Chapter 1

Review of Literature

1. Introduction

1.1. Heavy metals

The entire ecosystem affected by toxicity of well known environmental contaminants such as heavy metals (Leonard et al., 2004). It is evident from several growing facts that oxidative stress is the major mechanism behind heavy metal toxicity. Iron, copper, cadmium, mercury, nickel, lead and arsenic can oxidatively damage biological macromolecules such as nuclear proteins and DNA which can cause accumulation of altered nucleobase products in cultured cells (Chen et al., 2001). By generating radicals, they can deplete enzyme activities as well as cause damage to lipid bilayer (Stohs et al., 1995). Chelates of proteins, peptides and amino acids can be formed by heavy metals eventually leading to neurotoxicity, hepatotoxicity and nephrotoxicity in humans and animals.

1.2. Mechanism of action

Mostly redox inactive metals induce the oxidative stress by depletion of sulfhydryl reserves (Stohs et al., 1995). However, in case of redox-active metals, oxidative stress generated by Fenton-like reactions **(Fig. 1.1)** (Liochev, 1998).

$$M^{(n)} + O_2^{\cdot -} \longrightarrow M^{(n-1)} + O_2$$

$$2\,O_2^{\cdot -} + 2\,H^{+} \longrightarrow H_2O_2 + O_2$$

$$M^{(n-1)} + H_2O_2 \longrightarrow M^{(n)} + HO^{\cdot} + OH^{-}$$

Fig. 1.1. Mechanism of generating free radicals by metals through Fenton-like reaction (Liochev, 1998).

In mammalian tissues glutathione (GSH) present in millimolar (mM) concentration which constitutes for more than 90% of the total non-protein sulfur (Quig, 1998; Hultberg et al., 2001). Interactions of lead, mercury and cadmium with GSH through covalent bonds results into depletion of GSH causing the synthesis of more of GSH by cysteine via the γ-glutamyl cycle to overcome the deficiency, however, if metal exposure continues then GSH cannot be sufficiently replenished. Along with GSH several other antioxidant enzymes can also be inactivated by heavy metals by binding with sulfhydryl

groups located at their active sites. Besides that, cofactor of several enzymes could also be replaced by heavy metals and thereby inactivating them. The possible influences of action of heavy metals on cellular system are illustrated in **Fig. 1.2**.

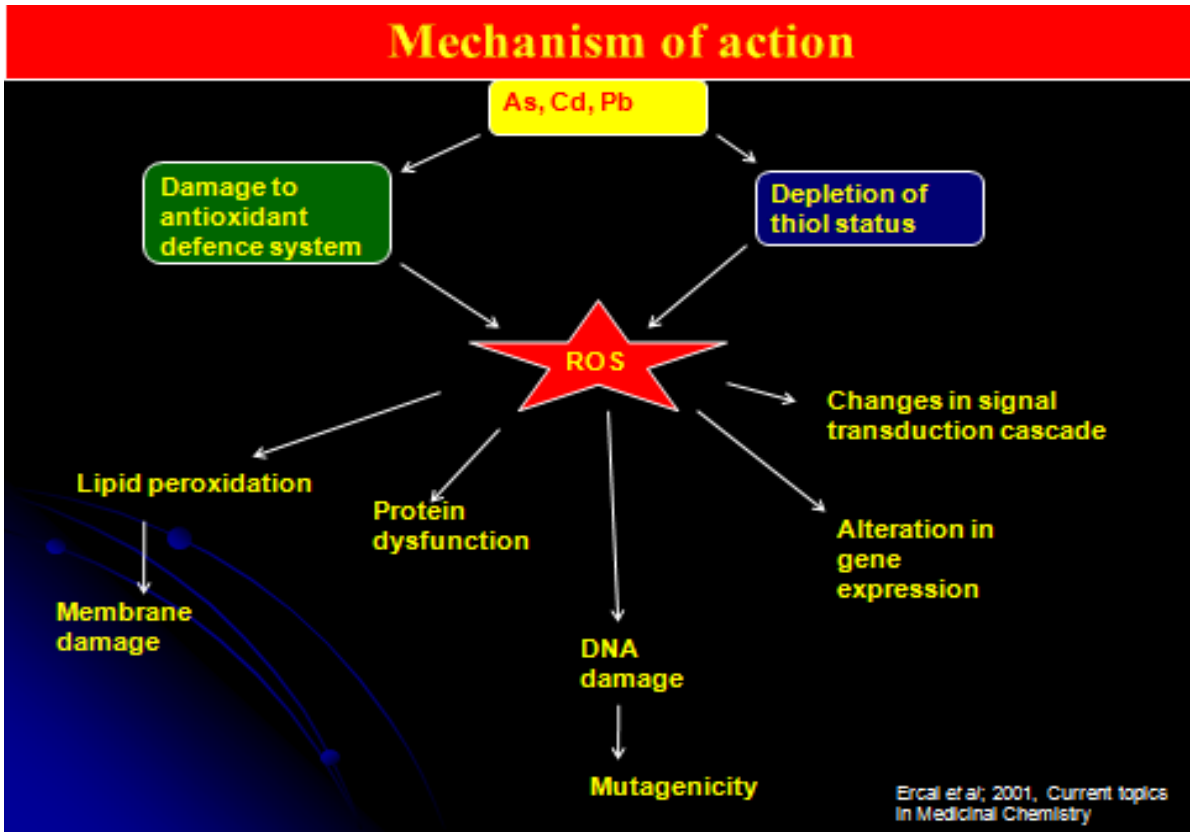

Fig.1.2. Possible mechanism for heavy metal induced oxidative stress.

1.3. Sources of hazardous metals and minerals

Both natural as well as anthropogenic sources can cause environmental pollution from hazardous metals (Marg, 2011). Most commonly, they enter into human body through contaminated food or water. The natural sources of heavy metals include the volcanic activities, forest fires and seepage from rocks into water. However, anthropogenic sources include gasoline additives, paints, ceramic glazes, folk remedies, battery/plastic recycling industry, brands of cosmetics like talcum powder, lipsticks, shampoos, 'kajal' and hair colours, ash dumps from thermal power plants (**Table 1.1**). Various contaminated metal sites in India are listed in **Table 1.2**.

Metal	Industry
Chromium (Cr)	Mining, industrial coolants, chromium salts manufacturing, leather tanning
Lead (Pb)	lead acid batteries, paints, E-waste, Smelting operations, coal- based thermal power plants, ceramics, bangle industry
Mercury (Hg)	Chlor-alkali plants, thermal power plants, fluorescent lamps, hospital waste (damaged thermometers, barometers, sphygmomanometers), electrical appliances etc.
Arsenic (As)	Geogenic/natural processes, smelting operations, thermal power plants, fuel burning
Copper (Cu)	Mining, electroplating, smelting operations
Vanadium (Va)	Spent catalyst, sulphuric acid plant
Nickel (Ni)	Smelting operations, thermal power plants, battery industry
Cadmium (Cd)	Zinc smelting, waste batteries, e-waste, paint sludge, incinerations & fuel combustion
Molybdenum (Mo)	Spent catalyst
Zinc (ZN)	Smelting, electroplating

Table 1.1. Sources of heavy metals (Marg, 2011).

Chromium	Lead	Mercury	Arsenic	Copper
Ranipet, Tamil Nadu	Ratlam, Madhya Pradesh	Kodaikanal, Tamil Nadu	Tuticorin, Tamil Nadu	Tuticorin, Tamil Nadu
Kanpur, Uttar Pradesh	Bandalamottu Mines, Andhra Pradesh	Ganjam, Orissa	West Bengal	Singbhum Mines, Jharkhand
Vadodara, Gujarat	Vadodara, Gujarat	Singrauli, Madhya Pradesh	Ballia and other districts, UP*	Malanjkahnd, Madhya Pradesh
Talcher, Orissa	Korba, Chattisgarh			

Table 1.2. Major heavy metal contaminated sites in India (Marg, 2011)

1.4. Cadmium (Cd)

1.4.1. Toxicokinetics

Cd is not well absorbed with the absorption rate of 25% through inhalation while 1–10% by oral exposure and <1% for dermal route of the dose (ATSDR, 2012). Absorption capacity varies with several factors; such as, Cd has a higher absorption capacity in

cigarette smoke due to small particle size. In gastrointestinal tract Cd absorption also increased with iron deficiency. After absorption, Cd gets distributed throughout the body with maximum deposition in liver and kidney without any metabolic conversions like reduction, oxidation or alkylation. Out of the total body burden of Cd, 0.007% is excreted in urine while 0.009% is excreted in feces per day.

1.4.2. Absorption

In the gastrointestinal tract, Cd absorption ranges between 3-5% with most of ingested Cd passes out without being absorbed (Vanderpool and Reeves, 2001; Morgan and Sherlock, 1984). In the gut, absorption of Cd decrease in presence of other metal ions such as iron, calcium, chromium, magnesium, and zinc. Subjects with iron deficiency showed upregulation of divalent metal transporter1 (DMT1) and metal transporter protein1 (MTP1) in the duodenum, thereby enhancing the absorption of Cd (Ryu et al., 2004; Kim et al., 2007).

1.4.3. Distribution

Once absorbed, Cd widely gets distributed to different tissues but primarily in the liver and kidney. Damage caused by Cd in liver and kidney is mainly proportionate to its quantity present in free form not bound to Metallothionein (MT). Body burden of heavy metals can be decreased via MT by forming excretable complexes, however, excess of metal could saturate MT which leading to accumulation of unbound free metal form and cause subsequent damage (Garcia-Nino and Pedraza-Chaverri, 2014) (**Fig. 1.3**). For short term exposure, the Cd levels are usually comparable in liver and kidney while for longer exposure, kidney accumulates more of Cd as compared to liver. During birth, Cd concentration is zero in both kidney and liver but rises to 40–50 μg/g wet weight by age of 50-60 in kidney and to 1–2 μg/g wet weight by age 20–25 in liver (Lauwerys et al., 1984; Chung et al., 1986; Hiratsuka et al., 1999). According to Nordberg-Kjellström model, the half life of Cd in kidney is 6-38 years, in liver 4-19 years and 9-47 years for other tissues.

