations may prove to be beneficial as a component of combination therapy in cancer patients under cyclophosphamide treatment (Haque et al., 2001). Phenolic compounds and the major components from the fruit juice of EO and from the branches, leaves and roots showed stronger inhibition against B16F10 cell growth than against HeLa and MK-1 cell growth. Norsesquiterpenoid glycosides from the roots showed significant antiproliferative activities (Zhang et al., 2004). Its beneficiary uses in a number of diseases are cancer, diabetis, heart diseses, liver treatment, ulcer, anemia, hypercholesterolemia, hyperthermia, ophthalmic disorder, dyspepsia, lung metastasis, healing dermal
wounds, dyslipidaemia pancreatitis, atherosclerosis, alzheimer's disease, fever, bronchitis, diarrhoea, jaundice.

USES OF *EMBLICA OFFICINALIS* IN DIABETES:

Oral administration of the extracts (100 mg/kg body weight) reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats significantly within 4 hours. EO and an enriched fraction of its tannoids are effective in delaying development of diabetic cataract in rats (Suryanarayan et al., 2007).

Aldose reductase (AR) has its involvement in the development of secondary complications of diabetes including cataract. EO is proved as an important inhibitor of AR. Exploring the therapeutic value of natural ingredients that people can incorporate into everyday life may be an effective approach in the management of diabetic complications (Suryanarayan et al., 2007).

EFFECTS OF *EMBLICA OFFICINALIS* ON LIVER:

EO fruits have been reported to be used for hepatoprotection in Ayurveda (Bhattacharya et al., 2000b). *Phyllanthus emblica* extract was investigated on ethanol induced rat hepatic injury. Protective roles of this against ethanol induced liver injury in rats are reported (Pramyothin et al., 2006). Oral administration of *Emblica officinalis* aqueous extract and ochratoxin also produced a significant increase in glutathione and ascorbic acid concentrations in mouse liver and kidney (Chakraborty and Verma, 2010).
A hydroalcoholic (50%) extract of fruit of EO (EO-50) decreased the severity of hepatic fibrosis induced by thioacetamide and carbon tetrachloride. EO-50 effectively reversed profibrogenic events possibly due to its antioxidative activity. Hepatoprotective effect of EO-50 against antituberculosis (anti-TB) drugs-induced hepatic injury has been reported. EO-50 exhibits hepatoprotective activity due to its membrane stabilizing, antioxidative and CYP 2E1 inhibitory roles (Tasduq et al., 2005). EO also inhibited hepatic toxicity in Wistar rats (Sultana et al., 2005). The extract of EO and Chyavanaprash were investigated for its hepatoprotective activity using carbon tetrachloride (CCl₄) induced liver injury in rats. Both extracts were observed to inhibit the hepatotoxicity produced by acute and chronic CCl₄ administration as seen from the decreased levels of serum and liver lipid peroxides (LPO), glutamate-pyruvate transaminase (GPT) and alkaline phosphatase (ALP). Chronic CCl₄ administration was also found to produce liver fibrosis as seen from the increased levels of collagen-hydroxyproline and pathological analysis. Both extracts were found to inhibit these elevated levels significantly, showing that the extract could reduce the induction of fibrosis in rats model (Jose and Kuttan, 2000).

**CARDIOPROTECTIVE ACTIVITY OF EMBLICA OFFICINALIS:**

The effects of chronic oral administration of fresh fruit homogenate of Amla on myocardial antioxidant system and oxidative stress induced by ischemic-reperfusion injury (IRI) were investigated on heart in rats. Chronic EO administration produces myocardial adaptation by augmenting endogenous antioxidants and protects rat hearts from oxidative stress associated with IRI (Rajak et al., 2004).
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radical (pro-oxidant) and anti-free radical (anti-oxidant) balance leading to oxidative stress which may result in tissue injury and subsequent diseases. Thus, the oxidant status in human reflects the dynamic balance between the anti-oxidant defense and pro-oxidant conditions and has been suggested as a useful tool in estimating the risk of oxidative damage.

