2. REVIEW OF LITERATURE

2.1 Smoking and its ill effects

The health threat posed by tobacco has been recognized by scientists since the 17th century, with the systematic collection of information on the health effects of tobacco beginning in the early 20th century [Haenszel W, Et Al; 1956]. As early as 1928, studies indicated a link between smoking and cancer [Lombard HL, Et Al; 1928].

A cigarette is an efficient, well-engineered nicotine delivery device that has proved to be deadly when smoked regularly. Nicotine from a smoked cigarette will reach the brain in as little as 7 seconds after inhalation [Maisto SA, Et Al; 2004]. A typical cigarette contains approximately 0.5 to 1.0 g of tobacco and, on average, 10 mg of nicotine [Royal College of Physicians; 2007] [Bethesda MD; 2006]. A cigarette is typically smoked in 10 puffs and within 5 minutes [Bethesda MD; 2006]. A typical smoker will absorb 1 to 2 mg of nicotine, but absorption can range from 0.5 to 3 mg [Karan LD, Et Al; 2003] [Lynch B, Et Al; 1994]. The elimination half-life of nicotine is 2 to 3 hours, meaning that the level of nicotine in the blood decreases by one half after a smoker stops smoking for that length of time [Lynch B, Et Al; 1994].

The role of nicotine in addiction has been extensively reviewed and reported [Royal College of Physicians; 2007] [Matta SG, Et Al; 2007] [Brigham J, Et Al, WHO; 2001]. The following is a brief overview of nicotine’s role in the development and maintenance of addiction:

The active ingredient for addiction is nicotine, a naturally occurring drug found in all the different forms of tobacco. Nicotine is highly addictive, as addictive as heroin and cocaine [National Center for Chronic Disease Prevention and Health Promotion; 1994]. All leading authorities, including WHO, Royal College of Physicians, and American Psychiatric Association (APA), [Royal College of Physicians; 2000] [American Psychiatric Association; 1994] [World Health Organization; 2001] have supported the three major conclusions of a 1988 report by
the Surgeon General of the United States [US Department of Health and Human Services; 1988] regarding nicotine and tobacco:
1. Cigarettes and other forms of tobacco are addictive.
2. Nicotine is the drug in tobacco that causes addiction.
3. The physiological and behavioural processes that determine tobacco addiction are similar to those that determine heroin and cocaine addiction.
All forms of tobacco have the potential to be addictive because they all contain nicotine, but cigarettes are the most efficient for delivering nicotine into the body [Royal College of Physicians; 2000].

Nicotine is an alkaloid found in abundance in the tobacco plant. Nicotine’s effects on the brain and on body systems have been reviewed extensively [Royal College of Physicians; 2007] [Matta SG, Et Al; 2007]. Nicotine is classified as a stimulant, but many people who use it report decreased arousal. Nicotine causes paradoxical effects, acting both as a stimulant and a depressant. As a stimulant, it has been shown to increase attention, memory, information processing, and learning [Matta SG, Et Al; 2007] [Benowitz NL; 1996]. It has also been shown to alleviate anxiety, depression, and pain. For these reasons, smokers often report that smoking is a stress reliever and that they are more apt to smoke in response to stressful situations or negative moods [Goldstein MG; 2003]. As noted above, inhalation of nicotine in the form of smoke provides the quickest delivery [Matta SG, Et Al; 2007] [Benowitz NL; 1996] with nicotine reaching the brain in approximately 7 seconds.34 Nicotine stimulates the dopaminergic pathways of the mesolimbic system in brain, an area which is involved in reinforcement for other drugs of abuse[Benowitz NL; 1998].

Nicotine binds to the nicotinic acetylcholine receptors in the brain (nAChRs), causing the release of dopamine in the nucleus accumbens [Jarvis MJ; 2004] and the subsequent release of neurotransmitters, resulting in a variety of physiological effects, including behavioural arousal and neural activation [Lynch B, Et Al; 1994]. Release of dopamine, norepinephrine, and serotonin is associated with pleasurable feelings and also with appetite suppression. The excess release of acetylcholine associated with nicotine consumption is related to improved attention [Rezvani AH, Et Al; 2001], increased vigilance in the performance of repetitive tasks, and memory
improvements [Lynch B, Et Al; 1994] [Rezvani AH, Et Al; 2001]. These pharmacological effects play a large role in maintaining smoking behaviour in the addicted smoker.

Nicotine improves mood. Smokers have commonly reported increased pleasure as well as reduced tension, anger, depression and stress after smoking a cigarette. It is unclear whether these effects are due to the effect of nicotine on the brain or to the alleviation of withdrawal symptoms. The perceived calming effect from the reduction of withdrawal symptoms may be what nicotine users find reinforcing. Some of these effects may be pharmacological, but some of the sedating psychological effect of smoking comes from the smoker’s perception of coping with stress successfully while smoking [Maisto SA , Et Al; 2004]. Nicotine also affects metabolism by decreasing appetite and increasing metabolic rate [Goldstein MG; 2003].

**Diagnostic Criteria for Nicotine Withdrawal**

[American Psychiatric Association; 1994]

A. Daily use of nicotine for at least several weeks.

B. Abrupt cessation of nicotine use or reduction in the amount of nicotine used, followed by four (or more) of the following signs within 24 hours:

1. dysphoric or depressed mood
2. insomnia
3. irritability, frustration, or anger
4. anxiety
5. difficulty concentrating
6. restlessness
7. decreased heart rate
8. increased appetite or weight gain.

C. Clinically significant distress or impairment in occupational, social, or any other important areas of functioning.
D. Symptoms not due to a general medical condition and not better accounted for by a mental disorder.

**Classification Of Smoking:**

I] Smoker can be classified based on number of cigarettes per day [World Health Organization; 2008]:

Daily smokers can be classified by the number of cigarettes they reported smoking per day:
Heavy: 25 or more cigarettes per day
Moderate: 15 to 24 cigarettes per day
Light: 14 or fewer cigarettes per day.

