SECTION-A

(Objective type questions. Each question carries 2 marks) 12 X 02 = 24

Q1. a).
   i) Ans: Structure of Lovastatin is

   ![Lovastatin Structure](image1)

   ii) Ans: Structure of Dehydroepiandrosterone is

   ![Dehydroepiandrosterone Structure](image2)

   iii) Ans. Structure of Cholestipol is

   ![Cholestipol Structure](image3)

   iv) Ans: Structure of Progesterone

   ![Progesterone Structure](image4)

   v) Ans: Structure of Ethinyl estradiol

   ![Ethinyl estradiol Structure](image5)
Q1. b) 
Ans- The IUPAC name of Methimazole is -1-methyl-3H-imidazole-2-thione.

Q1. c) 
Ans. TPO inhibitors are
Thiouracil/methylthiouracil/propylthiouracil/methimazole/carbimazole.

Q1. d) 
Ans. HMG Co-reductase inhibiotor drugs are Simbastatin/Pravastatin/Atorvastatin/Lovastatin

Q1. e) 
Ans. Nonsteroidal antifertility agents are Centchroman /Nafoxidine/Gossypol

Q1. f) 
Ans- applications of haemoglobin are to carry the respiratory gases O2 & CO and in the formation of biliverdin & bilirubin.

i) 
Ans applications of endorphins -In Pain Management and in feelings of well-being with sensations of pleasure

ii) 
Ans -In the treatment of Graves' disease and hyperthyroidism.

Section –B

(Descriptive questions. Each question carries 14 marks 14 X 04 = 56)

Q2.

Ans: Antifertility agents are the drug which used for preventing fertilization are called as anti-fertility agents. These are also known as contraceptive agents. Contraception is the method of preventing normal process of ovulation, fertilization and ovum implantation nothing but pregnancy. These drugs are classified into two classes; Steroidal anti-fertility agents and nonsteroidal - anti-fertility agents.

Steroidal anti-fertility agents

Estrogens:
1) Natural Oestrogens : Oestradiol, Oestriol, Oestrone.
2) Esterified Oestrogens : Oestradiol valerate, Oestradiol benzoate, Oestradiol dipropionate.
3) Conjugated Oestrogens: Equilin, estrone sodium sulfate, equilin sodium sulfate.
4) Semisynthetic Oestrogens : Ethinyl oestradiol, Mestranol.
5) Synthetic Oestrogen/ Diethylstilbestrol derivatives :Diethylstilbestrol, Dienoestrol, Stilboestrol and Benzestrol.
6) Phyto Oestrogens : Coumestrol from legumes, Diadzein from soybean, Genistein from soybean and species of clover
Progesterone and its derivatives: Progesterone, Hydroxy progesterone, Medroxy progesterone, Megestrol
Synthetic progestins: Trimegestone, Drospirenone
AntiAndrogen:
1)Testosterone derivatives: Ethisterone, Dimethisterone.
2)19-Nor testosterone derivatives: Norethindrone, Norethynodrel, Ethynodiol, Norgestrel, Norgestimate
Combination regimen: The combination oral contraceptives containing both an estrogen and a progestin. Ethinyl estradiol and mestranol are the two estrogens used, progestins are 19-nor compounds.

Non-steroidal anti-fertility agents

1)Centchroman
2)Nafoxidine
3)Diethylstilbestrol
4)Calcium channel blockers: Nifedipine
5)Male antifertility Agents:
   a) Gossypol
   b) Extract of Trypterigium hypoglacum and Trypterigium regeli.
   c) Extract of Azaractica indica

Mechanism of fertility control:

Orally effective suppression of ovulation accomplished by administration of progesterone and estrogen combination. Coordination of synthesis and secretion of follicle stimulating hormone (FSH) and leutinising hormone (LH) from the anterior pituitary gland originate in the hypothalamus. Releasing factors such as gonadotrophin releasing hormone stimulate FSH and LH production which facilitates ovulation (fertilization). Endogenous estrogen and progesterone are present in the blood at high levels subsequent to ovulation (fertilization) and during pregnancy.

These intract with estrogenic receptor and progestogenic receptor in the hypothalamus to down regulate GnRH production, ultimately reducing FSH and LH level during pregnancy preventing further ovulation. This process of feedback inhibition is mimicked by orally administered estrogen and progesterone combination.

Estrogen suppress ovulation by inhibition GnRH release due to suppression of hypothalamus releasing factor and progesterone causes pseudodeci dual changes in endometrium prevents implantation of the zygote. It also modifies cervical mucus which make it impenetrable to sperm. Both in combination prevent the FSH and LH peak in the mid cycle. The secretion become thick and decrease sperm motility.
Q3. Ans: Chemistry of corticosterone.