EO was studied against the cold stress-induced alterations in the behavioral and biochemical abnormalities. Verma and Chakraborty (2007) observed that aqueous and alcoholic extract of EO has powerful retarding effect on ochratoxin-induced haemolysis on RBC. Triphala administered orally about 1g/kg/animal body weight for 48 days significantly prevented cold stress-induced behavioral and biochemical abnormalities in albino rats. Thus Triphala supplementation can be regarded as a protective drug against stress (Dhanalakshmi et al., 2007).

The administration of ethyl acetate (EtOAc) extract of Amla or Sun Amla (Taiyo Kagaku Co., Ltd., Japan) reduced the elevated levels of urea nitrogen and serum creatinine in the aged rats. Oral administration of this extract significantly reduced thiobarbituric acid-reactive substance levels of serum, renal homogenate and mitochondria in aged rats, suggesting that Amla would ameliorate oxidative stress under aging. The increase of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 expressions in the aorta of aging rats were also significantly suppressed by EtOAc extract of Amla or Sun Amla extract. EtOAc extract of Amla or SunAmla reduced the COX-2 and iNOS expression levels by inhibiting NF-kappaB activation in the aged rats. Thus Amla would be a very useful antioxidant for the prevention of age-related renal disease (Yokozawa et al., 2007). Prefeeding of Amla
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appeared to reduce the hexachlorocyclohexane (HCH)-induced raise in renal gamma-glutamyl transpeptidase (GGT) activity. This shows the elevation of hepatic antioxidant system and lowering of cytotoxic products as which were otherwise affected by the administration of HCH (Anilakumar et al., 2007).

Elevation in xanthine oxidoreductase activity and lowering in superoxide dismutase activity was observed in the intestine of mice exposed to whole body gamma-irradiation (WBI), which, however, reverted back to those levels of sham-irradiated controls, when animals were fed with Triphala for 7 days prior to irradiation. This suggested the prevention of oxidative damage caused by whole body radiation exposure after feeding of animals with Triphala. Triphala protected whole body irradiated mice. Protection was mediated through inhibition of oxidative damage in cells and organs. It indicated that this drug has potential to develop into a novel herbal radio-protector for practical applications (Sandhya et al., 2006b).

Methanol was used to extract the dried fruit rind of Phyllanthus emblica and then separated into ethyl acetate, hexane and water fractions. Only the ethyl acetate phase showed strong NO scavenging activity in vitro, when compared with hexane and water phases. In the ethyl acetate extract gallic acid was found to be a major compound that showed highest NO scavenging activity (Kumaran and Karunakaran, 2006). Triphala due to its antioxidant properties was also found to restore the noise-stress induced changes (Srikumar et al., 2006).

Vitamin C in EO accounts for approximately 45-70% of the antioxidant activity (Scartezzini et al., 2006). Rats were examined for the antioxidant properties of Amla extracts and its effect on the oxidative stress in streptozotocin-induced diabetes was
(CAT) activities in hyperthyroid mice, exhibiting its hepatoprotective nature. It potentially ameliorates the hyperthyroidism with an additional hepatoprotective benefit (Panda and Kar, 2003).

EuMil is a polyherbal formulation composed of standardized extracts of *Ocimum sanctum, Withania somnifera, Asparagus racemosus* and EO, was used as an anti-stress agent to attenuate the various aspects of stress related disorders. It has significant anti-stress and adaptogenic activities, qualitatively comparable to *Panex ginseng*, against a number of behavioral, biochemical and physiological perturbations, induced by unpredictable stress, which has been proposed to be a better indicator of clinical stress than acute stress. The contribution of the individual constituents of EuMil (polyherbal formulation) in the adaptogenic action has been reported (Muruganandam et al., 2002). EO is used to protect the skin from the devastating effects of free radicals, non-radicals and transition metal-induced oxidative stress. It is suitable for use in, anti-aging, general purpose skin care products and as sunscreen (Chaudhuri, 2002; 2004). The fruits of EO contain tannoid principles that have been reported to exhibit antioxidant activity *In Vitro* and *in vivo*. Emblicanin-A (37%) and -B (33%) enriched fraction of fresh juice of EO fruits was investigated for antioxidant activity against ischemia-reperfusion -induced oxidative stress in rat heart. The study confirms the antioxidant effect of EO and also indicated that the fruits of the plant may exhibit a cardioprotective effect (Bhattacharya et al., 2002). The antioxidant activity of EO extract is associated with the presence of hydrolyzable tannins having ascorbic acid-like action have been also reported (Pozharitskaya et al., 2007).
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A number of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana identified for their interesting antioxidant activities. EO has been reported for its antioxidant activity (Scartezzini and Speroni, 2000). It contains tannoid principles comprising of emblicanin A, emblicanin B, punigluconin and pedunculagin, have been reported to possess antioxidant activity in vitro and in vivo (Bhattacharya et al., 2000a).