II] Smoker can also be classified based on habit criteria as [US Centers for Disease Control and Prevention; 2010] [Schane R.E, Et Al; 2010]:

A. Stimulation smokers.

1. This is a person who gets a lift from smoking.
2. A person feels perked-up, energetic and more awake.
3. A person could substitute a brisk work or moderate exercise and get the same feeling.

B. Handling-Oral gratification smokers.

1. This is a person who likes the trappings and the ritual of smoking (taping cigarette, striking matches, or having something to chew on.)
2. A person could substitute candy or gum or handle pens, pencils, coins, etc. and get the same feeling.

C. Pleasurable relaxation smokers.

1. About 2/3 of smokers smoke for positive feelings of contentment, victory, achievement and satisfaction.
2. They feel good about themselves when they smoke.
3. Often when they realize the harmful effects of the habit, they substitute drinking, eating, physical activities or social activities for the tobacco.
D. Crutch-tension reduction smokers.
   1. A person who uses the cigarette to manage stressful situations, negative
effects and feelings of fear, anger and/or anxiety.
   2. The cigarette is used as a tranquilizer and cigarettes are the crutch used to
manage tension producing situations.
   3. This person may find it easy to quit when things are going well, but might
be tempted to start again when things are In crisis- type situation.
   4. This person needs to find something else to do when tension strikes such as
eating, exercise, social activity, etc.

E. Craving-psychological and physical addiction smokers.
   1. This person has a physical need for nicotine and will go through withdrawal
if they don't get the nicotine that they crave.
   2. Contrary to popular belief, this type of smoker smokes more under stress
because stress reduces level of nicotine in the body. (Smoking will not reduce
in this case but stress reduces nicotine.)

F. Habit smokers.
   1. This person has developed behavioral patterns that will cause them to light
up as a response to a cue (getting in car, cup of coffee, etc. ).
   2. The enjoyment of smoking is usually gone for this person.

The complexity of tobacco smoke leads to some confusion about the specific
mechanisms by which lung cancer is caused. Among the numerous components of
tobacco smoke, 20 carcinogens have been shown to convincingly cause lung tumors
in laboratory animals or humans and are, therefore, most likely to be involved in lung
cancer induction. Among these, polycyclic aromatic hydrocarbons and the tobacco-
specific nitrosamine 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone are likely to
play major roles.
Figure: 2.1 Toxic Fumes Generated by burning of Cigarette

Figure: 2.2 Cigarette Smoke - Active and Passive Smoking
Figure: 2.3 Components of Cigarette Smoke
### Table: 2.1

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>The chemical is carcinogenic to humans.</td>
</tr>
<tr>
<td>Group 2A</td>
<td>The chemical is probably carcinogenic to humans.</td>
</tr>
<tr>
<td>Group 2B</td>
<td>The chemical is possibly carcinogenic to humans.</td>
</tr>
<tr>
<td>Group 3</td>
<td>The chemical is not classifiable as to its carcinogenicity to humans.</td>
</tr>
<tr>
<td>Group 4</td>
<td>The chemical is probably not carcinogenic to humans.</td>
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</tbody>
</table>

[World Health Organization, International Agency for Research on Cancer; 2002]

### Table: 2.2

<table>
<thead>
<tr>
<th>IARC classification (Overall evaluation of the degree of evidence for carcinogenicity based on human and animal evaluation)</th>
<th>EXAMPLES OF Tobacco smoke carcinogens</th>
</tr>
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<tbody>
<tr>
<td>Group 1</td>
<td>Arsenic</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
</tr>
<tr>
<td></td>
<td>Benzo(a)pyrene</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
</tr>
<tr>
<td></td>
<td>Chromium (Hexavalent)</td>
</tr>
<tr>
<td></td>
<td>3-methylcholanthrene</td>
</tr>
<tr>
<td></td>
<td>1-Nitroso-2-methylurea</td>
</tr>
<tr>
<td></td>
<td>Nickel</td>
</tr>
<tr>
<td></td>
<td>4-(N-Methyl-N-nitrosamino)-1-(3-pyridyl)-butane (NNK)</td>
</tr>
<tr>
<td>Group 2A</td>
<td>coal (coked)</td>
</tr>
<tr>
<td>Group 2B</td>
<td>Acrylonitrile</td>
</tr>
<tr>
<td></td>
<td>Acrylamide</td>
</tr>
<tr>
<td></td>
<td>Spermine</td>
</tr>
<tr>
<td></td>
<td>Styrene</td>
</tr>
</tbody>
</table>

[World Health Organization, International Agency for Research on Cancer; 2002]
**Figure: 2.4**

![Diagram showing the relationship between nicotine addiction and lung cancer](image)

Schematic linking nicotine addiction and lung cancer via tobacco smoke carcinogens and their induction of multiple mutations in critical genes. PAH = polycyclic aromatic hydrocarbons; NKK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

[Stephen S. Hecht; 1999]