Structure of corticosterone is

\[
\text{corticosterone (I)}
\]

1) Molecular formula of corticosterone is $C_{21}H_{29}O_4$
2) When react with strong reducing agent it form aldehyde or ketone. It suggest that corticosterone is $\alpha,\beta$ unsaturated ketone.
3) $\alpha,\beta$ unsaturated ketone react with $\text{HIO}_4$ to give formaldehyde and hydroxyl acid (II). Compound, suggest the presence of a primary alcohol and keto group.
4) It Shows absorbance $\lambda_{\text{max}}$ at 240mu suggest presence of n-Alcohol.
5) Cortecosterone react with the acetic acid give cortisone diacetate. The formation of diacetate suggest the presence of two hydroxyl groups.
6) The structure of corticosterone is confirm by following chemical reactions:

Reaction :1

\[
\text{Reactions:}
\]

\[
\text{corticosterone (I) } \xrightarrow{\text{strong reducing agent}} \text{11-dehydrocorticosterone}
\]

\[
\text{(II) } \xrightarrow{\text{CrO}_3} \text{(III)} \xrightarrow{\text{H}_2\text{-Pt/Alcohol}} \text{(IV)}
\]

\[
\text{HIO}_4
\]

\[
\text{H}_2\text{-Pt/Alcohol}
\]
Structure of corticosterone is further confirmed by its synthesis.

\[
\begin{align*}
&\text{Methyl ester of Bisnov} \\
&\text{12-Acetate} \\
&\text{Pyrolysis 320 C} \\
&\text{Grignard} \\
&\text{Br_2} \\
&\text{Pyridine} \\
&\text{H_2Pt} \\
&\text{Ac}_2\text{O} \\
&\text{OH-Br} \\
&\text{OHUBr} \\
&\text{Ac}_2\text{O} \\
&\text{K}_2\text{CrO}_3 \\
&\text{Diacetate} \\
&\text{Saponification} \\
&\text{Benzoylation} \quad \text{(ii)} \\
&\text{Oppene oxidation} \quad \text{(i)} \\
&\text{Oppene oxidation} \\
&\text{Hydrolysis} \quad \text{(i)} \\
&\text{Pb(AC)}_{14} \\
&\text{Oxytocin} \\
\end{align*}
\]

Q4.

Ans: Oxytocin: It is an octapeptide secreted by posterior pituitary along with ADH. Structure is given below:
In general it is represented as:

\[ \text{H}_2\text{N-Cys} \quad \text{Cys-Pro-Leu-Gly-NH}_2 \quad \text{Ty-Isol-Glu-Asp} \]

It is a mammalian neurohypophysial hormone that acts primarily as a neuromodulator in the brain, it is synthesized and released by neurons of the hypothalamic-neurohypophysial system.

It is synthesized within the nerve cell bodies in supraoptic and paraventricular nuclei of hypothalamus and transported down the axon and stored in the nerve ending within the neurohypophysis. Stored in neurons as complexes with their specific binding protein and released by appropriate stimuli. Its proportion can vary, depending upon the nature of stimuli.

**Actions of oxytocin:**

1. **Uterus:** It increases the forces and frequency of uterus contraction. In low doses full relaxation occurs and in high doses basal tone increases.

2. **Mammary glands:** It contracts the myoepithelium of mammary alveoli and initiates the “milk ejection reflex”.

Oxytocin plays roles in sexual reproduction, in particular during and after childbirth. It is released in large amounts after distension of the cervix and uterus during labor, facilitating birth, maternal bonding, and, after stimulation of the nipples, breastfeeding. Both childbirth and milk ejection result from positive feedback mechanisms.

3. **Brain:** In high doses produce vasodilation by direct action, fall in blood pressure, reflex tachycardia, and flushing occurs.

4. **Kidney:** In high doses it gives ADH like action, decreased urine output, and produced pulmonary oedema.

Recent studies have begun to investigate oxytocin’s role in various behaviors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors. For this reason, it is sometimes referred to as the “love hormone”. There is some evidence that oxytocin promotes ethnocentric behavior, incorporating the trust and empathy of in-groups with their suspicion and rejection of outsiders. Furthermore, genetic differences in the oxytocin receptor gene (OXTR) have been associated with maladaptive social traits such as aggressive behaviour.

**Uses:**

1) Induction of labour (postmaturely and prematurely)
2) Uterine inertia
3) Postpartum haemorrhage

**Insulin**

Insulin is a peptide hormone, produced by beta cells of the pancreas, and is central to regulating carbohydrate and fat metabolism in the body. Insulin causes cells in the liver, skeletal muscles, and fat tissue to take up glucose from the blood. In the liver and skeletal muscles, glucose is stored as glycogen, and in fat cells (adipocytes) it is stored as triglycerides.
Insulin stops the use of fat as an energy source by inhibiting the release of glucagon. With the exception of the metabolic disorder diabetes mellitus and metabolic syndrome, insulin is provided within the body in a constant proportion to remove excess glucose from the blood, which otherwise would be toxic. When blood glucose levels fall below a certain level, the body begins to use stored sugar as an energy source through glycogenolysis, which breaks down the glycogen stored in the liver and muscles into glucose, which can then be utilized as an energy source. As a central metabolic control mechanism, its status is also used as a control signal to other body systems (such as amino acid uptake by body cells). In addition, it has several other anabolic effects throughout the body.

When control of insulin levels fails, diabetes mellitus can result. As a consequence, insulin is used medically to treat some forms of diabetes mellitus. Patients with type 1 diabetes depend on external insulin (most commonly injected subcutaneously) for their survival because the hormone is no longer produced internally. Patients with type 2 diabetes are often insulin resistant and, because of such resistance, may suffer from a "relative" insulin deficiency. Some patients with type 2 diabetes may eventually require insulin if other medications fail to control blood glucose levels adequately. Over 40% of those with Type 2 diabetes require insulin as part of their diabetes management plan.