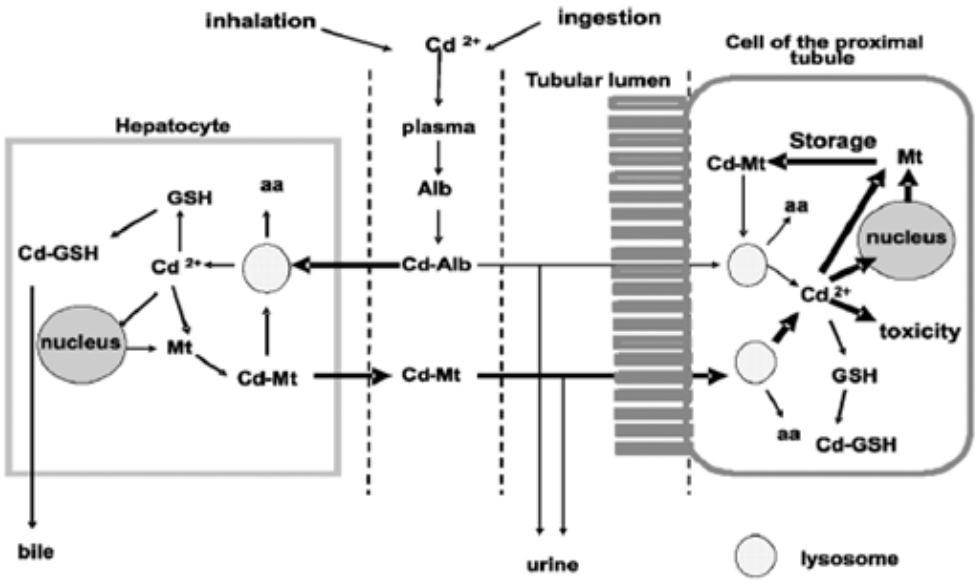

Fig. 1.3. Schematic accumulation pattern of Cd. Alb-Albumin; Mt- metallothionein; GSH-glutathione; aa-amino acid (Bernard, 2008).

1.4.4. Health effects:

Cd is potent carcinogen and by accumulating throughout the lifetime, it can cause anemia, osteoporosis, renal tubular injury, non-hypertrophic emphysema, eosinophilia, chronic rhinitis, and anosmia and could also affects the essential metal ions (Flora et al., 2008) (**Fig. 1.4**).

Cd could generate free radicals by replacing iron and copper from several proteins like ferritin, thereby increasing the levels of unbound iron or copper ions. These unbound metals give rise to oxidative stress by Fenton reaction (Casalino et al., 1997; Galan et al., 2001; Waisberg et al., 2003). Cd deactivates SOD by binding with imidazole group of the His-74, liver mitochondrial MnSOD by substituting for manganese and also causes increase in the levels of MDA (Ognjanovic et al., 2003; Yang et al., 2003; Djukic-Cosic et al., 2007). Cd can disable DNA repair by inhibiting mismatch repair leading to accumulation of errors within the cell (McMurray and Tainer, 2003) and forms intrastrand bifunctional AT adducts by covalently binding with DNA.

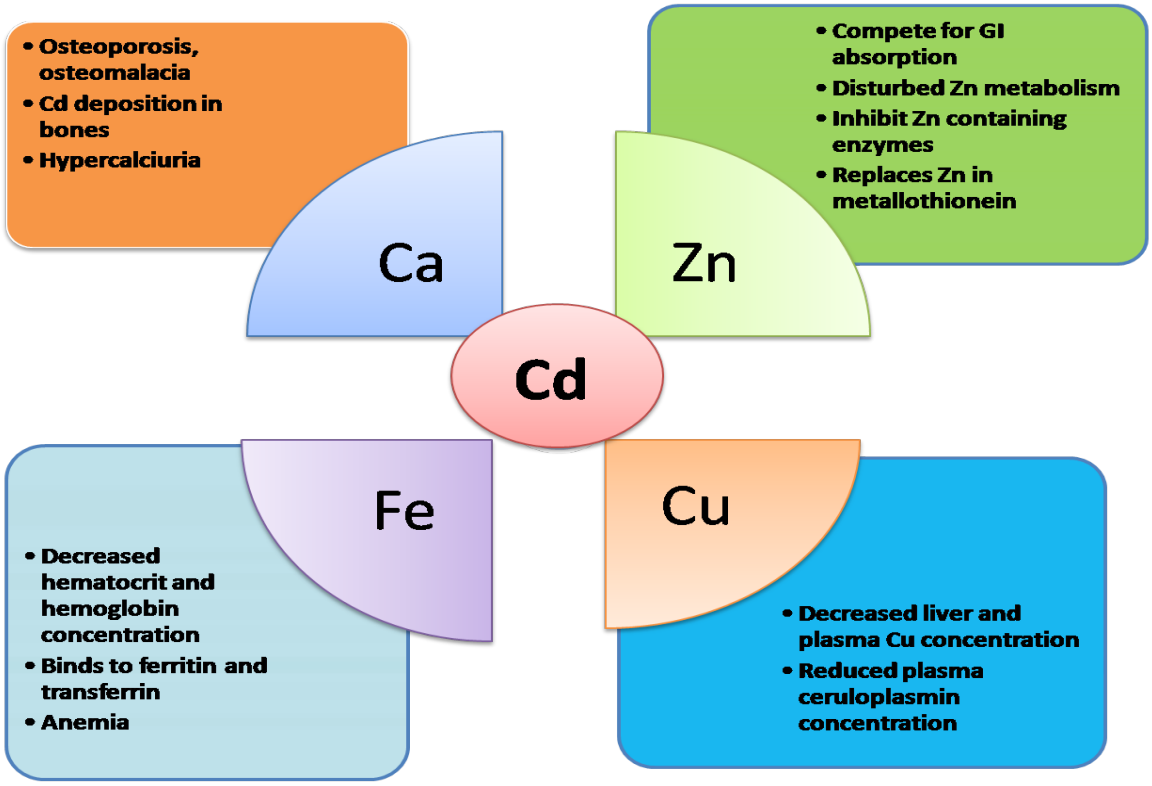

Fig.1.4. Effects of Cd on essential nutrients in body (Flora et al., 2008).

1.5. Lead (Pb)

1.5.1. Toxicokinetics

Inhalation and oral exposure are the major routes of Pb absorption rather than dermal route (ATSDR, 2007). From intestine, Pb get absorbed and distributed primarily in following three major cubicles: blood, soft tissues (liver, kidney, bone marrow and brain) and mineralizing tissues (bones and teeth). The half-life of Pb in blood is 30 days and in bone 27 years. Organic Pb gets metabolized in liver by P-450 enzymes. Major routes of excretion include the urine or feces while the minor routes consist of hair, nails, saliva, sweat, and breast milk. Pb can be transmitted from mother to fetus by crossing placenta or from mother to infants through breast milk. Bone resorption is increased in conditions such as menopause, lactation, pregnancy, and osteoporosis and eventually increases the Pb levels in blood.

1.5.2. Absorption

Gastrointestinal absorption of Pb is found to be 40-50% in infants and children of ages between 2 weeks to 8 years while it is observed to be 3-10% in adults (Watson et al., 1986). Similar to Cd, the intake of Pb also influenced by dietary calcium intake (Mahaffey et al., 1986).

1.5.3. Distribution

Mostly Pb is found bound with proteins in RBC and not with RBC membrane. In plasma, 40-75% of Pb bound with plasma proteins abundantly with albumin but it can also bind with γ-globulins. In serum, it remains usually bound with sulfhydryl rich compounds such as cysteine, homocysteine (Al-Modhefer et al., 1991). In children, 73% of total body burden of Pb accumulates is bone while 94% in human adults. Pb can accumulates in increasingly concentration in bone throughout the lifetime and also acts as pool to maintain blood Pb levels long after the exposure. Due to high bone deposition of Pb, it can transfer to fetus by resorption of maternal bone for formation of fetal skeleton (Gulson et al., 2003). Maximum levels of Pb deposits in liver among several soft tissues. Amount of Pb in various soft tissues is as follows: liver, 33%; skeletal muscle, 18%; skin, 16%; dense connective tissue, 11%; fat, 6.4%; kidney, 4%; lung, 4%; aorta, 2%; brain, 2% and other tissues, <1% (Oldereid et al. 1993).

1.5.4. Health effects

Pb toxicity is one of the well known environmental and occupational hazards (Flora et al., 2006). Several dysfunctions can be induced by Pb in laboratory animals and humans via affecting central and peripheral nervous systems, cardiovascular system, haemopoietic system, reproductive system, kidneys, and liver (Lancranjan et al., 1975; Sharma et al., 1980; Khalil-Manesh et al., 1993; Bressler et al., 1999; Lanphear et al., 2000; Damek-Poprawa et al., 2004). Pb could primarily affect the heme synthesis pathway either by inhibiting the heme and haemoglobin synthesis or by inducing changes in the RBC morphology and survival (**Fig. 1.5**). ALAD (δ-aminolevulinic acid dehydratase) is a cytosolic sulfhydryl enzyme involved in synthesis of heme formation by converting two molecules of ALA into prophobilinogen. ALAD activity is inhibited by low blood Pb levels of about 15 μg/dl causing activation of ALA synthetase by feed back

inhibition resulting into accumulation of ALA which can induce ROS generation in sufficient level to inhibit the activity of this enzyme (Noriega et al., 2003; Chia et al., 2004; Saxena et al., 2005; Flora et al., 2007a; Zhao et al., 2007). The hydroxyl radical thus generated can leads to formation of thiyl radicals by reacting with cysteine containing proteins. Thereafter, these thiyl radicals can react with GSH to form certain intermediates which are capable of reacting with molecular oxygen to form glutathionylated protein and superoxide ion (**Fig. 1.6**). ALA induces neurological dysfunction in rats by inhibiting K^+-stimulated release of γ-aminobutyric acid (GABA) or by preventing binding of GABA to synaptic membranes. Along with ROS, Pb could elevate Ca^{2+} levels, thereby inducing apoptosis by a fall in mitochondrial potential and cytochrome c release (Flora et al., 2007b).

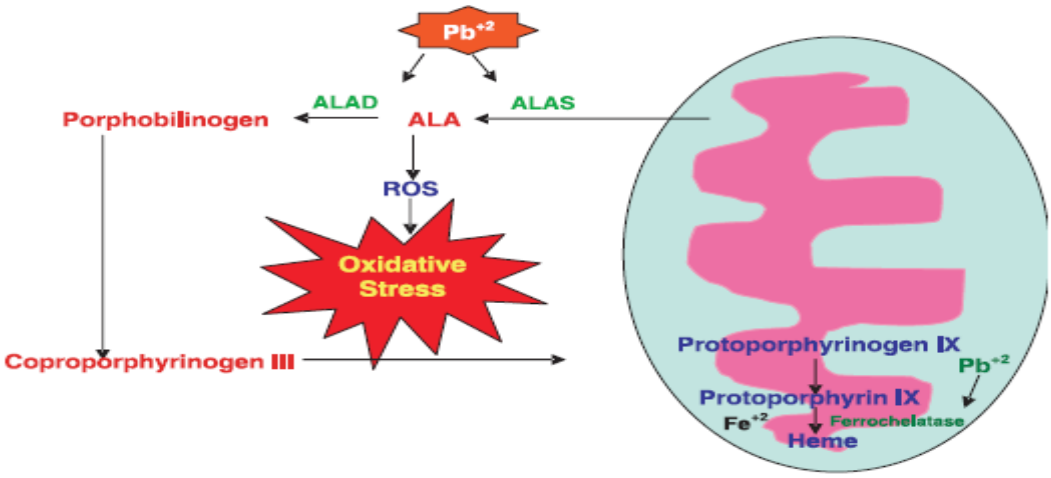

Fig.1. 5. Effect of Pb on heme biosynthesis pathway (Flora et al. 2008).