**Table: 2.3**

<table>
<thead>
<tr>
<th>Type</th>
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</tr>
</thead>
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<tr>
<td>Polycyclic aromatic hydrocarbons</td>
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</tr>
<tr>
<td>Acetamides</td>
<td>5</td>
</tr>
<tr>
<td>N-Nitrosoamines</td>
<td>7</td>
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<tr>
<td>Aromatic amines</td>
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</tr>
<tr>
<td>Heterocyclic aromatic amines</td>
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</tr>
<tr>
<td>Aldehydes</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous repair compounds</td>
<td>15</td>
</tr>
<tr>
<td>Inorganic compounds</td>
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</tr>
<tr>
<td>Total</td>
<td>55</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Carcinogen class</th>
<th>Compound</th>
<th>Amount in mainstream (ppm)</th>
<th>Sidestream (ppm)</th>
<th>Representative lung neoplasms in species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td>Benzo[a]pyrene</td>
<td>4-22</td>
<td>2-6</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td></td>
<td>Benzo[b]fluoranthene</td>
<td>6-21</td>
<td>2-6</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td></td>
<td>Benzo[k]fluoranthene</td>
<td>6-12</td>
<td>2-6</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td></td>
<td>Benzo[a]pyrene</td>
<td>1-6-2</td>
<td>0.2-0.4</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td></td>
<td>Indole, 1,2,3-pyrazine</td>
<td>4-20</td>
<td>2-4</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td></td>
<td>Thiophene, thiophene 2,3-dione</td>
<td>6</td>
<td>2-4</td>
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<tr>
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<td>1,2,4,5-Tetrahydronaphthalene</td>
<td>0.8</td>
<td>0.1</td>
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</tr>
<tr>
<td></td>
<td>2B,8-Dimethoxycarbazole</td>
<td>0.7</td>
<td>0.1</td>
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<tr>
<td>N-Nitrosoamines</td>
<td>4-Methylmethanesulphonamide</td>
<td>4-20</td>
<td>2-4</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td></td>
<td>1,1-Nitrosobis(2-naphthylamine)</td>
<td>1</td>
<td>0.1</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td>Miscellaneous organic compounds</td>
<td>1-Methyl-2-naphthalen-4-amin</td>
<td>0.13</td>
<td>10-20</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td></td>
<td>Terephthalic acid</td>
<td>0.2-0.5</td>
<td>0.1-0.2</td>
<td>Mouse, rat, hamster (44-47)</td>
</tr>
<tr>
<td>Inorganic compounds</td>
<td>Nickel</td>
<td>0.5-20</td>
<td>3-5</td>
<td>Mouse, rat (53)</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>0.2-0.5</td>
<td>0.1-0.2</td>
<td>Mouse, rat, hamster (53)</td>
</tr>
<tr>
<td></td>
<td>Cobalt</td>
<td>0.1-0.2</td>
<td>0.1-0.2</td>
<td>Mouse, rat, hamster (53)</td>
</tr>
<tr>
<td></td>
<td>Polonium-210</td>
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<td>1.0-40</td>
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<td>Ammonium</td>
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<td>0.1-0.2</td>
<td>Mouse, rat, hamster (53)</td>
</tr>
<tr>
<td></td>
<td>Hydrazine</td>
<td>1.0-6</td>
<td>0.1-0.2</td>
<td>Mouse, rat, hamster (53)</td>
</tr>
</tbody>
</table>

[Stephen S. Hecht; 1999]
2.2 Free Radicals and its consequences

Free radicals are defined as molecules or fragments of molecules containing one or more unpaired electrons [Halliwell B; 1987]. Highly reactive species capable of damaging lipids, carbohydrates, proteins and/or nucleic acids, as well as causing loss of molecular functions [Halliwell B; 1995]. Reactive oxygen species (ROS) represent the more abundant free radicals in mammalian cells and include, mainly, superoxide anions (O2−), hydroxyl radicals (•OH) [Pastor N, Et Al; 2000] and peroxide radicals (ROO•) [Burcham, P.C; 1998]. It should also be taken into account that the levels of other molecules with unpaired electrons but also harmful and ROS-related, such as hydrogen peroxide (H2O2) [Shacter E, Et Al; 2002]. The sources of ROS are the electron transport system in the mitochondria, the Krebs cycle, different oxidases (including xanthine oxidase, NADPH oxidase, certain arachidonic acid oxygenase activities) and the radicals released from immune cells [Boveris A; 1973].
An increase in free radicals levels may lead to an increase in different cellular defense systems or if the damage is irreversible may lead to cell death. Moreover, oxidative stress or redox status shifts may cause cell transition from quiescent to proliferative status as well as growth arrested or cell death activation according to the duration and extent of the redox imbalance [Ekshyyan O, Et Al; 2005].

Although several mechanisms of cell death has been characterized, classifying them is difficult because more than one single mechanism can be activated by the same signal [Hara MR, Et Al; 2007]. Main cell death mechanisms include:
(a) apoptosis which is a process of programmed cell death, characterized by shrinkage of cells, chromatin condensation, caspases activation and DNA fragmentation;
(b) necrosis is an uncontrolled event caused by loss of cell homeostasis where cell volume increases [Higuchi Y, Et Al; 2002], and
(c) autophagy is a process where degradation of cellular components through the lysosomal machinery is observed.

Cells possess antioxidant systems to control the redox state and, thereby, survival. Antioxidant defenses include superoxide dismutases; ceruloplasmin; GSH, and enzymes involved in GSH homeostasis, such as glutaredoxins, GSH peroxidase, GSH reductase as well as GSH transferases; catalase; thioredoxins; peroxiredoxins; sulfiredoxins; vitamins; metal-complex proteins, etc. [Federico A, Et Al; 2007]. In consequence, down regulation of these antioxidant defenses can lead to increased ROS levels, redox status alterations, and cell damage; thus increasing the risk of developing pathologies such as cancer, neurodegeneration, etc [Franco R, Et Al; 2009].

2.3 Serum Ceruloplasmin

Ceruloplasmin is a glycoprotein with a polypeptide chain including 1046 amino acid residues. Ceruloplasmin belongs to the group of acute phase reactants and is a principal copper containing protein of plasma. It therefore is a major copper carrier in plasma and serves as a link between the hepatocyte and other cells. It has been difficult to isolate but it is now generally agreed that it is a single polypeptide
chain containing 6 to 7 copper atoms per molecule. The high content of copper ions gives ceruloplasmin a beautiful blue color (L. caeruleus = blue) [Pamela beilli, Et Al; 2002] [Senra Varela A, Et Al; 1997].