The human insulin protein is composed of 51 amino acids, and has a molecular weight of 5808 Da. It is a dimer of an A-chain and a B-chain, which are linked together by disulfide bonds.

Insulin's name is derived from the Latin *insula* for "island". Insulin's structure varies slightly between species of animals. Insulin from animal sources differs somewhat in "strength" (in carbohydrate metabolism control effects) in humans because of those variations. Porcine insulin is especially close to the human version.

The structure of insulin is:

```
Gln-Gly
Ile
Val
Glu
His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys
Gln
Asn
Val
Arg
Phe
Thr-Lys-Pro-Thr-Tyr-Phe-Phe-Gly
```

The actions of insulin (indirect and direct) on cells include:

- Control of cellular intake of certain substances, most prominently glucose in muscle and adipose tissue (about two-thirds of body cells)
- Increase of DNA replication and protein synthesis via control of amino acid uptake
- Modification of the activity of numerous enzymes.
- Increased glycogen synthesis – insulin forces storage of glucose in liver (and muscle) cells in the form of glycogen; lowered levels of insulin cause liver cells to convert
glycogen to glucose and excrete it into the blood. This is the clinical action of insulin, which is directly useful in reducing high blood glucose levels as in diabetes.

- Increased lipid synthesis – insulin forces fat cells to take in blood lipids, which are converted to triglycerides; lack of insulin causes the reverse.
- Increased esterification of fatty acids – forces adipose tissue to make fats (i.e., triglycerides) from fatty acid esters; lack of insulin causes the reverse.
- Decreased proteolysis – decreasing the breakdown of protein
- Decreased lipolysis – forces reduction in conversion of fat cell lipid stores into blood fatty acids; lack of insulin causes the reverse.
- Decreased gluconeogenesis – decreases production of glucose from nonsugar substrates, primarily in the liver (the vast majority of endogenous insulin arriving at the liver never leaves the liver); lack of insulin causes glucose production from assorted substrates in the liver and elsewhere.
- Decreased autophagy - decreased level of degradation of damaged organelles. Postprandial levels inhibit autophagy completely.
- Increased amino acid uptake – forces cells to absorb circulating amino acids; lack of insulin inhibits absorption.
- Increased potassium uptake – forces cells to absorb serum potassium; lack of insulin inhibits absorption. Insulin's increase in cellular potassium uptake lowers potassium levels in blood. This possibly occurs via insulin-induced translocation of the Na+/K+-ATPase to the surface of skeletal muscle cells.
- Arterial muscle tone – forces arterial wall muscle to relax, increasing blood flow, especially in microarteries; lack of insulin reduces flow by allowing these muscles to contract.
- Increase in the secretion of hydrochloric acid by parietal cells in the stomach
- Decreased renal sodium excretion.

Insulin also influences other body functions, such as vascular compliance and cognition. Once insulin enters the human brain, it enhances learning and memory and benefits verbal memory in particular. Enhancing brain insulin signaling by means of intranasal insulin administration also enhances the acute thermoregulatory and glucoregulatory response to food intake, suggesting that central nervous insulin contributes to the control of whole-body energy homeostasis in humans.

Diseases and syndromes

There are several conditions in which insulin disturbance is pathologic:

- Diabetes mellitus – general term referring to all states characterized by hyperglycemia
- Type 1 – autoimmune-mediated destruction of insulin-producing β-cells in the pancreas, resulting in absolute insulin deficiency
- Type 2 – multifactoral syndrome with combined influence of genetic susceptibility and influence of environmental factors, the best known being obesity, age, and physical inactivity, resulting in insulin resistance in cells requiring insulin for glucose absorption. This form of diabetes is strongly inherited.
- Other types of impaired glucose tolerance.
- Insulinoma - a tumor of pancreatic β-cells producing excess insulin or reactive hypoglycemia.
- Metabolic syndrome – a poorly understood condition first called Syndrome X by Gerald Reaven, Reaven's Syndrome. It is currently not clear whether these signs have
a single, treatable cause, or are the result of body changes leading to type 2 diabetes. It is characterized by elevated blood pressure, dyslipidemia (disturbances in blood cholesterol forms and other blood lipids), and increased waist circumference (at least in populations in much of the developed world). The basic underlying cause may be the insulin resistance that precedes type 2 diabetes, which is a diminished capacity for insulin response in some tissues (e.g., muscle, fat). It is common that morbidities, such as essential hypertension, obesity, type 2 diabetes, and cardiovascular disease (CVD) develop.

- Polycystic ovary syndrome – a complex syndrome in women in the reproductive years where anovulation and androgen excess are commonly displayed as hirsutism. In many cases of PCOS, insulin resistance is present.

Q5.

Ans: Antidepressant drugs are classified as:

1) MAO Inhibitors: Phenelzine, Isocarboxazide, Tranylcypromine, Amiflamine.