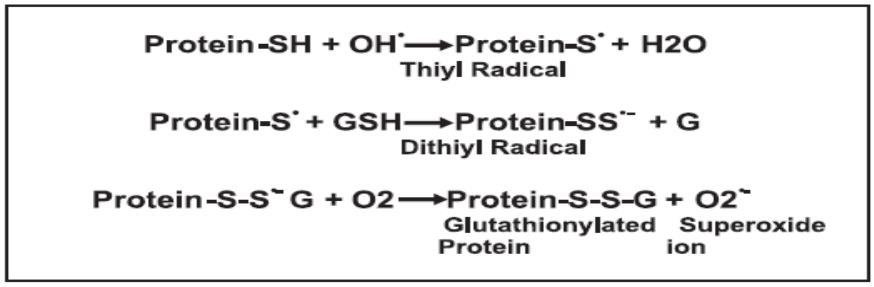

Fig.1.6. Reaction of hydroxyl radical with sulfhydryl group of protein and subsequent generation of superoxide radical (Flora et al., 2008).

Various studies suggest that the inhibition of activities of antioxidant enzymes by Pb such as SOD, catalase, glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD), and antioxidant molecules like GSH (Chiba et al., 1996) (**Fig.1.7**). Hence, enhanced rate of lipid peroxidation is found in brain of Pb exposed rats (Saxena et al., 2006).

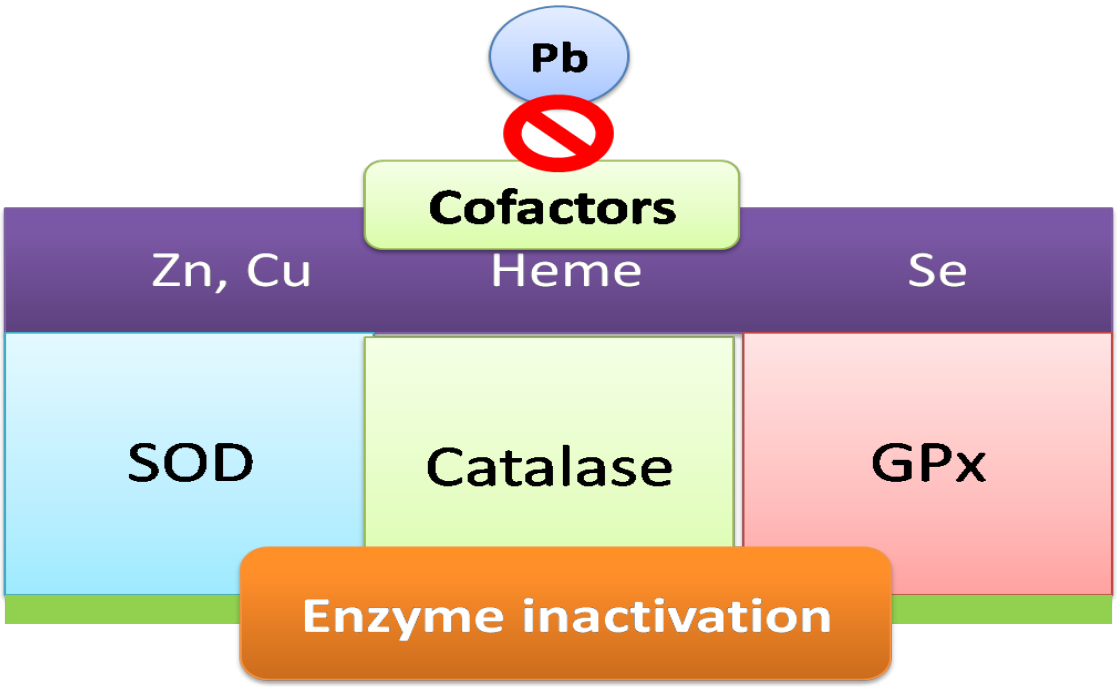

Fig.1.7. Effect of Pb on antioxidant enzymes (Flora et al., 2008).

1.5.5. Pb induced immunotoxicity

Immune dysfunction is considered as sensitive indicator of Pb toxicity (Theron et al., 2012). Pb exposure leads to increased production of endogenous glucocorticoids and catecholamines which may contribute to neutrophil dysfunction, most likely by interfering with adhesion of neutrophiles to vascular endothelium. Pb has interfering effect on the induction of iNOS and spreading of macrophages which is opposite to the effects on cytokine production. Pretreatment of Pb in bone marrow derived macrophages resulted into tenfold increase in levels of TNF-α, IL-6, IL-12, and prostaglandin E2 (PGE2) but decrease in IL-10 most likely by activation of protein kinase C (Flohe et al., 2002). In rodents, Pb treatment enhances the susceptibility to endotoxin (**Table. 1.3**) and parasitical infections.

Group	Treatment	Mortality	Organ lesions
		%	
1	None	0	0.1
2	*Salmonella abortivoequina* 3390 S_3 endotoxin (Westphal), 1 μg	80	0.5
3	*S. abortivoequina* 3390 S_3 endotoxin (Lüderitz), 1 μg	90	0.8
4	*Escherichia coli* O26:B6, B endotoxin (Boivin), 1 μg	90	0.8
5	*E. coli* O26:B6, W endotoxin (Westphal), 1 μg	90	0.7
6	*S. abortivoequina* (Difco), 1 μg	100	0.9
7	*S. enteritidis*, 1 μg	90	1.0
8	*Shigella flexneri*, 1 μg	100	1.0
9	*Serratia marcescens*, 1 μg	100	1.0
10	*Salmonella typhosa* 0901, 1 μg	100	0.8
11	Mixed fecal flora (fresh), 0.1%	60	0.6
12	Mixed fecal flora (boiled), 0.1%	10	0.4
13	Mixed fecal flora (fresh), 1%	80	0.9
14	Mixed fecal flora (boiled), 1%	60	0.6

Table 1.3. Percentage of mortality and the mean intensity of the organ lesions on endotoxin treatment in rats pre-sensitized with Pb (Selye et al., 1966).

Antibody production decrease on Pb treatment and also results in switching of B lymphocytes from producing IgM and IgG antibody isotype involved in protecting against infectious agents to IgE which primarily deals with allergic and hypersensitivity responses (Basaran and Undeger, 2000). Pb disrupts the critical Th1/Th2 lymphocyte balance necessary to maintain the host resistance to infectious disease by suppression of the Th1 cytokine IFN-γ and elevation of Th2 cytokine IL-4 can induce Th2 immune response and results into increased susceptibility to pathologic agents, allergic hypersensitivity and Th2 dominated autoimmune diseases (Hemdan et al., 2005). Thus, the cumulative effect of Pb results in the inhibition of antibody formation against specific pathogens and thus impairs eliciting protective immune response. Research conducted on the children under 3 years of age in Thailand and Philippines showed that interferon-γ is essential to initiate and maintain protective immune response induced by vaccines against influenza (Forrest et al., 2008), but significantly diminished by Pb exposure (Lawrence & McCabe., 2002; Lee and Dietert., 2003).

1.6. Cd and Pb coexposure

Chronic low exposure of Cd and Pb is a major public health issue (Wang and Fowler, 2008). Low level exposure of Cd and Pb can cause adverse renal effects (Fadrowski et al., 2010; Fowler, 2009). Several epidemiological studies have stated that isolated environmental or occupational exposure to Cd rarely occurs, however, it is

frequently associated with Pb exposure (Ekong et al., 2006; Johri et al., 2010). Coexposure of Cd and Pb can generate reactive oxygen species leading to liver and kidney damage as well as induces apoptosis henceforth impairing their function. Additive effect of Cd and Pb is neurotoxic too by disabling blood brain barrier (Yuan et al. 2014a; Yuan et al. 2014b; Tobwala et al 2014;Matovic et al. 2015).

The combination of Cd and Pb can show additive or synergistic interactions or complex new effects which are not observed by either of single metal treatment (Wang and Fowler, 2008). Their mechanism of toxicity mainly involves inhibition of sulfhydryl group containing enzymes and induction of ROS production (Navas-Acien et al., 2009). In rats coexposure to Pb and Cd exacerbates cytotoxicity in proximal tubular cells (Wang et al., 2011). Another study on rats showed that dietary administration of Cd and Pb together resulted in significant reduction of Pb in kidneys, blood and femur as compared to Pb alone (Mahaffey et al., 1977; Mahaffey et al., 1981). The reduction in Pb levels might be because of alterations in the gastrointestinal tract induced by Cd such as shortening of villi and atrophy of microvilli which in turn prevent Pb absorption. In contrast, on oral administration of low to moderately high levels of Cd and Pb to mice increased Cd levels in kidney compared with kidney Cd in mice fed on Cd (Exon et al., 1978). The combination had also caused marked reduction in haemoglobin or hematocrit levels (Thawley et al., 1977). Urinary ALA was intermediate for the mixture while increased for Pb alone but not Cd alone.

Neurological changes were observed by feeding rats on high levels of Cd and Pb for 60 days and found that Cd inhibits the Pb induced disturbances in dopamine and serotonin turnover (Nation et al., 1989; Nation et al., 1990). Brain Pb levels were not decreased with Cd coadministration as that of blood where Pb levels were decreased but the combination has additive effect on decreasing the body weight. Testicular effects of Cd and Pb showed synergism with marked effect on seminiferous tubules and decreased epdidymal sperm counts compared to control (Saxena et al., 1989). In rats, low dose oral coexposure of Cd and Pb suggested an additive or synergistic effect on systolic blood pressure (Kopp et al., 1980). In contrast, lack of interactive or additive effects were also

seen with Cd and Pb where renal dysfunction in workers exposed to Cd and Pb was similar to that in workers exposed to Cd alone (Buchet et al., 1981).

1.7. Arsenic (As)

1.7.1. Toxicokinetics

As is well absorbed by both the oral and inhalation routes while dermal route is not well characterized (ATSDR, 2007). Once absorbed, arsenates (As^{5+}) oxidized to arsenites (As^{3+}) and then methylated. These steps repeated several times until formation of dimethylated and trimethylated As metabolites and then eventually forming trimethyl arsine gas (Bentley et al., 2002) (**Fig.1.8**). Enzymic methylation of As(+3) form primarily takes place in liver by As(+3) methyltransferase to form MMA and DMA. The extent and rate of methylation varies with different species. Mostly, As is excreted in the urine in following mixture of As(+3), As(+5), Monomethylated arsenicals and Dimethylated arsenicals with Dimethylated arsenicals is the primary form in the urine. However, certain amount also gets excreted in feces.