ACTIVE ROLES OF EMBLICA OFFICINALIS IN IMMUNOMODULATION:

Immune activation is an effective as well as protective approach against emerging infectious diseases. Albino rats were used to assess the immunomodulatory activities of Triphala on various neutrophil functions like adherence, phagocytic index, avidity index and nitro blue tetrazolium. Oral administration of Triphala appears to stimulate the neutrophil functions in the immunized rats and stress induced suppression in the neutrophil functions were significantly prevented by Triphala (Srikumar et al., 2005). EO and Evolvulus alsinoides (Shankhpushpi) were assessed for its immunomodulatory activity in adjuvant induced arthritic (AIA) rat model. Complete Freund’s Adjuvant (CFA) was injected in right hind paw of the animals induced inflammation. Lymphocyte proliferation activity and histopathological severity of synovial hyperplasia were used to study the anti-inflammatory response of both the extracts. Both the extracts showed a marked reduction in inflammation and edema and caused immunosuppression in AIA rats, indicating that they may provide an alternative
approach for the treatment of arthritis (Ganju et al., 2003). Immu-21 is an Ayurvedic polyherbal formulation containing extracts of EO, Ocimum sanctum, Withania somnifera and Tinospora cordifolia. Its immunomodulatory activity was studied on proliferative response of splenic leukocytes to T cell mitogens, concanavalin (Con)-A and phytohemagglutinin (PHA) and B cell mitogen, lipopolysaccharide (LPS) in vitro by (3H)-thymidine uptake assay in mice. Pretreatment with Immu-21 selectively elevated the proliferation of splenic leukocyte to B cell mitogen, LPS and cytotoxic activity against K 562 cells in mice (Nemmanni et al., 2002). EO has been reported to inhibit Cr-induced free radical production and also restored the anti-oxidant status back to control level. It also inhibited apoptosis and DNA fragmentation induced by Cr. It relieved the immunosuppressive effects of Cr on lymphocyte proliferation and even restored the IL-2 and gamma-IFN production (Sai et al., 2002).

**ANTIPYRETIC AND ANALGESIC ACTIVITIES OF EMBLICA OFFICINALIS:**

Extracts of EO fruits possess potent anti-pyretic and analgesic activities. A single oral dose of ethanolic extract and aqueous extract (500 mg/kg, i.p.) showed significant reduction in hyperthermia in rats induced by brewer's yeast. Both of these extracts elicited pronounced inhibitory effect on acetic acid-induced writhing response in mice in the analgesic test (Perianayagam et al., 2004). This may be due to the presence of tannins, alkaloids, phenolic compounds, amino acids and carbohydrates.
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CYTOPROTECTIVE, ANTITUSSIVE, GASTROPROTECTIVE PROPERTIES OF EMBLICA OFFICINALIS:

EO has been reported for its cytoprotective and immunomodulating properties against chromium (VI) induced oxidative damage. It inhibited chromium induced immunosuppression and restored gamma-IFN production by macrophages and phagocytosis (Sai et al., 2003).

EO was tested for its antitussive activity in conscious cats by mechanical stimulation of the laryngopharyngeal and tracheobronchial mucous areas of airways. Antitussive activity of EO was more effective than the non-narcotic antitussive agent dropropizine but less effective than shown by the classical narcotic antitussive drug codeine. It is supposed that the dry extract of EO exhibits the antitussive activity not only due to antiphlogistic, antispasmolytic and antioxidant efficacy effects, but also to its effect on mucus secretion in the airways (Nosal’ova et al., 2003).