2) Tricyclic antidepressant:
   a) Iminodibenzyl derivative-Imipramine, Trimipramine, Doxipine, Dothipine, Clomipramine
   b) Dibenzocycloheptane- amitritlyline, protrytline, nortrytline.
   c) Dibenoxepine derivative-Doxepine

3) Second generation Drugs
   a) Bicyclics : viloxamine, zimeldine
   b) Tricyclics : Amoxapine, imprindole
   c) Tetra cyclic: maprotiline, mianserine

4) Benzodiazepine-Alprazolam

5) Selective serotonin reuptake inhibitor
   Floxitine, fluvoxamine, paroxetine, Sertraline, Citalopram

6) Atypical antidepressantsa
   Trazodone, Mianserin, Mirtazapine, Duloxitine,Bupropion,amineptine

Structure of despiramine:

\[
\begin{align*}
\text{N} & \quad \text{CH}_2\text{CH}_2\text{CH}_2\text{NHCH}_3 \\
& \quad \text{5-propanamine-10,11-dihydro-N-methyl-5H-dibenz[b,f]azepine}
\end{align*}
\]
Synthesis: despiramine is synthesizes by pyrolysis of the methanesulfonate of 4,4’-diaminobibenzyl result in cyclization with formation of 10,11 –dihydro-5H-dibenz(b.f)azepine. This condensation with N-3chloropropyl,N-methylbenzylamine in the presence of alkali to form n-benzylated despiramine, which following debenzylated through reduction cleavage.

\[
\text{N} \quad \text{N} \quad \text{ClCH}_2\text{CH}_2\text{CH}_2\text{N(CH}_3\text{)}_2\text{Cl} \quad \text{C}_6\text{H}_5\text{NH}_2 \quad \text{NH}_2
\]

Debenzylation cyclization

PHENELZINE SULPHATE

\[
\text{CH}_2\text{CH}_2\text{NHNH}_2
\]

1-phenethylhydrazine, sulfate

Synthesis: Phenethyl alcohol reacted with thionyl chloride to give phenethyl chloride which is then added to hydrazine hydrate to yield phenelzine.

Q6.

Ans: Radiopharmaceuticals:

Radiopharmaceuticals are the preparations intended for in vivo use and contain radionuclide in the form of simple salt or complex. These may exist as solid, liquid, gas or pseudogas. The radiopharmaceutical is intended to target certain tissues, binding sites, and/or biochemical pathways. Depending on its specific physicochemical and radiation properties, a radiopharmaceutical can be used for either diagnostic or therapeutic purposes. A radiopharmaceutical preparation designed for therapeutic purposes must contain enough radioactivity to produce the intended tissue effects.

Pharmaceutical applications of radioprotective agents.

Reduction of radiation exposure is the primary goal for a radiation protective agents. Amifostine and thiols (cysteine, 2-mercaptoethylamine, cystamine) are important radioprotective agents.

The thiol metabolite is responsible for most of the cytoprotective and radioprotective properties of amifostine. It is readily taken up by cells where it binds to and detoxifies reactive metabolites of platinum and alkylating agents as well as scavenges free radicals.
Other possible effects include inhibition of apoptosis, alteration of gene expression and modification of enzyme activity. Structure of amifostine is:

Exposure to ionising radiation results in mutagenesis and cell death, and the clinical manifestations depend on the dose and the involved body area. Reducing carcinogenesis in patients treated with radiotherapy, exposed to diagnostic radiation or who are in certain professional groups is mandatory. The prevention or treatment of early and late radiotherapy effects would improve quality of life and increase cancer curability by intensifying therapies. The four distinct types of radiation damage - cellular depletion, reactive gene activation, tissue disorganisation, stochastic effects, bystander effects and classifies the radioprotective agents into four relevant categories:

a) protectants against all type of radiation effects
b) death pathway modulators
c) blockers of inflammation, chemotaxis and autocrine/paracrine pathways
d) antimitagenic keepers of genomic integrity,
e) agents that block bystander effects.

Radioprotectives (a) blocking the formation of free radicals; (b) blocking the free radicals, once formed, with free radical scavengers; (c) enhancing the deoxyribonucleic acid (DNA) repair process; (d) inhibiting death signalling pathways; (e) preventing the acute release of cytokines and growth factors by fibroblasts and endothelium during radiotherapy; (f) breaking the vicious cycle that continuously stimulates fibroblast and endothelium dysfunction; (g) preventing the survival of somatic mutated cells that repopulate the tissue with initiated cells; (h) preventing the DNA damage and hypomethylation of bystander cells.

Type A1 radioprotectant

The first step in radiation interaction with the cell is the formation of free radicals as a result of ionisation of water and other molecules. Reduction of oxygen tension in the body extremities to protect tissues by using tourniquet techniques has been proposed in the past for normal tissue protection. Since the early 1960s, reports have proposed that compounds such as hydroxytryptamine that reduce oxygen consumption could have a role as radioprotectants. Amifostine, the only radioprotectant currently approved for clinical use, has such a role, although its major cytoprotective pathway is its activity as a free radical scavenger.

Another approach to reduce the cellular oxygen consumption is amplifying the activity of the hypoxia-inducible factors-1 alpha and 2 alpha (HIF-1α and HIF-2α, respectively).