Turn over of copper is very less in ceruloplasmin. Once it is secreted by liver, it neither gains nor loses copper ions, it can donate copper if some process exists to internalize the ceruloplasmin molecule into a cell in which the copper can be removed from the protein. Plasma copper consists of nondialyzable fraction (95%) that is attached to ceruloplasmin and a dialyzable fraction (5%) loosely bound to albumin and histidine. Copper is believed to be transported in the dialyzable form from the gut to the liver, where copper is incorporated into ceruloplasmin apoprotein, which is sialized and released into the bloodstream. Increased absorption of copper leads to increased synthesis of ceruloplasmin [Senra Varela A, Et Al; 1997].

Ceruloplasmin is the most potent free radical inhibitor both in tissue homogenates and in simple lipid emulsions is the copper protein ceruloplasmin. Copper alone was ineffective as was the apoprotein (that is, ceruloplasmin from which the copper atoms had been removed). To clinical and enzyme chemists the findings may seem paradoxical. One of the most celebrated qualities of ceruloplasmin in vitro is its oxidase like activity. Oxidases are a family of enzymes which catalyze the direct oxidation by atmospheric oxygen of various organic substrates. The reactions are in many ways similar to free radical oxidation; indeed, in biological mixtures enzymatic and non-enzymatic changes may be difficult to separate. At first sight therefore oxidase-like and antioxidant activity seem the exact opposites. One possible explanation hinges on the role of the transitional metal, Iron [Pamela beilli, Et Al; 2002].

In its free form iron is one of the most effective antioxdation catalysts. Its catalytic effectiveness depends on its readiness to change from one valency state to another, donating or abstracting the single electron required for free-radical generation. Ceruloplasmin is not only an oxidase of various organic compounds but also ‘ferroxidase’, it converts reduced (ferrous) to oxidized (ferric) iron. If the resulting shift in equilibrium prevented iron from behaving like a free transitional metal its potential as an autooxidation catalyst would be abolished. The explanation is
supported by much indirect evidence. In particular, in the absence of iron, free-radical oxidation can be induced in many biological and non-biological mixtures by ultraviolet irradiation and this is not inhibited by ceruloplasmin [Pamela beilli, Et Al; 2002].

The effectiveness of ceruloplasmin as an antioxidant, points to a possible biological role. The protein is one of the acute phase reactants whose concentration in plasma rises after tissue injury. Most acute phase proteins can be thought of as protecting the organism as a whole from the possible ill-effects of local damage. One of the ill-effects could be the release of free-radical oxidation products. This suggests that the organism might respond by raising the antioxidant efficiency of plasma.

2.4 Serum Glutathione

Glutathione (GSH, \( \gamma \)-glutamyl-cysteinylic-glycine) is the most abundant non-protein thiol in eukaryotic cells. The synthesis of this tripeptide is catalyzed by two cytosolic enzymes: \( \gamma \)-glutamate-cysteine ligase (first step), and GSH synthetase (second step; which combines \( \gamma \)-glutamyl-cysteine with glycine to generate GSH). GSH is involved in cell protection against free radicals and in many cellular functions being particularly relevant in regulating carcinogenic mechanisms [Ames B.N, Et Al; 1995]; sensitivity against ionizing radiation and some cytokines; sensitivity against xenobiotics; DNA synthesis and cell proliferation [Meister A; 1991].

Lipid peroxidation (LPO) in cancer, because of uncontrolled free radical involvement, results in several changes in cancer cells [Eriksson LC, Et Al; 1992]. Glutathione (GSH) and GSH peroxidase (GS-Px) salvage the cell from LPO damage, particularly from damage to the membranes [Masotti L, Et Al; 1988]. The salvaging process occurs when peroxo-polyunsaturated fatty acids (peroxy-PUFAs) are converted to hydroxy-FAs by GS-Px at the expense of GSH [Sevanian A, Et Al; 1983], rather than allowing LPO to go to completion with the production of short-chain FAs and MDA.
GSH and GS-Px also prevent other oxyradical damage, especially that inflicted on proteins and DNA. They acted as members of the antioxidant system of the cell by degrading hydrogen peroxide before it is converted to a hydroxyl radical (OH) [Halliwell and Gutteridge; 1992]. Hydroxyl radicals can cause mutagenic damages to DNA by hydroxylation of the bases of DNA, especially guanine (G), to form 8-hydroxy-G [Floyd RA; 1990]. This mutagenic event causes a change in base pairing from a G:C base pair to a 8-OH-G:A pair, which will eventually lead to a substitution of G by T in the subsequent progeny strand, causing a G to T transversion [Kasai and Nishimura; 1991].

The GSH/GSH-s-transferase (GST) system detoxifies mutagens, including those derived from ROS. In tumours, the GST P1-isoenzyme is strongly and persistently expressed [Campbell JAH, Et Al; 1991] but it was reported that the A- and M-isoenzymes appeared to be down-regulated or simply not expressed in many tumours [Gajewska and Szczypka; 1992]. Studies suggest that the expression of GST-P1 may be linked directly to malignant transformations and may be regulated by oncogenes such as c-jun and c-fos [Tsuchida and Sato; 1992].

GSH synthesis is upregulated during oxidative stress and inflammation. Oxidants like hyperoxia, ozone, hydrogen peroxide, etc. cause short-term falls in intracellular GSH which associate with higher oxidized glutathione (GSSG) levels; this is followed by increases in GSH levels and/or upregulation of γ-glutamate-cysteine ligase mRNA in in vivo and in vitro models [Paget M.S, Et Al; 1998]. Therefore, oxidants and oxidant-generating systems (if their levels do not compromise cell viability) can upregulate GSH synthesis-linked genes, thereby providing paradoxically a protective mechanism against oxidative stress.