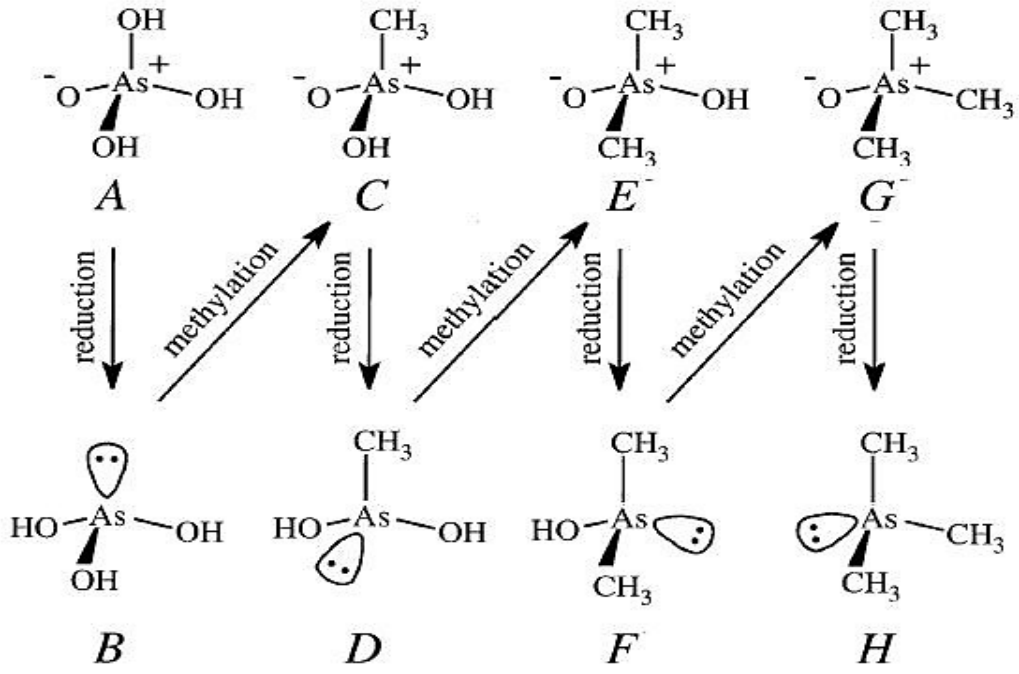

Fig. 1.8. Reduction and oxidative methylation of Arsenic (Bentley et al., 2002): (A) arsenate; (B) arsenite; (C) methylarsonate; (D) methylarsonite;(E) dimethylarsinate; (F) dimethylarsinite; (G) trimethylarsine oxide; (H) trimethylarsine. The top line shows the As(V) species. The vertical arrows indicate the reduction to As(III) species (bottom line) and the diagonal arrows indicate the methylation reaction conducted by SAM.

1.7.2. Absorption

In gastrointestinal tract, As absorption is approximately 95% with 55-87% of daily oral intake excreted as urine (Kumana et al., 2002; Zheng et al., 2002).

1.7.3. Distribution:

As was estimated in all tissues from subjects residing in As exposed area (ATSDR, 2007). Mostly, tissues have same concentration of As around 0.05–0.15 ppm while higher levels found in hair 0.65 ppm and nails 0.36 ppm. In internal organs, As deposition might be low due to methylation process in body. Methylation of As is primarily carried out in liver by enzyme As(+3) methyltransferase. Ingestion of 8 g of As trioxide leads to deposition of 147 µg/g of As in liver while 27 µg/g in kidney and 11–12 µg/g in muscle, heart, spleen, pancreas, lungs, cerebellum (Benramdane et al. 1999). Low amount of As is also found in brain 8 µg/g, skin 3 µg/g and 0.4 µg/g in hemolyzed blood.

1.7.4. Health effects:

Phosphorylation reactions are inhibited via arsenate (As^{5+}) by acting as phosphate analogue. Biochemical pathways also get inhibited by arsenite (As^{3+}) due to high affinity for sulfhydryl groups of proteins (Valko et al., 2005). Although, most of the arsenate after absorption get reduced to arsenite, both of them have toxic effects. As toxicity is mainly contributed due to its high biological reactivity and also known to have carcinogenic effects causing lung, skin, liver, bladder, and kidney tumours (Waalkes et al., 2004). It can alter neurotransmitter levels and also act on the transcriptional factors of various signal transduction pathways (Namgung et al., 2001; Son et al., 2001).

As reduces GSH levels by several ways; by utilizing it as electron donor for reducing pentavalent arsenicals to trivalent, due to its high affinity for GSH and by oxidation of GSH with free radicals generated by As (Wang et al., 1996). Along with GSH, As intoxication also reduces the level of antioxidant enzymes. Apoptosis can also be induced by As by generation of reactive oxygen species as well as alterations in the signal cascade and the disturbed antioxidant levels, eventually resulting into cellular apoptosis (Shi et al., 2004).

1.7.5. Biotransformation of As in humans

In mammalian species including humans, biomethylation of inorganic arsenic (iAs) involves two enzymatically catalyzed reactions: the reduction of all species having AsV and the oxidative methylation of all species having AsIII (Cullen et al. 1984). During biotransformation of As in humans, four major methylated As species are formed including methylarsonic acid ($CH_3As^VO_3^{2-}$, MAsV), methylarsonous acid ($CH_3\ As^{III}O_2^{2}$, MAsIII), dimethylarsinic acid [$(CH_3)_2As^VO_2^-$; DMAsV], and dimethylarsinous acid [$(CH_3)_2As^{III}O^-$; DMAsIII] (Thomas et al. 2007) (**Fig. 1.9**). As3MT using *S*-adenosyl methionine as a methyl group donor and a dithiol containing reductant catalyses the formation of all methylated metabolites of iAs (Waters et al. 2004a). However, in case of rats, two additional methylated arsenicals are formed involving trimethylarsine oxide [$(CH_3)_3As^VO$; TMAsVO), and volatile trimethylarsine [$(CH_3)_3$AsIII; TMAsIII] (Waters et al. 2004b) (**Fig. 1.9**). In urine of residents of Bangladesh, a new sulfur containing derivative of DMAsV, dimethylthioarsinic acid (DMTA), has also been found (Raml et al., 2007).

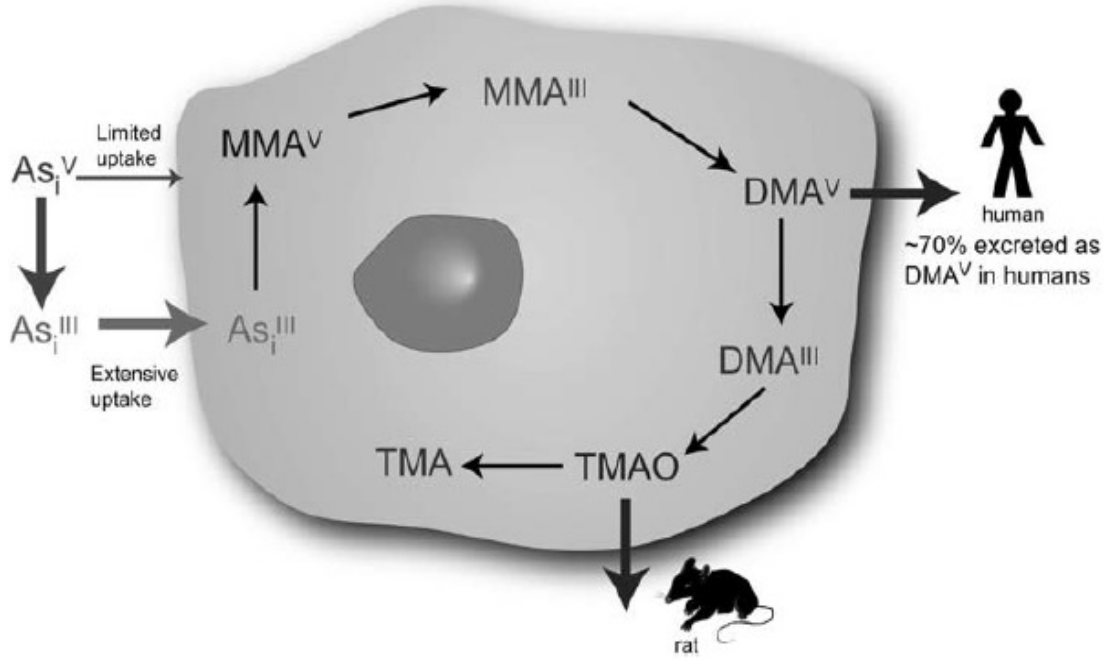

Fig. 1.9. Intracellular metabolism of inorganic As (Cohen et al., 2006)

There are few other factors also determine biomethylation process such as the cofactors for AS3MT activity e.g., thioredoxin reductase (TR) and thioredoxin (TRx) and transport systems for the uptake or efflux of iAs and other methylated forms (**Fig. 1.10**). GLUT2 and MRP2 (Multidrug resistance associated protein2) significantly affect the methylation of iAs by regulating the retention of iAs or other metabolites inside the cells. Furthermore, in hepatocytes AQP9 (Aquaporins 9) and an organic anion transport protein (OATP) are also involved in the uptake of iAsIII. Pentavalent methylated arsenicals can be reduced to their trivalent forms in a reaction with GSH (Delnomdedieu et al.1994). GSH transferase (GST) helps in reduction of pentavalent methylated arsenicals to trivalent forms and eventually formation of the GSH complexes with iAsIII, MAsIII, or DMAsIII (Liu et al. 2001; Brambila et al. 2002; Zhou et al.2005). These complexes of trivalent arsenicals with GSH are then exported out into bile through the MRP2 and P-gp (P-glycoprotein) (Hoffmaster et al., 2004; Planchamp et al. 2007). Bidirectional permeases GLUT2 and AQP9 can transport iAsIII and MAsIII along a concentration gradient but they cannot transport DMAsIII. The basolateral form MRP3 might be involved in the transport of complexes of GSH with trivalent arsenicals from the liver to the blood. OATP-C which is bidirectional basolateral transporter (Mahagita et al. 2007) could involve in efflux of iAs species (iAsV and iAsIII) into the blood while role for exporting methylated arsenicals is not certain.