Cobalt chloride (CoCl2), the iron chelator desferrioxamine, and the organomercurial compound mersalyl are important inducers of HIFs, which they achieve by mimicking hypoxia, probably by reducing the ubiquitination of HIF. Clioquinol, a copper (Cu) (II)/zinc (Zn) (II) chelator, also inhibits the degradation of HIF-1α and leads to expression of
downstream genes in normoxic cells. Isoflurane, a volatile anaesthetic drug, can also upregulate HIF-1α and enhance HIF-1-responsive genes. Okadaic acid and vanadate also appear to promote HIF-1α expression.

The activity of prolyl-hydroxylase (PHD), the direct sensor of hypoxia, is decreased under hypoxic conditions. Failure of hydroxylation of HIF results in reduced ubiquitination and HIF accumulation. Inhibitors of PHDs have been developed. Tilorone is a low molecular weight antiviral and a potent activator of the HIF pathway in neuronal cell lines, enhancing the expression of downstream target genes.

Baicalein suppresses ubiquitination of HIF-1α by inhibiting the HIF-specific hydroxylases and increasing the target gene transcription in tissue culture cells.

**Type A2 radioprotectants**: free radical scavengers

Generation of free radicals in the nuclear environment leads to a direct reaction with DNA chains and causes strand breakage. Use of free radical scavengers at this stage would reduce the number of free radicals that damage DNA or organelles. All cells contain a certain level of endogenous scavengers, the expression of which can be induced under stressful conditions. Zn, Cu or the mitochondrial manganese (Mn) superoxide dismutase (SOD) are important enzymes converting oxygen radicals to hydrogen peroxide.

Glutathione is also an important endogenous scavenger bearing a thiol group. Several compounds can induce the expression of endogenous scavengers, such as N-acetyl-cysteine.

The dephosphorylated thiolic form of amifostine is a potent exogenous free radical scavenger with established clinical position in the protection against platinum toxicities and the prevention of radiation xerostomia, and in the protection of radiation proctitis and oesophagitis.

Fullerenes are crystal forms of carbon molecules that are neither graphite nor diamond. They consist of a spherical, ellipsoid or cylindrical arrangement of dozens of carbon atoms. The addition of a hydroxy moiety on each carbon of a 60-fullerene gives 60-fullerenol, a water-soluble molecule with potent free radical scavenger activity. The administration of a high dose of this compound resulted in radioprotection against total body irradiation (TBI) of 8 Gy, similar to that provided by amifostine.

Cerium oxide (CeO2) nanoparticles have also been shown to protect the gastrointestinal epithelium and lung tissue against radiation damage in mice by acting as free radical scavengers and increasing the endogenous production of SOD.

Nitroxides have also been tested as free radical scavengers. The lead compound of oxidised forms of nitroxides, tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), has shown radioprotective efficacy and have paramagnetic properties allows the study of the accumulation and clearance using MRI, which may be useful in optimising the administration time before radiotherapy to prevent tumour protection.
**Type A3 radioprotectants:** DNA repair boosters

Oxidation of the WR-1065 thiolic product of amifostine in cells leads to a disulphidic form, WR-33278, with DNA repair activity. An effect of the molecule on the polyamine-induced compaction and aggregation of DNA has been reported as decreasing DNA strand break accumulation. WR-33278 has structural similarities to the polyamines putrescine, spermidine and spermine, and is transported at the same velocity by the polyamine transport system. Polyamines are integral components of the cell chromatin structure and are involved in DNA repair mechanisms. Inhibitors decreasing their intracellular concentration result in enhanced radiation cell killing and mutagenic effect. In supercoiled plasmid DNA experiments, WR-33278 has protected DNA even following neutron exposure, by scavenging of hydroxyl radicals and by reducing the accessibility of radiolytic attack sites via the induction of packaging of DNA in liquid crystalline condensates. Enhancement of the topoisomerase I-mediated unwinding of supercoiled DNA is a process mediated by WR-33278. Both WR-1065 and WR-33278 molecules have the ability to remove the platinum adducts from DNA.

Resveratrol (3,4-trihydroxy-trans-stilbene) is a polyethanol extract from red grapes that is available as a dietary supplement; several studies have shown that it increases life expectancy in experimental animals and prevents carcinogenesis.

Direct molecular interference in DNA repair machinery by targeting multiple enzymes involved in the process may also prove critical in accelerating the restoration of DNA damage. Oxoguanine DNA glycosylase (OGG1) initiates base excision repair of oxidised purine bases and exhibits 8-OH-G glycosylase activity. Although this enzyme does not interfere with DNA double-strand breaks and cell death, it seems to have an important antimutagenic effect, as shown in cell lines exposed to ultraviolet light. OGG1 seems also to prevent oxidative damage of mitochondrial DNA. Butin, a 7,3′,4′-trihydroxydihydroflavone, protects cells against hydrogen peroxide-induced damage of DNA by enhancing the transcriptional activity of OGG1 and the expression of phosphorylated Akt, a regulator of OGG1, which makes the compound a candidate antimutagenic agent.