2.5 Serum Beta-Carotene

Beta-carotene is in the carotenoid family of pigments that occur naturally in plants. Carotenoids and especially beta-carotene are an important source of vitamin A and are also postulated to protect human cells from the reactive species that may induce cancer and atherosclerosis [Gaziano IM , Et Al; 1990] [Riemersana RA, Et Al;
There is also recent evidence that carotenoids can induce cell-mediated tumor lysis [Schwartz JL, Et Al; 1990]. Their ability to accumulate in lipid-rich atherosclerotic plaque makes selective removal of plaque possible with laser radiation tuned to the carotenoid absorption peak [Murray A, Et Al; 1989] [Prince MR, Et Al; 1986] [Prince MR, Et Al; 1988]. In addition, a carotenoid metabolite, retinoic acid, recently has been shown to play a fundamental role in cell differentiation and organogenesis [Durston Al, Et Al; 1989].

The amount of beta-carotene absorbed is a small fraction of the ingested dose and is highly variable from individual to individual. Beta-carotene absorption into the body is known to increase when it is taken in association with a high-fat diet. The extent to which fl-carotene absorption can be enhanced by adminstering it in association with the entire day’s fat consumption is not accurately known. Beta carotene, in the small intestine [Paik j, Et Al; 2004] is not efficiently metabolized to vitamin A (retinol) and it is mostly absorbed intact along with the metabolized parts.

In the intestinal mucosa, retinal is reduced to vitamin A (retinol) by retinaldehyde reductase and free retinol is taken up by the enterocytes, involving both diffusion and protein-mediated facilitated transport. In cells, retinol is complexed with cellular retinol-binding protein type-II and this complex is believed to help re-esterification process of retinol by the enzyme retinol acyltransferase and free retinol is also esterified by acyl-CoA and acyltransferase and these esters are then incorporated into chylomicrons and finally secreted into the lymph [Takase S, Et Al; 2000] [Silalahi J; 2002] and transported to the target tissues. It may be suggested that the different metabolic patterns of vitamin A and β-carotene play significant roles in cancer modulation by these compounds at variable degrees.

The protective benefits of beta carotene are probably related to its role as an antioxidant in decreasing free-radical damage and its ability to quench singlet oxygen species, which is a reactive and unstable molecule. Beta carotene has immunoregulatory properties, retarding the development of cancer cells [Ranganathan K, Et Al; 2004]. Ingestion of beta carotene quickly increases the number of CD4 helper T-lymphocytes and significantly increases their response to mitogens. Hence it seems that beta carotene supplementation might enhance the immunoresponse of patients who are deficient in beta carotene [Bendich A, Et Al; 1986]. Stahelin [Stahelin HB, Et
Al; 1991] demonstrated a dose-dependent effect of beta carotene by comparing the relative risk of developing cancer with serum beta carotene levels. Subjects who had lower levels of beta carotene had a higher risk than those with average or higher levels.

The influence of vitamin A on detoxification enzymes like non-specific carboxylesterase and glutathion S-transferase has been studied and was concluded that vitamin A has a potential role in the regulation of detoxification enzymes [Kasree B, Et Al; 2003]. Role of vitamin A on the biotransformation may be critical in delaying or inhibiting the cancerous process. Studies have shown that carcinogens like methylcholanthrene, 2-acetylaminofluorine had a higher hepatic microsomal cytochrome P-450 level in vitamin A deficient rats [Marill J, Et Al; 2000]. Thus vitamin A and its metabolites may play a crucial role in metabolizing various carcinogens to less harmful chemicals and they, thereby, provide some anticarcinogenic effects. Moreover, vitamin A is known to be a potent inducer of apoptosis [Simoni D, Et Al; 2001].

Beta-carotene undergoes metabolism to retinol (vit. A1), which is required for normal cell differentiation of stem cells in epithelial tissue [Robert K. Murray, Et Al; 2003]. Beta-carotene in the intestinal mucosa converts into 8/, 10/ and 12/-apo-carotenals by asymmetric cleavage with the enzyme carotene deoxygenase [Dianne Robert Soprano, Et Al; 2004]. This is then oxidized into two isomers of retinoic acid. Two major active metabolites all-trans-retinoic acid and 9-cis- retinoic acid have high affinity for ligands to the protein receptor RARs and 9- cis-retinoic acids also possess high affinity for the RXRs [Robert K. Murray, Et Al; 2003]. These two receptors proteins form homodimer (RXR/RXR) and heterodimer (RXR/RAR) [Alfanson R. gennaro; 2000]. They function as retinoic acid inducible transcriptional regulatory protein and bind to DNA sequences probably within the promoter region of genes to control the gene function. The parts of these sequences are commonly known as retinoic acid response element (RARE) and retinoid X response element (RXRE) [Dianne Robert Soprano, Et Al; 2004] [Cheung B, Et Al; 2003]. Thus they play an important role in the transcriptional control of various genes by causing DNA to be inaccessible to the transcriptional machinery. The abnormal transcriptional regulation of the retinoic receptors (RARs) α, β and γ results in various patho-physiological
conditions including cancer. Loss of normal RAR function in the presence of physiological levels of retinoic acid is reported to be associated with various forms of cancer. Translocation involving RAR-α gene is reported to be a hallmark of acute promyelocytic leukaemia [Cheung B, Et Al; 2003] [Takahashi N; 2002]. Again a loss in RAR-β expression occurs in a variety of premalignant and malignant lesions [Toulouse A, Et Al ; 2000] [Teraishi F, Et Al; 2003].