EO (ethanolic extract) was investigated for its antisecretory and antiulcer activities using various experimental models in rats, including pylorus ligation Shay rats, indomethacin, and necrotizing agents. It was then reported that Amla extract exhibit antisecretory, cytoprotective and antiulcer properties (Al-Rehaily et al., 2002).

MEMORY ENHANCING EFFECTS OF EMBLICA OFFICINALIS:

Amla churna produced a dose-dependent improvement in memory of young and aged rats. It reversed the amnesia induced by scopolamine and diazepam. Amla churna may prove to be a useful remedy for the management of Alzheimer’s disease
due to its multifarious beneficial effects such as memory improvement and reversal of memory deficits (Vasudevan and Parle, 2007a,b).

MANAGEMENT OF OPHTHALMIC DISORDERS WITH *EMBLICA OFFICINALIS*:

Ophthacare is a herbal eye drop preparation containing basic principles of different herbs viz *Carum copticum*, *Terminalia belerica*, EO, *Curcuma longa*, *Ocimum sanctum*, *Cinnamomum camphora*, *Rosa damascena* and *Meldespumapum*. Clinical trial was conducted in patients suffering from different ophthalmic disorders namely, conjunctival xerosis, conjunctivitis, acute dacryocystitis, degenerative conditions and postoperative cataract patients with a herbal eye drop preparation. In most cases improvement was observed with the treatment of the herbal eye drop. During the course of study no side effects were observed and the eye drop was well tolerated by the patients. Ophthacare exhibits beneficial role in a number of inflammatory, infective and degenerative ophthalmic disorders (Biswas et al., 2001).

ROLES OF *EMBLICA OFFICINALIS* IN REDUCING CHOLESTEROL AND DYSLIPIDEMIA:

Cu (2+) -induced LDL oxidation and cholesterol-fed rats were used to investigate the effects of Amla on low-density lipoprotein (LDL) oxidation and cholesterol levels *in vitro* and *in vivo*. It was concluded that Amla may be effective for hypercholesterolemia and prevention of atherosclerosis (Kim et al., 2005). EO and *Mangifera indica* contain flavonoids which reduce the levels of lipids in serum and tissues of rats induced hyperlipidemia. Both causes the degradation and elimination of cholesterol (Anila and Vijayalakshmi, 2002).
**EMBLICA OFFICINALIS AS SNAKE VENOM NEUTRALIZER:**

EO and *Vitex negundo* were explored for the first time for antisnake venom activity. *Naja kaouthia* and *Vipera russellii* venom was antagonized by the plant extracts significantly both *in vivo* and *in vitro* studies. *V. russellii* venom-induced coagulant, haemorrhage defibrinogenating and inflammatory activities were significantly neutralized by both plant extracts. No precipitating bands were formed between the snake venom and plant extract which confirmed that the plant extracts possess potent snake venom neutralizing capacity and need further investigation (Alam and Gomes, 2003).

**ANTIMICROBIAL AND ANTIMUTAGENICITY ACTIVITIES OF EMBLICA OFFICINALIS:**

EO has been reported for the antimicrobial activities (Srikumar et al., 2007). The plant have been reported to posses potent antibacterial activity against *Escherichia coli*, *K. ozaenae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *S. paratyphi A*, *S. paratyphi B* and *Serratia marcescens* (Saeed and Tariq, 2007).

Water, chloroform and acetone extracts of Triphala were investigated to evaluate an antmutagenic effect using an Ames histidine reversion assay having TA98 and TA100 tester strains of *Salmonella typhimurium* against the direct-acting mutagens, 4-nitro-o-phenylenediamine (NPD), sodium azide and the indirect-acting promutagen, 2-aminofluorene (2AF), in the presence of phenobarbitone-induced rat hepatic S9. The results with chloroform and acetone extracts showed inhibition of mutagenicity induced by both direct and S9-dependent mutagens (Kaur et al., 2002).