A key enzyme of base excision repair is the human apurinic/apyrimidinic endonuclease (HAP1/APE1); the mitochondria-targeted APE1 exhibits robust DNA repair activity. Overexpression vectors of mitochondria-targeted truncated APE1 and full-length APE1 significantly protect normal cells from oxidative stress. Poly-(adenosine diphosphate-ribose) polymerase (PARP-1) is a nuclear enzyme that is possibly involved in DNA base excision repair. PARP-1 is involved in suppressing imprecise repair of endogenous DNA damage, leading to deletion mutation during ageing. PARP-1 amplifies a signal for rapid recruitment of repair factors, enabling efficient restoration of genome integrity. Boosting the activity of such enzymes by gene therapy techniques could have an important role in the prevention of radiation mutagenesis or toxicity.

**Type B1 radioprotectants:** inhibitors of death signalling pathways

The DNA damage and, presumably, the endoplasmic reticulum and mitochondria damage induced by radiation-free radicals immediately trigger the pro-apoptotic cell response. The
p53 gene, as a guardian of genomic integrity, is rapidly upregulated, producing the wild type p53 protein, which is considered as the first step of apoptosis. The gap 1 phase cell-cycle arrest that follows (first death check point) provides the necessary time for the DNA repair process to occur. If the cell considers the repair to be effective then proliferation follows; if not, the cell progresses to the second death check point (G2 arrest), followed by the release of mitochondrial caspases and death. The B-cell lymphoma 2 (bcl-2) family of proteins, being the guardians of this latter check point, can regulate the cell decision towards survival. The anti-apoptotic activity of Bcl-2 is prominent in cancer cells.

Blockage of the p53 function will break the apoptotic pathway and prevent death, ignoring sometimes important DNA damage. Sodium orthovanadate (Na<sub>3</sub>VO<sub>4</sub>) and Pifithrin-α (imino-tetrahydrobenzothiazol-tolylethanone hydrobromide) are potent p53 inhibitors.

**Type B2 radioprotectants: growth factors**

Depopulation of the early responding component of tissues (epithelial and blood cells) induces accelerated proliferation of the relevant stem cells, in order to balance the cellular loss. The pick of this Type I early radiation toxicity appears 15–20 days after exposure to radiation. Specific growth factors acting directly on the stem cell population of epithelial or haematological tissues enhance the rate of repopulation and toxicity healing.

Haematopoietic growth factors such as erythropoietin (EPO) and granulocyte (G) or granulocyte–macrophage (GM) colony-stimulating factor (CSF) have an established position in the treatment of anaemia and neutropenia following chemotherapy or radiochemotherapy. GM-CSF may also have a role in the healing of acute radiation mucositis. Second-generation thrombopoietin (TPO) mimetic agents such as romiplostim and eltrombopag are important agents activating the TPO receptor and have been approved for the treatment of immune thrombocytopenic purpura. The keratinocyte growth factor palifermin has been approved for the treatment of severe oral mucositis that occurs after high-dose chemotherapy and radiotherapy followed by stem cell rescue.

Becaplermin, a recombinant platelet-derived growth factor B-chain homodimer, has shown important activity in wound healing and seems also to have an important role in osteogenesis. Intracoronary infusion of recombinant human (rh) VEGF, a potent angiogenic factor, induces revascularisation and improves angina in patients with stable exertional angina. Repeated local application of the rhVEGF telbermin in chronic diabetic foot ulcer has been applied in Phase I trials. An eventual role of such topical gel formulations to accelerate healing of severe radiation mucositis is postulated as VEGF overexpression, and has been shown to occur during the healing of oesophageal acute radiation damage.

A peptide derived from the receptor-binding domain of fibroblast growth factor (FGF)-2, FGF-P, is a potent mitogen that promotes stem cell renewal, progenitor cell differentiation and epithelial proliferation. 5-day administration of this peptide in mice immediately after sub-TBI (to avoid death by myelosuppression) shows enhanced proliferation of crypt cells of the small intestine, preservation of the gastrointestinal function and prevention of weight loss,
suggesting an eventual role of this approach in preventing acute gastrointestinal radiation syndrome.

**Type C1 radioprotectants**: blockers of radiation inflammation and chemotaxis

Following irradiation, the slowly proliferating cell compartments of tissues suffer a reactive upregulation and downregulation of genes, changing the functional profile of the cells: an event that results in early Type II radiation toxicity. Agents targeting this effect may be extremely useful in reducing acute vascular dysfunction and leukocyte chemotaxis and infiltration during radiation pneumonitis and colitis, as well as in preventing acute breast, laryngeal/pharyngeal or even brain oedema. Type C1 radioprotectants could block the appearance of Type II toxicities when given during or immediately after the end of a radiotherapy course. These are also likely to act prophylactically against the subsequent development of Type III toxicities by preventing the establishment of autocrine/paracrine pathways. Moreover, they may have a role in the treatment of Type III toxicities by interfering in immunological pathways together with Type C2 radioprotectants.

Infliximab, an anti-TNFα agent, reduces the infiltration of the synovium by TNFα-producing inflammatory cells. Baicalein, a component of Scutellaria radix, blocks IkBα degradation followed by downregulation of IL-6. Anakinra, an IL-1 receptor antagonist, is also an effective agent that inhibits inflammatory pathways and exhibits potent antirheumatoid activity.