Various trans-cripational factors like COUP-TF and two orphan receptors have been reported to regulate RAR-β expression in malignant cells [Lin B, Et Al; 2000] [Lin F, Et Al; 2002]. The orphan receptor TR3 is deeply involved in the regulatory process of ATRA. It has been observed that TR3/RXR-α heterodimer formation in the nucleus [Ye X, Et Al; 2004] and subsequent translocation in the cytoplasm down regulate antiapoptotic protein like Bcl-2 and Bcl-xl [Fujimura S, Et Al; 2003] [Pettersson F, Et Al; 2002] and induces apoptotic protein Bax that results in ATRA induced apoptosis in the cancerous cell [Palozza P, Et Al; 2002]. It was also found that ATRA, 9-cis-retinoic acid reduce the expression of apoptotic proteins and cell cycle protein cyclin A, D1, cdk4 and cdk2 which causes large growth inhibition by slow down G2 and M phases of cell division [Hatoum A, Et Al; 2001]
Figure: 2.6 Mode of anticancer action of vitamin A and beta carotene

[BiswaJit Mukherjee, Et Al; 2011]
2.6 Serum Copper

Copper is an essential trace element for most organisms. Critical proteins such as zinc–copper superoxide dismutase, cytochrome oxidase, lysyl oxidase and several transcription factors require copper for activity. Free copper is a potent oxidant. Cells therefore rigorously limit the amount of free copper by binding copper to other molecules. Chaperone proteins shuttle copper through the cell to copper-requiring enzymes in different cellular compartments. Copper toxicity can exist either because of the ingestion of excessive amounts of copper or because of genetic defects that interfere with copper homeostasis. Ingestion of toxic amounts of copper leads to acute gastrointestinal symptoms of nausea, vomiting and diarrhea. Ceruloplasmin is a copper-containing protein secreted by the liver into the blood. The copper in Ceruloplasmin accounts for about 90% of the total plasma copper.

The essential role that angiogenesis plays in tumor development was initially hypothesized by Dr Judah Folkman. In the absence of new blood vessel formation, solid tumors must receive necessary oxygen and nutrients by diffusion, restricting growth to 1–2 mm [Folkman J; 1971]. Dr Folkman further postulated that tumor cells elaborate a growth factor termed ‘tumor angiogenesis factor’ (TAF), which would behave as an angiogenic switch [Folkman J; 1974]. Once activated, TAF would promote vessel formation, allowing tumor growth, invasion and metastasis. Thus, blockade of angiogenesis via TAF inhibition might serve as a novel anti-neoplastic strategy. TAF activity is controlled several proangiogenic mediators including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and transforming growth factor-b (TGF-b) [Scappaticci FA; 2002]. These pro-angiogenic factors are counterbalanced by inhibitors of angiogenesis such as thrombospondin, angiostatin and endostatin. Tumors exploit an imbalance between the pro-angiogenic and anti-angiogenic factors to allow growth and metastasis [Hanahan & Folkman; 1996] [Iruela-Arispe & Dvorak; 1997].

Endothelial cell migration is an essential early step in angiogenesis and copper has been shown to induce migration of aorta endothelial cells in bovine [McAuslan BR, Et Al; 1983]. Furthermore, a heparin–copper complex has been shown to stimulate capillary migration in vitro as well as angiogenesis in vivo [Alessandri G, Et
Angiogenin is secreted by vascular endothelial cells and aortic smooth muscle cells in addition to fibroblasts and tumor cells. It has been shown to induce new vessel formation. As copper is known to participate in angiogenesis, the copper binding to angiogenin results in an increased affinity between angiogenin and endothelial cells, thus promoting new vessel formation [Rybak SM, Et Al; 1987]. Human acidic FGF1 has been demonstrated to bind copper using a copper-affinity HPLC column [Watanabe T, Et Al; 1990]. FGF1 is an angiogenic factor which requires secretion into the extracellular compartment for activity. This protein contains no signal sequence for endoplasmic reticulum (ER)–Golgi mediated secretion, the mechanism of FGF secretion and copper plays a role in the formation of a multi-protein complex implicated in the release of FGF1 in response to heat shock [Landriscina M, Et Al; 2001]. Similarly, IL-1a undergoes copper-dependent secretion into the extracellular compartment. VEGFs are a family of angiogenic proteins which are essential in vasculogenesis and hypoxia-induced angiogenesis [Bikfalvi A, Et Al; 2002]. Recent data suggest that copper may be a required cofactor of VEGF mediated angiogenesis.

Copper plays an important role in angiogenesis. Studies suggest several mechanisms through which copper may exert this effect:

1. Copper may act through binding of angiogenic growth factors and increasing their affinity for endothelial cells, as seen in case of angiogenin;
2. Copper may control the secretion of angiogenic cytokines, as demonstrated with FGF1 and IL-1a;
3. Copper may induce expression of angiogenic growth factors such as VEGF.

Thus, therapy aimed at depleting copper may be a successful anti-neoplastic strategy which may target multiple angiogenic growth factors.

### 2.7 Serum Iron

Iron is an essential nutrient for body. Iron is a vital trace element participating in numerous biological and cellular processes such as oxygen transport, electron transfer and DNA synthesis as well as cell cycle progression and growth. Iron absorption from the diet occurs mainly in the duodenum by a tightly regulated mechanism. Most of the iron absorbed, is utilized for erythropoiesis and any excessive
iron is stored mainly in the liver. Iron absorption is inversely regulated by body iron levels, decreasing in conditions of iron excess and increasing during iron deficiency. Iron metabolism is regulated by the hepatic hormone hepcidin and its expression is controlled by many factors like iron stores, hypoxia, inflammation, anaemia and erythropoiesis [Anita CG Chua, Et Al; 2010]

It has a central role in metabolism. It is also an essential component in DNA synthesis as well as in respiratory and oxidative metabolism. These central and essential functions relate to the properties of unremitting proliferation and a more anaerobic metabolism that may contribute to a selective advantage of neoplastic cells over non-neoplastic cells. Clinical correlations have been made linking cellular iron content to the development of cancer in human [Graham, Et Al; 1977].