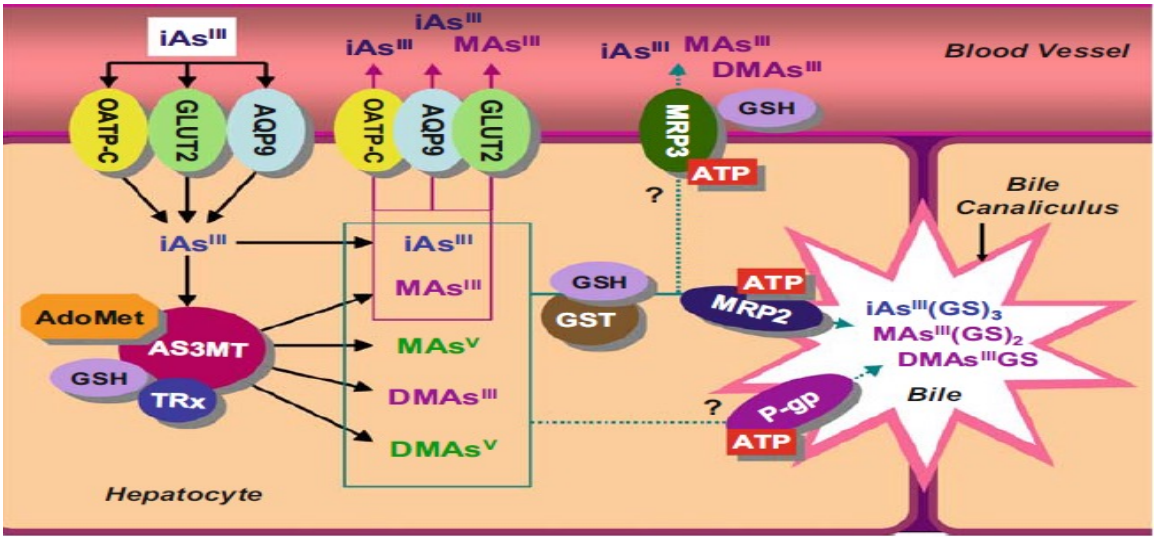

Fig. 1.10. Molecular components involved in the uptake and efflux of iAsIII and its methylated metabolites from liver to the blood and bile (Drobna et al., 2010).

1.7.6. Arsenic resistance in Prokaryotes

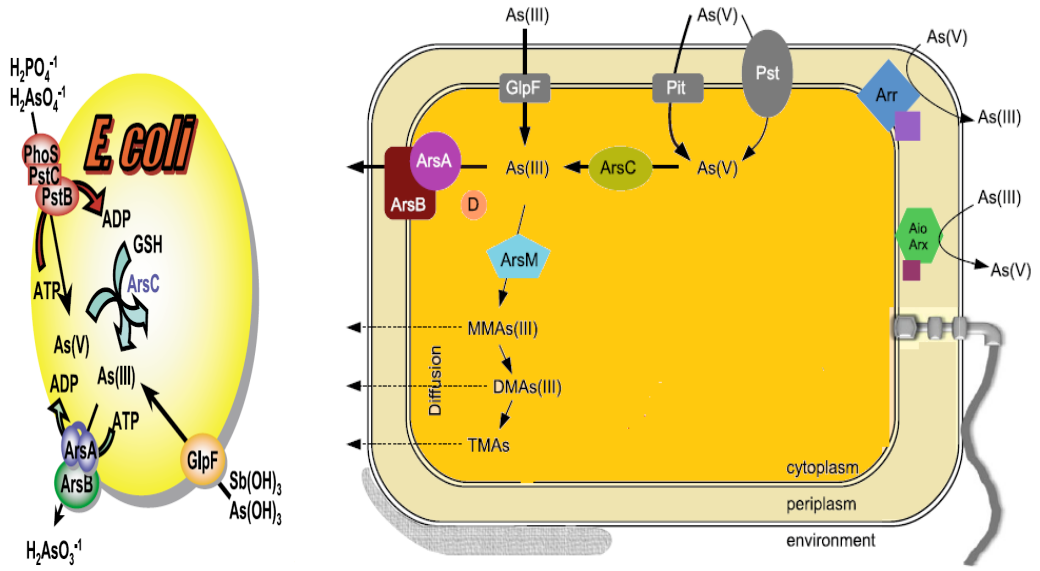

Fig. 1.11. Arsenic resistance mechanism in (A) *E. coli* (B) Biomethylation into volatile gas TMAs (Trimethyarsine) (Rosen., 2002; Kruger et al., 2013).

The process of As methylation was observed in aerobic and anaerobic bacteria as well as in photosynthetic organisms (Rosen, 2002; Kruger et al., 2013). In *E. coli*, arsenate (AsV) is taken inside the bacterial cell by phosphate transporters Pit and Pst while arsenite (AsIII) with aquaglyceroporins (GlpF in *E. coli*, Aqp7 and Aqp9 in mammals and Fps1p in yeast) (**Fig. 1.11a**). Thereafter, arsenate is reduced to arsenite by the bacterial ArsC utilizing glutathione and glutaredoxin as reducing agent. Arsenite is then extruded out from the cells by ArsB alone or by the ArsAB ATPase. Alternatively, As can be methylated which is considered as a detoxification mechanism, however, some of the intermediates have found to be more toxic compared to inorganic As (**Fig. 1.11b**). As resistance was developed in an As-hypersensitive strain of *E. coli* by cloning an As(III) S-adenosylmethionine(SAM) methyltransferase gene (*arsM*) of *Rhodopseudomonas palustris* leading to the production of trimethylarsine gas (Qin et al., 2006). Notably, this leads to the conclusion that the increased volatility of methylated arsenicals would compensate for the toxic intermediates that forms during the process. During the process of biomethylation in prokaryotes arsenate first reduced to arsenite which then undergoes oxidative methylation by ArsM using S-adenosylmethionine, followed by reductive steps utilizing glutathione forming intermediates like monomethyl

arsenite (MMAs III), dimethyl arsenite (DMAsIII) and eventually forming volatile trimethyl arsine (TMAs).

1.8. Dislipidemia

Heavy metal exposure can produce several adverse health effects in the biological systems. Heavy metal effect on the perturbations in lipid metabolism in different compartments of organisms is another not well discussed outcome in literature. Pb exposure causes impaired lipid metabolism by disturbing the activities of enzymes involved in the metabolism of these lipids (Ademuyiwa et al., 2009). Auto mechanics and painters who are highly exposed to Pb are on high risk of atherogenesis and cardiovascular diseases (Nriagu et al., 1997; Ademuyiwa et al., 2005). Pb leads to an increase in plasma concentrations of cholesterol, triacylglycerol, and phospholipids (Kristal-Boneh et al., 1999; Hamadouche et al., 2009). Higher cholesterol levels in plasma might be due to the following reasons: (a) change in cholesterol distribution between the plasma and the tissues; (b) increase in cholesterol absorption from diet; (c) increase in cholesterol synthesis; (d) decrease in excretion of cholesterol in the form of neutral sterols; and (e) decrease in transformation of cholesterol to bile acids (Kilic, 1993). Pb can also cause overproduction of very low density lipoprotein (VLDL), thereby increasing the burden of triacylglycerol rich lipoproteins (Hassan and Jassim, 2010).

Cd is also reported to alter the TC, TG, HDL-C and LDL-C metabolism resulting into dyslipidemia. Higher cholesterol levels in serum is explained by Cd induced increase in the activity of rate limiting enzyme of cholesterol synthesis HMG-CoA (hydroxy-3-methylglutaryl-coenzyme A reductase) along with decrease in LDL receptor gene expression (Prabu et al., 2013; Ugbaja et al., 2013). Increase in serum FFA induced by inhibition of β oxidation via Cd. Lower level of plasma HDL-C is found due to altered metabolism of the major HDL apoprotein. Furthermore, impaired catabolism of TG-rich particles induced by Cd leading to increase in serum triglyceride.

Dislipidemia can also be caused by As exposure resulting into an increase in plasma triglycerides, total cholesterol, free fatty acids levels, and a decrease in HDL

cholesterol levels (Afolabi et al., 2015; Miltonprabu & Sumedha, 2015). Higher cholesterol levels were observed in animals exposed to As due to increase in the activity of rate limiting enzyme of cholesterol synthesis *i.e.* 3-hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) reductase leading to cholesterol accumulation. As treatment also inhibits the activity of cytochrome P450 enzyme present in the endoplasmic reticulum *i.e.* cholesterol-7α-hydroxylase which is involved in the biosynthesis of bile acids, hence preventing the exclusion of cholesterol from the body (Gesquière et al., 1999; Kojima et al., 2004; Sawada et al., 2005).

As can also induce hypertriglyceridemia by reducing activity of LPL (lipoprotein lipase) which is involved in catabolism of TGs thus, releasing FFAs from chylomicrons and VLDL thereby disturbing the circulation of TGs (Muthumani and Miltonprabu, 2015). LDL can be aggregated due to reduction in LPL activity induced by As and subsequent LDL receptor defects in liver due to exposure. Serum free fatty acid levels were increased by As through inhibition of β-oxidation due to decrease in mitochondrial function. HDL helps in removing excessive cholesterol in presence of LCAT, and its activity is also reduced on As treatment leading to a decrease in HDL levels.

1.9. Metallothionein

Metallothioneins (MT) are cysteine-rich, low-molecular-weight, metal-binding proteins which get induced in response to various toxicologic and physiologic stimuli (Klaassen et al., 1999; Thirumoorthy et al., 2011). They have several beneficial properties for instance, detoxification of heavy metals, homeostasis of essential metals such as zinc and copper, antioxidant by acting as free-radical scavenger, protect against oxidative stress and DNA damage, helps in angiogenesis, cell survival and apoptosis. Altogether, MT controls three elementary processes: 1) exchange and binding of heavy metals such as zinc, Cd or copper 2) apoptosis, and 3) the release of gaseous mediators such as nitric oxide and hydroxyl radical. MT-null mice fed on Cd resulted into development of nephrotoxicity. Different isoforms of MT have also been found such as MT-I, MT-II, MT-III and MT-IV. They have different roles in body which are as follows: MT-I and II are present throughout the body in all cells and involved in detoxification of heavy metals, immune function and several G.I. tract functions. MT-III is found mainly in brain

and involved in the development, organization and programmed death of brain cells. MT-IV is present in the skin and upper G.I. tract and plays role in regulating pH of acid produced in stomach, in discriminating taste and texture of the tongue as well as provides protection against sunburn.

1.10. Combination therapy: chelators, antioxidants, probiotics, prebiotics, synbiotics

1.10.1. Chelation: Concept

Chelation therapy is one of the most widely used medical treatment for heavy metal toxicity. Chelators form complex with heavy metals by binding with them using chemical groups like –SH, –S-S, $–NH_2$, =NH, –OH, $–OPO_3H$, or >C=O, thereby helps in excreting them out (**Fig. 1.12**).