**Type C2 radioprotectants**: blockers of autocrine/paracrine pathways

Protracted gene activation and stromal cell functional deregulation leads to the establishment of autocrine/paracrine loops, stimulating fibroblast and endothelial proliferation and leading to structural disorganisation and organ failure (Type III radiation toxicity). Identification of the key molecules involved in this cycle and of specific inhibitors would allow the breakage of the loop, interruption of damage progression and, possibly, reversal of the process. Type C2 radioprotectants could be useful during the immediate post-radiotherapy period to prevent the establishment of late radiation sequelae or could be used in the treatment of the already established Type III toxicities.

Lung hypoxia is a common event, with maximum levels reached within 6 months following irradiation, as shown in experimental studies applying pimonidazol localisation appraisal. Blockage of the prolyl- and asparaginyl hydroxylase activity and subsequent accumulation of hypoxia-inducible factors 1 and 2 is, therefore, expected to dominate Type III radiation toxicities.

Platelet-derived growth factor (PDGF) isoforms have an important role in stimulating the proliferation and migration of myofibroblasts during fibrosis. PDGF action is directed towards PDGFα and -β receptors with tyrosine kinase activity, present on the surface of stimulated fibroblasts. Administration of imatinib or SU9518 (an agent that blocks PDGF receptors) results in prolongation of survival of mice receiving 20 Gy to the lungs, and
reduces the radiomorphological signs of lung fibrosis. Imatinib also inhibits bleomycin-induced lung fibrosis.

Inhibitors of TNFα such as infliximab, a chimeric monoclonal immunoglobulin G1 (IgG1) antibody against the tumour necrosis factor used in Crohn's disease and rheumatoid arthritis, downregulate both basic FGF (bFGF) and VEGF in the serum of patients. Administration of infliximab in a patient with lung fibrosis and pulmonary hypertension associated with advanced systemic sclerosis has resulted in stabilisation of lung function tests and pulmonary arterial pressures that progressively worsened after cessation of therapy. Anti-TNFα treatment protects normal brain vasculature against radiation.

The combination of pentoxifylline and vitamin E in the prevention and treatment of fibrotic lesions has been tested in clinical trials. Pentoxifylline inhibits TNFα and leukotriene synthesis, and reduces inflammation. Prolonged treatment with this combination appears to reduce radiation skin fibrosis. Other clinical trials on breast, lung and pelvic tissue fibrosis have provided inconclusive results.

Hepatocyte growth factor (HGF) is a tyrosine kinase, a product of the c-Met gene, with angiogenic properties. HGF appears to be important in reparative lung response following injury. Activation of NF-κB by HGF has been reported and abrogation of NF-κB activity by IκBα repressors have resulted in loss of HGF/scatter factor-mediated protection of renal cells against doxorubicin. HGF promotes the proliferation of ECV304 cells and inhibits radiation-induced apoptosis of endothelial cells. Intramyocardial injection of adenoviral vectors transferring the HGF gene protect against experimental radiation-induced heart disease. Continuous infusion of HGF in mice attenuates bleomycin-induced lung damage; furthermore, administration of HGF after establishment of bleomycin fibrosis reverses the fibrotic process and accelerates the proliferation of alveolar epithelial cells. HGF plasmids, when injected into the liver and transferred by electroporation, significantly protect rats against radiation-induced liver damage. Retinoic acid, an active metabolite of vitamin A that acts on specific receptors on cells, prevents fibrosis by counteracting the activity of TGFβ and stimulating HGF promoter activity and HGF receptor phosphorylation.

There is some limited evidence for the protective effect of angiotensin-converting enzyme inhibitors, especially captopril and an angiotensin II Type 1 receptor blocker, on radiation-induced pulmonary injury. Finally, cyclo-oxygenase selective inhibitors may also have a role in preventing radiation pneumopathy.

**Type D radioprotectants: keepers of genomic integrity**

Patients undergoing radiotherapy are exposed to inhomogeneous low-dose whole-body radiation (ranging from 0.004% to 1% of the dose fraction as a consequence of the scattering of the radiation outside the portals used). Type D radioprotectants are important in decreasing the overall cancer incidence induced by medical applications. Such agents may also be useful in protecting, for example, radiologists, nuclear industry workers, aircraft crew and astronauts against occupational exposure.
**Type E radioprotectants:** protecting bystander cells

They help in the reduction of radiotherapy sequelae, enhance its antitumour efficacy and, most importantly, reduce the risk of secondary carcinomas.

Gap junctions allow the flow between cells of small molecules (1000–1500 Da) such as calcium ions, nucleotides and peptides, and this route is considered a major pathway of bystander effect manifestation within an organ. Targeting gap junctions and their constituent proteins, namely connexins, may prove important in blocking the bystander effects within a partially irradiated organ (e.g. the lung or liver). Gamma-hexachlorocyclohexane (lindane) induces gap junction endocytosis, a process that is activated by the ERK pathway. TGFβ-3 treatment downregulates connexin 43 and induces the ERK pathway. Phorbol ester 12-O-tetradecanoylphorbol-13-acetate and chlorohydroxyfuranones, by-products of drinking water chlorination, also appear to inhibit gap junction.