Iron is one of the most abundant and necessary transition metals in the body. Level of serum iron, ferritin, total iron binding capacity (TIBC) and transferrin saturation are the important diagnostic measures of iron deficiency or excess [Shakhawat Hossain, Et Al; 2007]. But, in these forms it is involved in profuse production of free radicals and reactive oxygen species which can be extremely harmful.

The potential role of iron in cancer etiology is supported by several possible mechanisms by which iron can provoke DNA damage and lead to carcinogenesis. As transitional metals, iron can generate the reactive oxygen species including hydroxyl radical. Iron binding sites on macromolecules serve as centers for repeated production of hydroxyl radicals generated via the Fenton reaction [V N Bhattathiri; 2005]. Iron and oxygen together constitute a biologically highly damaging mixture due to increased formation of free radicals. Normally, chances of these are reduced by sequestration in storage or transport proteins and action of ‘acute-phase’ proteins such as ceruloplasmin, haptoglobins, etc., involved in iron metabolism. Free radical mediated damage is known to be the root cause of many inflammatory, degenerative and neoplastic diseases [V N Bhattathiri; 2005]. These reactive oxygen species can attack DNA and cause DNA mutation; thus contributing to the pathological process of cancer [Toykuni, 1996]. Iron excess plays a definitive role in these processes. Presence of iron salts can decrease the protective effect of natural antioxidants like vitamin E and contribute to carcinogenesis. In sufficient concentrations, it can make
even ascorbate, an antioxidant, have pro-oxidant properties. Carcinogens are considered to be activated not only by the more usually accepted enzymic pathways but by free-radical reactions catalysed by iron in the vicinity of critical sites [Bhattathiri VN; 2005]. Carcinogenesis also is affected by inflammation which is exacerbated by iron [Anita CG Chua, Et Al; 2010].

2.8 Serum Zinc

Essential element zinc (Zn) has a role in many biochemical reactions as a micro-source. Zn stimulates gene transcriptions and cell multiplications. Increased levels of Zn concentration might help multiplication of tumor cells. While Cu and Zn are necessary for activation of RNA and DNA polymerase enzymes, Cu and Zn also have a role as co-factors of antioxidant enzymes. In addition to this, Zn is necessary for the optimum performance of the immune system. Zn is essential for the development of nucleic acid and protein synthesis. In addition, Zn ensures the stability of the fullness of the ‘d’ orbital, and this makes oxidation-reduction impossible in any environment containing Zn [Alper Boz, Et Al; 2005]. Zinc has a critical role in body’s defense against oxidative stress caused by excessive smoking. It acts as a free radical scavenger in view of their ability to scavenge OH, O2-, and NO radicals [Seema Joshi, Et Al; 2004].

Zn is required for the activity of numerous enzymes associated with proper immune function and for the conformation of many transcription factors that control apoptosis, cell proliferation, apoptosis and signaling pathways. Zn is known to undergo rapid ligand exchange reactions and is used as an information carrier in signal transduction pathways. Accordingly, Zn-deficiency predisposes to disease by adversely affecting the immune system by increasing oxidative stresses as well as by increasing the generation of inflammatory cytokines. Studies have shown that Zn-deficient diet creates precancerous condition by inducing the proliferation and gene expression changes including overexpression of the cyclooxygenase-2 (Cox-2) and the proinflammation-genes S100 calcium binding protein a8 (S100a8) and a9 (S100a9). Znreplenishment inhibits tumorigenesis by inducing apoptosis and reversing cellular proliferation. Dietary Zn-replenishment reverses overexpression of S100A8 in Zn-deficient states and modulates the link between S100A8 and its
receptor RAGE (receptor for advanced glycation end products) and downstream NF-kB/COX-2 signaling, thereby attenuating inflammation and reverses preneoplasia. Chronic inflammation, being a hallmark of cancer, Zn has an inflammation-modulating role in cancer initiation/reversal [Louise Y.Y. Fong, Et Al; 2011].

Zinc is known to play an important role in immune functions. Mild zinc deficiency is associated with decreased thymulin activity and decreased production of IL-2. Inasmuch as IL-2 plays a central role in the expansion and maintenance of thymocytes and peripheral T cell populations, the generation of anti-viral and anti-tumor specific cytotoxic T cells, delayed type hypersensitivity responses, and up regulation of NK and T cytolytic activities, it is conceivable that even a mild deficiency of zinc could lead to enhanced susceptibility to infections and malignancies by impairing production of this cytokine [Ananda S. Prasad, Et Al; 1998].

Zinc influences cancer development or prevention in several ways. Its role in DNA synthesis, and in T-cell cytolytic activity are well established and its deficiency may lead to progression or recurrence of malignancy. Dietary zinc deficiency increases the methylbenzylnitrosamine induced formation of 06-methylguanine in the esophageal DNA of the rat and these adducts are known to induce guanine to adenine point mutations which are responsible for certain carcinogen- induced tumors. Animal studies have shown that zinc administration may slow the progression of induced tumors and studies in humans also show that administration of zinc and other micronutrients may have therapeutic effects in patients with oral precancerous lesions [Ananda S. Prasad, Et Al; 1998].

2.9 Review of studies

In a study evaluating the serum values of ceruloplasmin in 144 patients with cancer and 103 normal controls by reciever operating characteristic curve analysis [rocca], it was concluded that serum ceruloplasmin was significantly elevated in advanced stages of solid malignant tumours. Finally, the results suggest that serum ceruloplasmin is characteristic of good diagnostic marker [Senra Varela A, Et Al; 1997].
In a study conducted in 149 patients with lung cancer and compared with 19 healthy controls as well as 23 patients with non-malignant lung diseases, an increased mean serum copper levels were found which seemed to reflect the stage of the lung disease, with asymptomatic patients showing lowest values and patients with metastatic symptoms showing the highest values [Huhti E, Et Al; 1998].