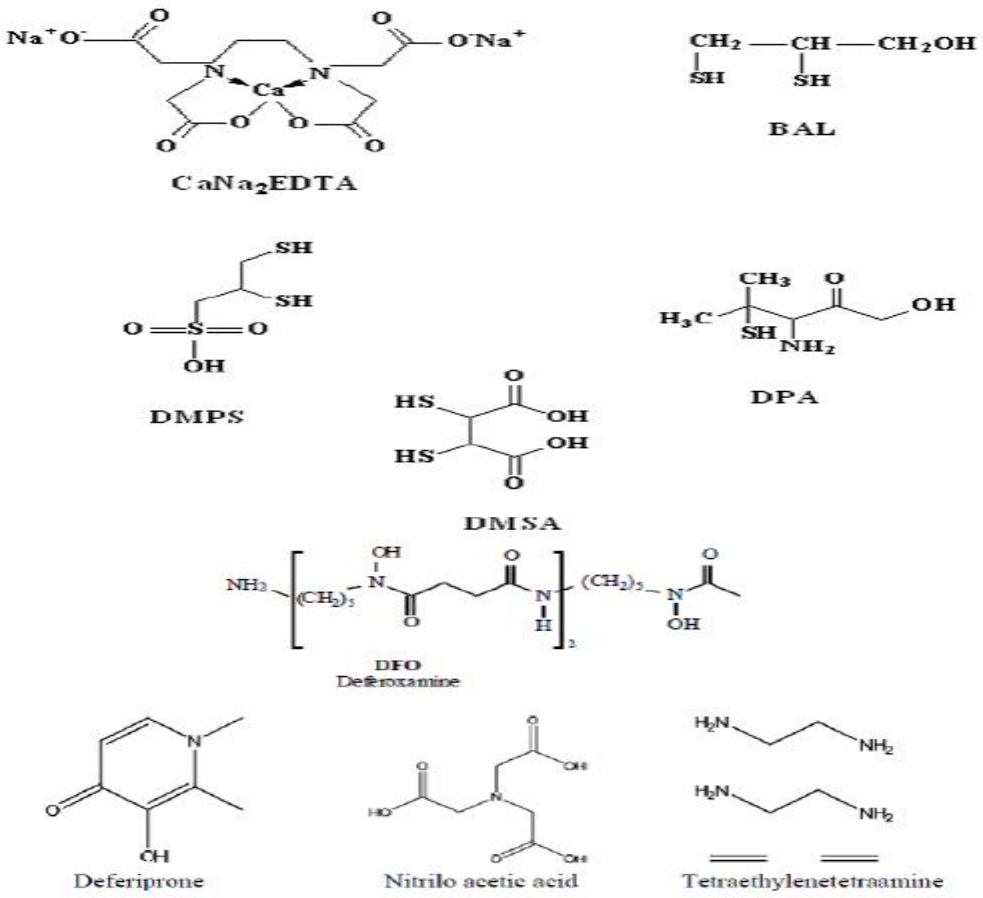

Fig. 1.12. Structures of various chelating agents used to treat heavy metal poisioning (Flora and Pachauri, 2010).

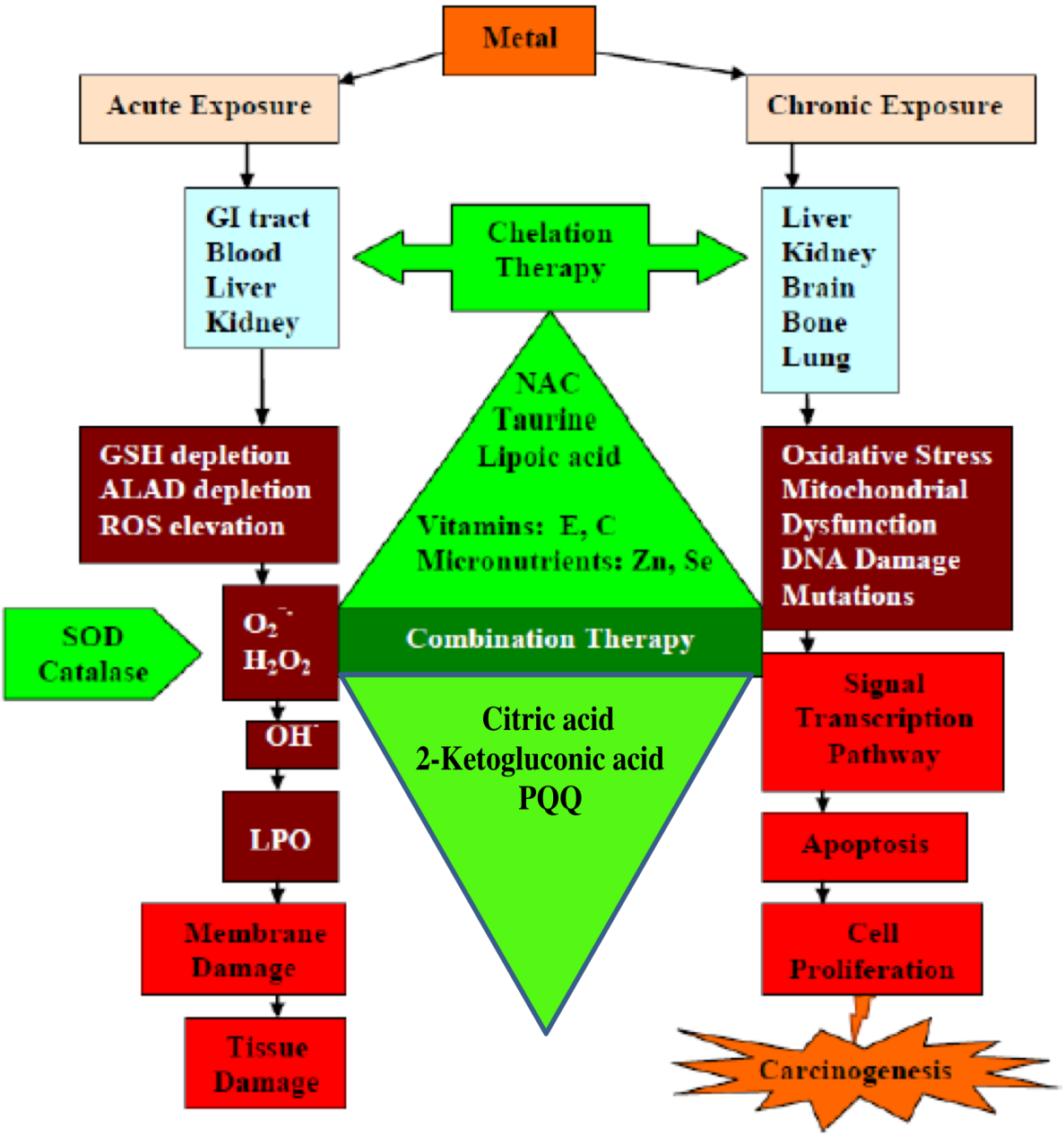

Fig. 1.13. Combination therapy involving chelation therapy and antioxidant treatment for heavy metal exposure (modified from Flora and Pachauri, 2010).

1.10.2. Antioxidants with chelators

Oxidative stress generated by heavy metal toxicity can be better dealt by including antioxidants with chelation therapy (**Fig. 1.13**). Antioxidant treatment along with chelators found to be more beneficial in removing Pb from tissues and restabilizing the disturbed biochemical parameters (Flora and Pachauri, 2010). Recoveries were observed

in animal models using combination therapies involving antioxidants such as lipoic acid, *N*-acetylcysteine, gossypin, and melatonin. The chain breaking mechanism of antioxidants is mentioned in **Fig 1.14**.

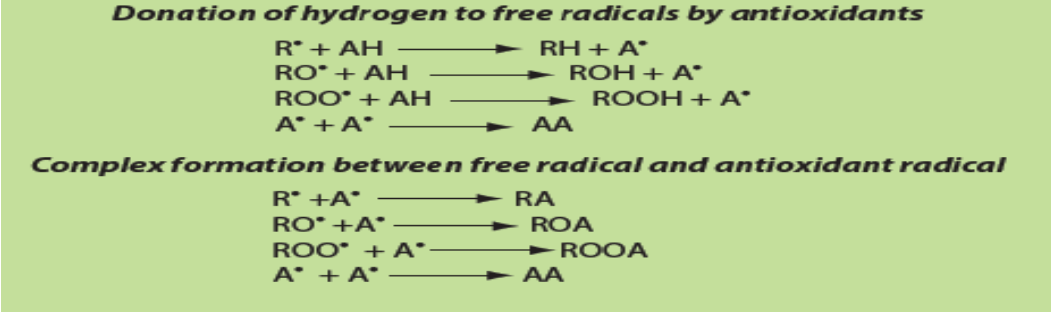

Fig.1.14. Chain breaking mechanism of antioxidants (Flora et al., 2012). AH: Antioxidant, A•: Antioxidant radical; ROO•, RO•, R•,: Free Radicals.

MiADMSA and captopril in the combination was found to be effective against apoptosis which is caused via Pb (Flora and Pachauri, 2010). As induced damage can be prevented by combined administration of *N*-acetylcysteine and succimer as well as combination of vitamin C and MiADMSA. Likewise, *N*-acetylcysteine along with MiADMSA/DMSA and the thiol chelator with natural antioxidant C*entella asiatica* was significantly effective in Pb intoxication rats.

1.10.3. PQQ (Antioxidant)

PQQ (Pyrroloquinoline Quinone) acts as a cofactor for enzymes in prokaryotes (Rucker et al., 2009; Akagawa et al., 2016; Itoh et al., 2016). It is considered as a potent antioxidant molecule with redox-oxidation cycle of 20,000 (**Fig.1.15**). PQQ also shows effects such as growth promoting, anti-diabetic and its supplementation has been reported to improve reproductive system, neonatal growth, mitochondriogenesis, cognitive, immune system, and neurological and

Compound	Potential Number of Catalytic Cycles
PQQ	20,000
Quercetin	800
Catechin	75
Epicatechin	700
Norepinephrine	200
Epinephrine	100
DOPA	20
6-OH-DOPA	20
Ascorbic Acid	4

Fig. 1.15. Redox cycling for PQQ (Rucker et al., 2009)

cardiac ischemic health (**Fig. 1.16**)

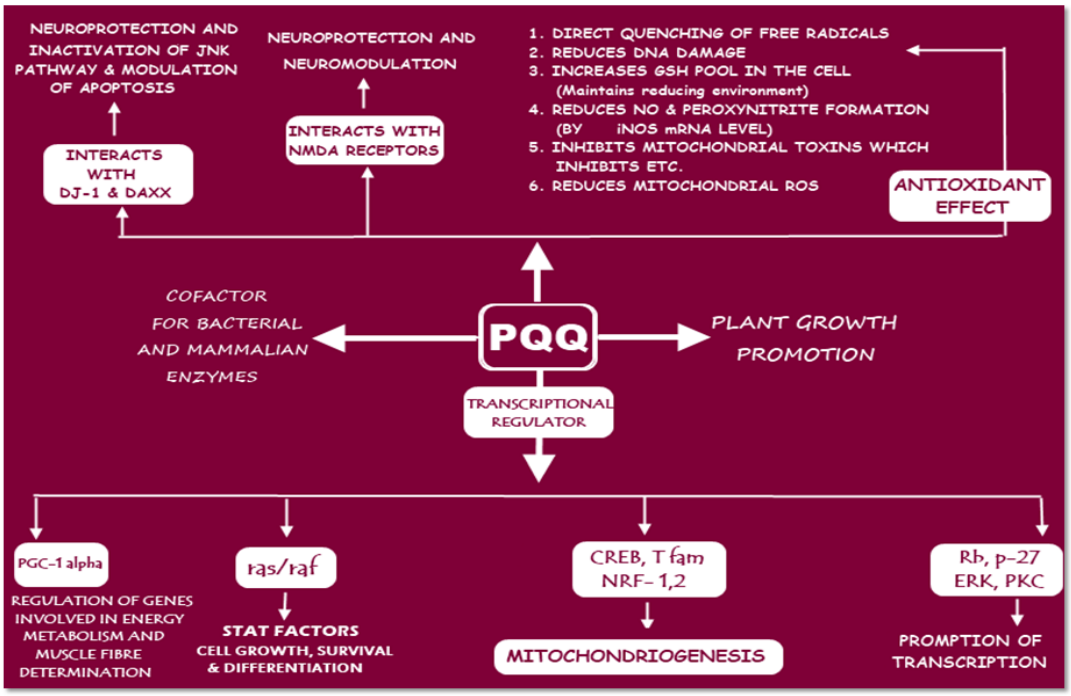

Fig. 1.16. Different functions of PQQ (Rucker et al., 2009).