The long-range abscopal effects are postulated to be mediated through large molecules (1000–10 000 kDa) such as lipid peroxide products and cytokines (IL-1, IL-6, TGFβ and TNFα). Such cytokines induce nitric oxide synthase 2 and increased nitric oxide content in target cells. Injection of Cu/Zn-SOD or nitric oxide synthase inhibitors such as L-NAME lead to reduced expression of bystander effects. Macrophages appear to be the source of the long- and short-range bystander signals through cytokine production. Whether abrogation of macrophage activation may have a role in protection against Type D radiation toxicities is an emerging hypothesis. Macrophage migration inhibitory factor (MIF) is a macrophage-produced cytokine that induces TNFα secretion and nitric oxide production, also contributing to the recruitment of leukocytes. Its activity can be blocked with monoclonal antibodies or by targeting MIF tautomerase activity using small molecules such as (S,R)-3-(4-hydroxyphenyl)-4, 5-dihydro-5-isoxazole acetic acid methyl ester. Inhibitors of protein kinase-C, such as 1-(5-isouquinolinesulphonyl)-2-methylpiperazine dihydrochloride, also display important inhibitory activity on macrophage activation.

Because hypomethylation of DNA is a bystander effect of radiation that may be important in genomic instability and carcinogenesis, agents that can restore this effect may also be useful Type V radioprotectants. CpG dinucleotides are major sites of DNA methylation in mammals. During somatic cell differentiation, DNA methylation occurs and represses germline-specific genes, a process catalysed by DNA methyltransferases (DNMTs). Demethylation is an important subsequent step in permitting expression of tissue-specific genes and occurs either by inhibition of DNMTs or as an active process involving the DNA repair-related demethylase. The latter process is largely obscure, although it seems to be related to DNA repair machinery, including DNA glycosylases. Growth arrest and DNA-damage-inducible protein GADD45α is a non-enzymatic factor involved in base excision repair that actively promotes DNA demethylation. Cytidine deaminase also appears to be involved, converting 5-methylcytosine into a thymine; this is followed by excision and replacement of methylated nucleotides. DNA demethylation correlates with extensive histone modification and exchange that is facilitated by histone chaperone proteins such as histone cell cycle regulation defective homolog A and nucleosome assembly protein. It is expected
that explanation of the process of demethylation will help in the development of strategies to block radiation-induced demethylation in bystander cells.

Q7.

**Ans. i) Enkephalins:**

Enkephalins are pentapeptides involved in regulating nociception in the body. Discovered in 1975, two forms of enkephalin were revealed, one containing leucine ("leu") and the other containing methionine ("met"). Both are products of the proenkephalin gene.

-Met-enkephalins has Tyr-Gly-Gly-Phe-Met.
Met-enkephalins are endogenous opioid peptide neurotransmitter found naturally in the brains of many animals, including humans. It regulate the memory and emotional condition.
- Leu-enkephalins has Tyr-Gly-Gly-Phe-Leu.
Leu-enkephalin has agonistic actions at both the µ- and δ-opioid receptors, with significantly greater preference for the latter. It has little to no effect on the κ-opioid receptor.

Structure of met enkephalin is:

![Structure of met enkephalin](image1)

Structure of leu enkephalin is:

![Structure of leu enkephalin](image2)

**ii) Dynorphins:**

Dynorphins are a class of opioid peptides that arise from the precursor protein prodynorphin. When prodynorphin is cleaved during processing by proprotein convertase 2 (PC2), multiple active peptides are released: dynorphin A, dynorphin B, and α/β-neo endorphin. Structure of dynorphin A is:

![Structure of dynorphin A](image3)
Dynorphins exert their effects primarily through the κ-opioid receptor and act as modulators of pain response, maintain homeostasis through appetite control and circadian rhythm, weight control, boosting immune system and regulation of body temperature.

iii) Abortifacients:

An abortifacient (Latin: that which will cause a miscarriage) is a substance that induces abortion. These are classified as natural abortifacient and synthetic abortifacient.

Natural abortifacient include brewer's yeast, vitamin C, bitter melon, wild carrot, blue cohosh, pennyroyal, nutmeg, mugwort, slippery elm, papaya, vervain, common rue, ergot, saffron and tansy. Animal studies have shown that pomegranate may be an effective abortifacient.

Synthetic abortifacient: two prostaglandins, \( \text{PGE} \) and \( \text{PGE} \alpha \), have been used as abortifacients. \( \text{PGE} \alpha \) is injected into the amniotic sac, whereas \( \text{PGE} \) is given by vaginal suppository to induce abortion. Saline induced abortions have also been used previously. The oral combination of mifepristone and prostaglandin, misoprostol, have been recommended for inducing an abortion. Common abortifacients used in performing medical abortions include mifepristone, which is typically used in conjunction with misoprostol in a two-step approach. There are also several herbal mixtures and oral contraceptive used as abortifacient.

Structure of mifepristone is:

![Structure of mifepristone](image)

11β-\([p-(\text{Dimethylamino})\text{phenyl}]\)-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one

Structure of misoprostol is:

![Structure of misoprostol](image)

Methyl,7-\([(1R,2R,3R)-3-hydroxy-2-\{(1E)-4-hydroxy-4-methyloct-1-en-1-yl\}-5-\text{oxocyclopentyl}]\text{heptanoate}.