In a study serum levels of ceruloplasmin, copper, zinc, total iron and total protein was estimated in a total of 92 patients with squamous cell carcinoma and oral leukoplakia and age and sex matched controls. Serum copper and ceruloplasmin level significantly increased in oral leukoplakia & cancer in both sexes. A significant decrease in serum total iron & proteins was observed only in carcinomas. The level of zinc decreased significantly in patients with leukoplakia and cancer. Thus, the study shows that serum ceruloplasmin, copper, zinc and iron have diagnostic value as biomarkers in differentiating malignancies from normal [Jaydeep A, Et Al; 1997].

In a study glutathione concentrations in human epidermoid carcinoma tissues were measured by high performance liquid chromatography and the mean glutathione content of 26 epidermoid carcinoma intratumor tissue specimens was significantly higher than that in adjacent non-tumor tissue parts. Abnormal proliferative activities may probably be the cause for elevated glutathione levels in cancer tissues. This indicate that glutathione level of oral tissues may be a useful marker in oral cancer, which is in agreement with findings in cervical squamous cell carcinoma, lung squamous cell carcinoma and other squamous cell carcinomas [Wong Dy, Et Al; 1994].

In a study, epidemiological statistics have linked low serum β-carotene levels to elevated risk of lung and other cancers as well as β-carotene to diminished preneoplastic lesions in intervention trials. β-carotene should block tumor promotion given a radical quenching mechanism, but more typically action is at the site of progression and an even later role in invasion has not been ruled out. Immunoenhancement has been attributed as cause for some antineoplastic actions of carotenoids whereas others may be attributed to conversion to retinoids and subsequent gene regulation. [Eunice J. Rousseau, Et Al; 1992]
In a study Serum ceruloplasmin, C3 complement and albumin in 119 male smokers and 65 male non-smoker; from a military unit in Bangkok were investigated. In smokers the serum ceruloplasmin concentration was found to be significantly higher than in non-smokers. However, in smokers the serum albumin concentration was statistically significantly lower than in non-smokers. Significant associations were found between quantity of cigarettes smoked, ages and albumin levels. The correlation between serum ceruloplasmin and C3 complement concentrations was significantly positive. An association was found between the quantity of cigarettes smoked and albumin, as well as a significant relationship was found between smoking and the quantities of cigarettes smoked to serum ceruloplasmin levels and the quantity of cigarettes smoked were taken as independent variables, while the serum ceruloplasmin levels as a dependent variable. The high concentrations of the acute-phase protein, i.e. ceruloplasmin, might suggest that this might constitute a risk of developing atherosclerosis or cardiovascular disease in smokers. [Tungtrongchitr R, Et Al; 2002]

In a study conducted to investigate the relationship between smoking and antioxidant nutrient intake and status, smokers (n 44) and non-smokers (n 44) male students aged between 22 and 28 years were selected. Intakes of dietary carotenes, vitamin C and Zn was lower among smokers but only the difference in Zn intake was significant statistically. The difference was not significant for either serum vitamin E (alpha-tocopherol) or vitamin A (retinol) level between smokers and non-smokers. The plasma vitamin C level was significantly lower in smokers than that in non-smokers (P = 0.0004). Significantly lower serum Cu (P = 0.04) and higher serum Zn levels (P = 0.003) was found in smokers. Further, the results showed a significant dose-response relationship between smoking and vitamin C status. Linear-regression analysis between dietary intake and plasma vitamin C values in non-smokers showed a significantly positive correlation (r 0.50; P = 0.0005). On the contrary, such association was not observed in smokers. These findings suggest an imbalance in antioxidant nutrient intake and status may be caused due to smoking. [Faruque MO, Et Al; 1995]

In a study higher levels of plasma-oxidized low-density lipoprotein (LDL) and lipid peroxide (LPO), and higher oxidizability of LDL were observed in smokers than
in nonsmokers. In smokers higher levels of serum iron and lower levels of plasma vitamin E were observed than in nonsmokers. Serum iron was shown as an independent determinant for both plasma-oxidized LDL and lag time of LDL oxidation by stepwise multiple regression analysis. The expression of 12-LO was enhanced threefold and its activity 1.5-fold by iron loading. Moreover, catalase expression decreased by 50% and its activity significantly reduced by 75% by iron loading. Enhanced oxidative stress in smokers may be due to increased iron levels. Iron-induced modulation of activity and expression of catalase and 12-LO may be relevant to increased iron-related oxidative stress as observed in smokers. [Yoshida H, Et Al; 2004]

In a study designed to investigate the influence of cigarette smoking on serum Se, Zn, Cu, vitamin C and lipid profile, 140 healthy volunteer men (70 Smokers and 70 non-smokers) were considered and the determination of serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-cholesterol) and triglyceride (TG) were performed by a Cobas Integra analyzer. Low-density lipoprotein cholesterol (LDL-cholesterol) was calculated. Serum vitamin C analysis was determined by a high-performance liquid chromatograph and serum concentrations of trace elements were measured by atomic absorption spectrophotometry. To identify differences between the cigarette smoker and non-smoker groups the statistical method of t-test was used. The serum vitamin C, zinc and selenium levels of smokers were significantly (P<0.05) lower than those of non-smokers. Smokers had significantly (P<0.05) higher serum copper concentration when compared with non-smokers. Smokers had significantly higher levels (P<0.05) of serum total cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol) and triglyceride levels than those of non-smokers. On the contrary, the smokers had significantly lower levels of serum HDL-cholesterol (P<0.05) than that of non-smokers. These findings suggest that an imbalance between antioxidant nutrient status and free-radical load may be caused by cigarette smoking which may initiate the deterioration process associated with cardiovascular disease; thereby, increasing the risk these diseases. [Khalid S. Al-Numair; 2006]