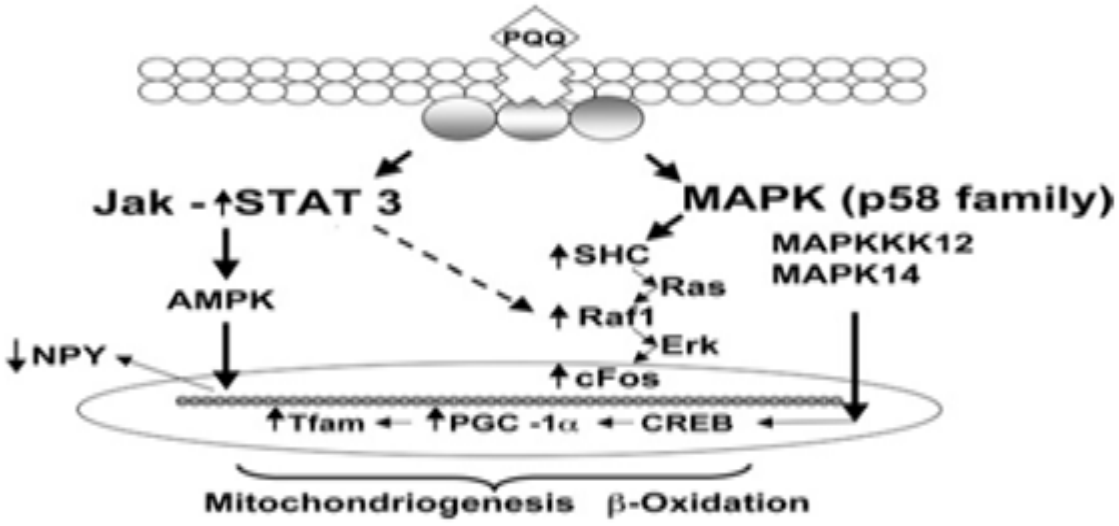

Fig. 1. 17. Proposed pathway for PQQ action (Tchaparian et al., 2010).

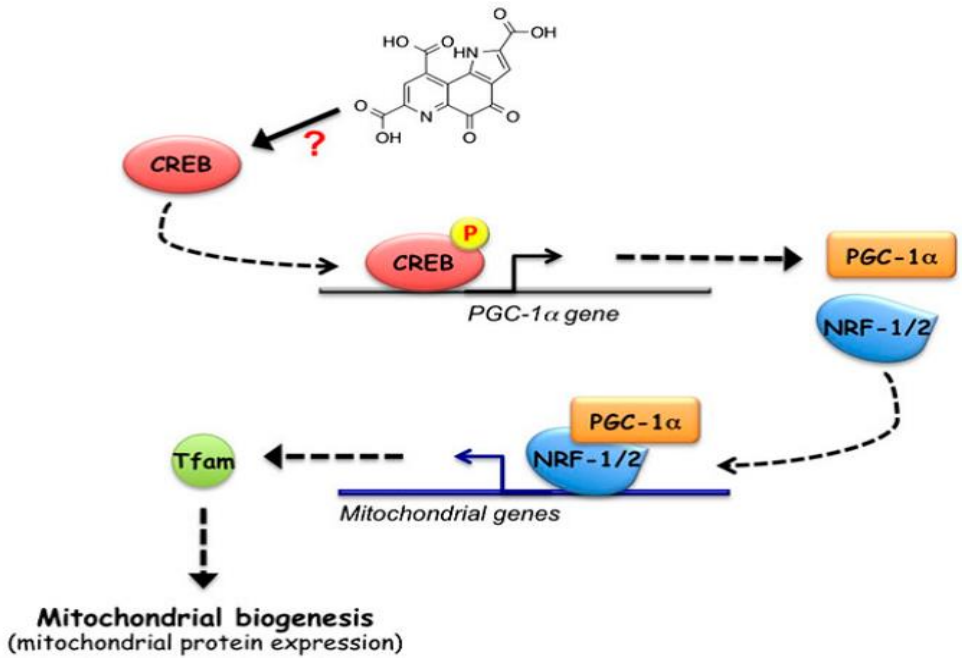

Fig. 1.18. Proposed mechanism for PQQ induced mitochongriogenesis (Akagawa et al., 2016).

PQQ is anticipated to effect cell signaling by binding with certain cell surface receptors (**Fig. 1.17**) (Tchaparian et al., 2010). PQQ regulates JAK/STAT3 pathway by upregulation of STAT3 which controls apoptosis, cell proliferation and differentiation. 5' AMP-activated protein kinase (AMPK) was proposed to be affected by down-regulation of NPY. Inter-relationships between MAPK/ERK signaling and STAT3 attributed by cross-talk between Raf-1 and STAT3 with the up-regulation of c-Fos, Raf-1 and Ras. The MAPK p58 family (MAPKKK12 and MAPK14) was also supposed to involved, eventually these signaling pathways results into activation of PGC-1α, PPARα (peroxisome-proliferator-activated receptor α) and CREB (cAMP response element binding protein).

PGC-1α is well known transcriptional coactivator which is primarily involved in energy metabolism, muscle fiber type determination, controlling blood pressure, cholesterol homeostasis, obesity, reduction in reactive oxygen species and protection from mitochondrial toxins (Puigserver, 2005; Muoio and Koves, 2007). PQQ interacts with PGC-1α as well as ras which is an oncogene involved in growth and development (Kumazawa et al., 2007). It also interacts with ERK which are protein kinases involved in

ras-signaling pathway to activate various activators, co-activators, and transcription factors as well as with raf which is also involved in the chain of events from ras to STATs.

PQQ activates PGC-1α mediated signaling through activation of AMPK or mitogen-activated protein kinases (MAPKs) (Stites et al., 2006; Bauerly et al., 2006; Zhu et al., 2006; Debray et al., 2008; Akagawa et al., 2016). Thereafter, these kinases activates the transcription factor CREB that ultimately binds with certain DNA sequences known as cAMP response elements, thus, eventually enhancing PGC-1α expression. PGC-1α coactivates the nuclear respiratory factor (NRF-1 and 2) by binding with it on the mitochondrial transcription factor A (Tfam) promoter to enhance Tfam levels which helps in regulating amplification of mitochondrial DNA and mitochondrial biogenesis (**Fig. 1.18**). PQQ is also known to influence the activity of DJ-1 which is important for oxidative stress responses within cell as well as for Parkinsons disease development (Rucker et al., 2009). Altogether, as shown in **Fig. 1.19**, PQQ controls proliferation, apoptosis, and mitochondriogenesis by affecting the signals such as ras, DJ-1 and various kinases.

Recently, the ligand-independent activation of epidermal growth factor receptor (EGFR) signaling have been reported through redox cycling of PQQ (Akagawa et al., 2016). In the presence of reductants such as ascorbate and glutathione, PQQ undergoes redox cycling to produce O_2^- and H_2O_2. The H_2O_2 thus generated inactivates protein tyrosine phosphatase 1B (PTP1B) by the oxidation of catalytic cysteinyl thiol (Cys-215) to the respective sulfenic acid (–SOH), sulfinic acid ($–SO_2H$), and sulfonic acid ($–SO_3H$). PTP1B negatively regulates the EGFR by catalyzing tyrosine dephosphorylation of activated EGFR, hence inhibiting the further EGFR signalling. Inhibition of PTP1B promotes the EGF-independent activation (tyrosine phosphorylation) of EGFR and eventually activation (serine/threonine phosphorylation) of ERK ½ (**Fig. 1.20**).

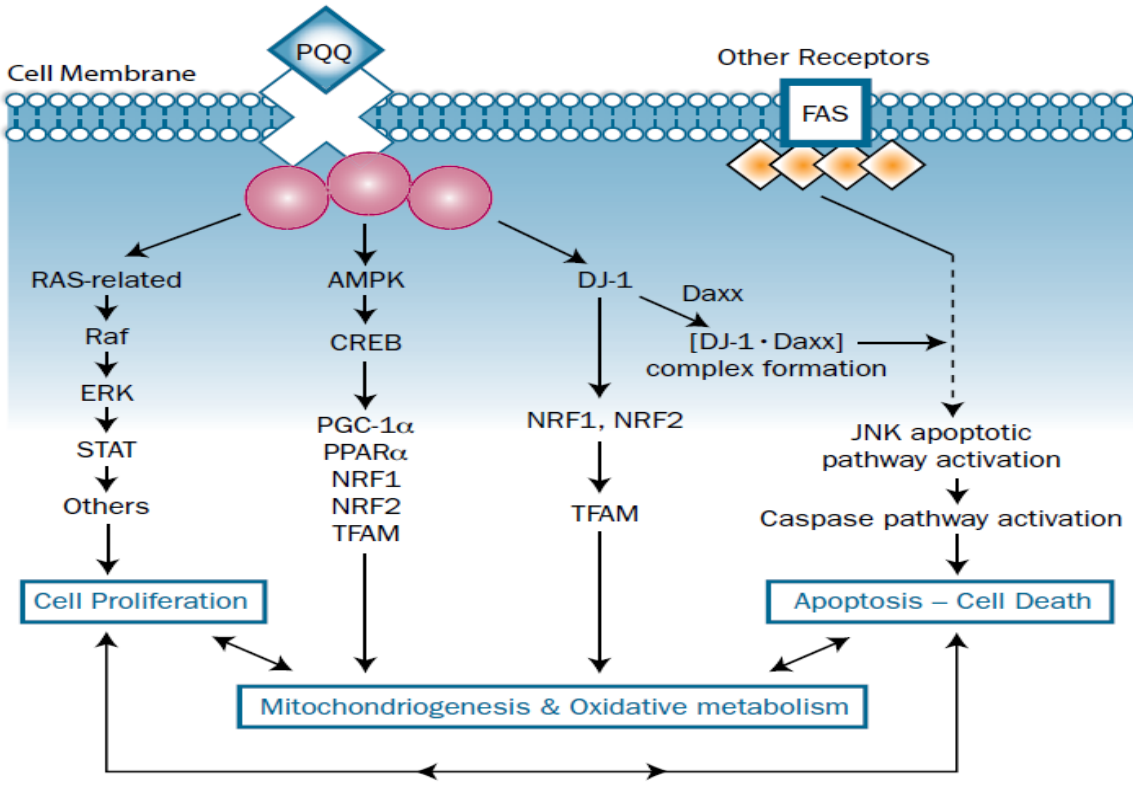

Fig. 1.19. Effects of PQQ on cell signaling (Rucker et al., 2009)

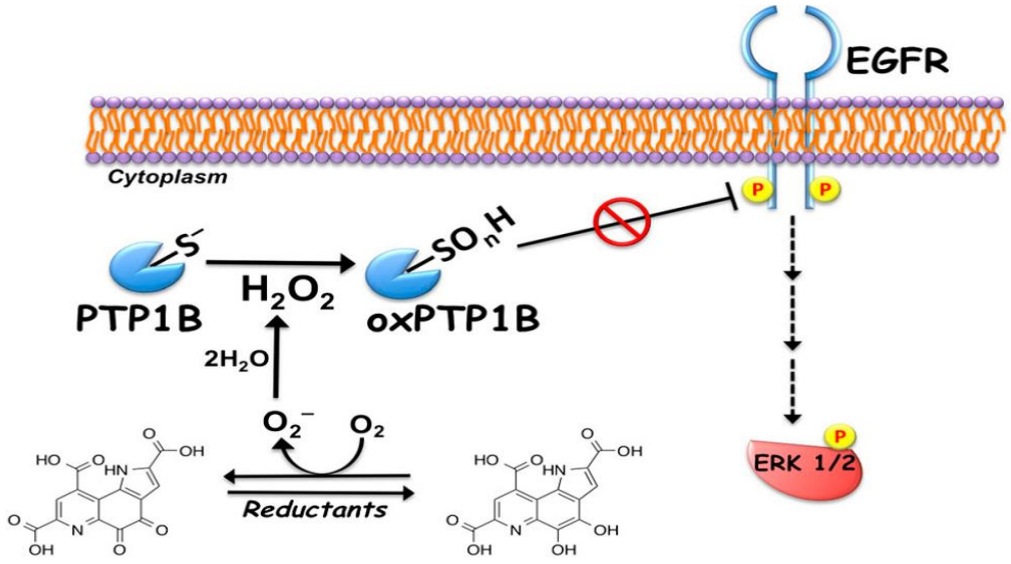

Fig. 1.20. Proposed mechanism for the PQQ mediated ligand-independent activation of EGFR signalling through redox cycling (Akagawa et al., 2016).

PQQ altered the levels of thioredoxin which is an oxidoreductases, helps in antioxidative defence and in catalyzing the formation of inter and intra molecular disulfide bonds in proteins, thus protects proteins from the oxidation damage (Zhang et al., 2002; Hirakawa et al., 2009). Quercetin, hydroxytyrosol, and resveratrol induces changes in mitochondrial number and function in mM levels while PQQ at nano to micromolar levels. PQQ also reported to act as anti-inflammatory molecule by suppressing NO, PGE2, iNOS, COX-2, TNF-a, IL-1b, IL-6, MCP-1, MIP-1a, nuclear translocation of NF-kB, phosphorylation level of p65, p38 and JNK MAP kinase pathways in LPS stimulated primary microglia cells (Yang et al., 2014). PQQ has also shown anti-fibrotic effect in Balb/C mouse models of liver fibrosis (Jia et al., 2015).

1.10.4. Probiotics

In the intestinal tract from proximal to distal side the density of the bacterial colonization increases reaching upto 10^{11} bacteria/gram feces in the colon (Faderl et al., 2015). The intestinal microbiota constitutes 1,000 different species with their collective genome found to contain 100 times more genes than the entire human genome. Gut microbiota in the form of short chain fatty acids provide energy to gut epithelial cells, produce essential vitamins, perform immunomodulation via their metabolites and also competes with potential pathogens for nutrients and niches, thereby provides "colonization resistance" (**Fig. 1.21 and 1.22**).

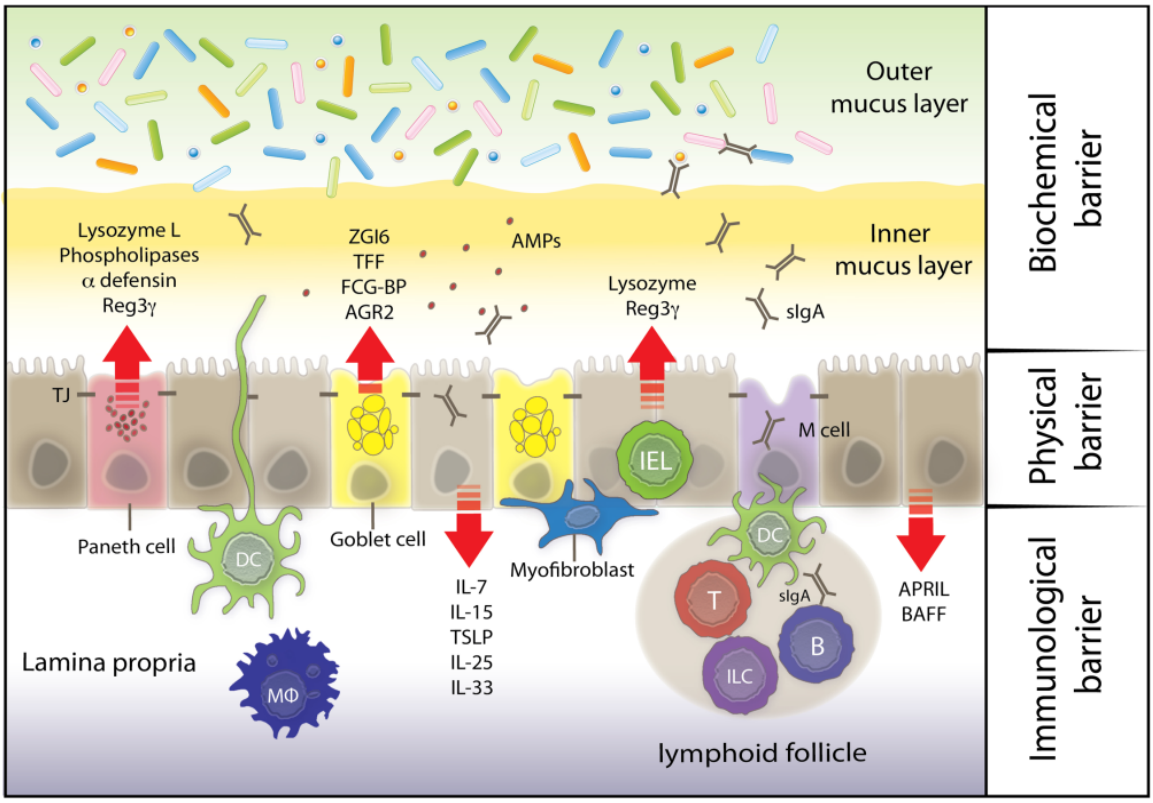

Fig. 1.21. A multilayered barrier system in gut (Faderl et al., 2015).

Probiotics are defined as a non-pathogenic living microorganisms when administered in adequate amounts confer health benefits to host (Nagpal et al., 2012). They are active component of human and veterinary medicine. Some of the beneficial effects associated with consumption of probiotics include stimulation and development of the immune system, improvement of intestinal health by the regulation of microbiota, reducing symptoms of lactose intolerance, synthesizing and enhancing the bioavailability of nutrients and decreasing the risk of several other diseases (**Fig. 1.23**).

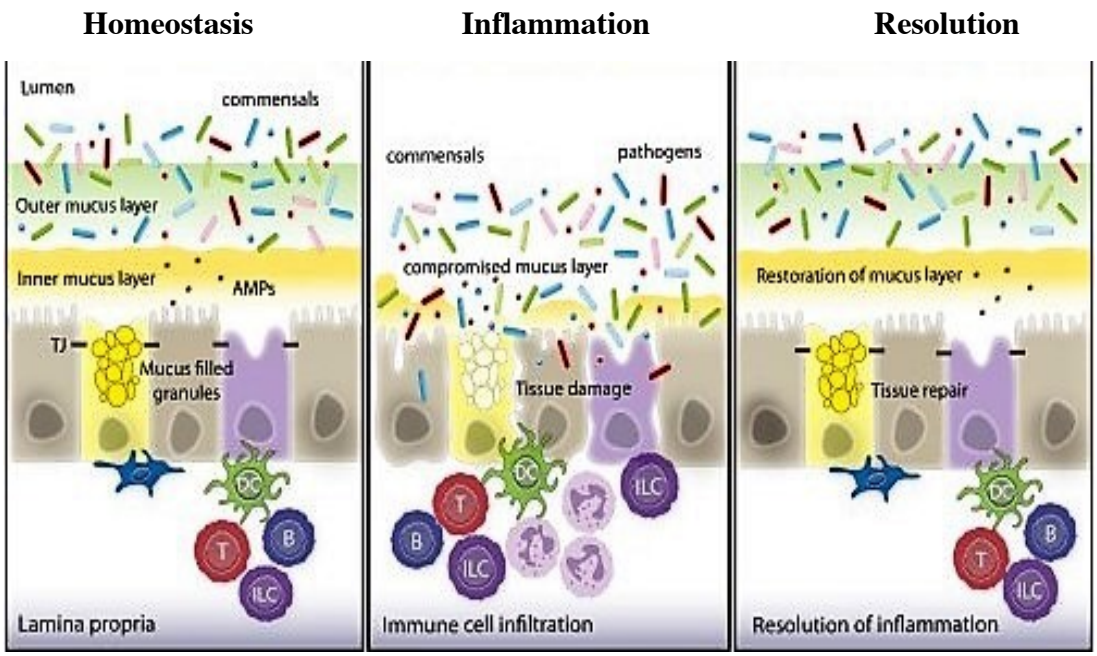

Fig.1.22. Effect of colonic mucus layer on the segregation of luminal bacteria from the intestinal mucosa during homeostasis, inflammation and resolution (Faderl et al., 2015).

Some of the major health benefits of probiotics are summarized in **Table 1.4**. Several important probiotic bacteria have been introduced in the market for the public health (**Table 1.5**).

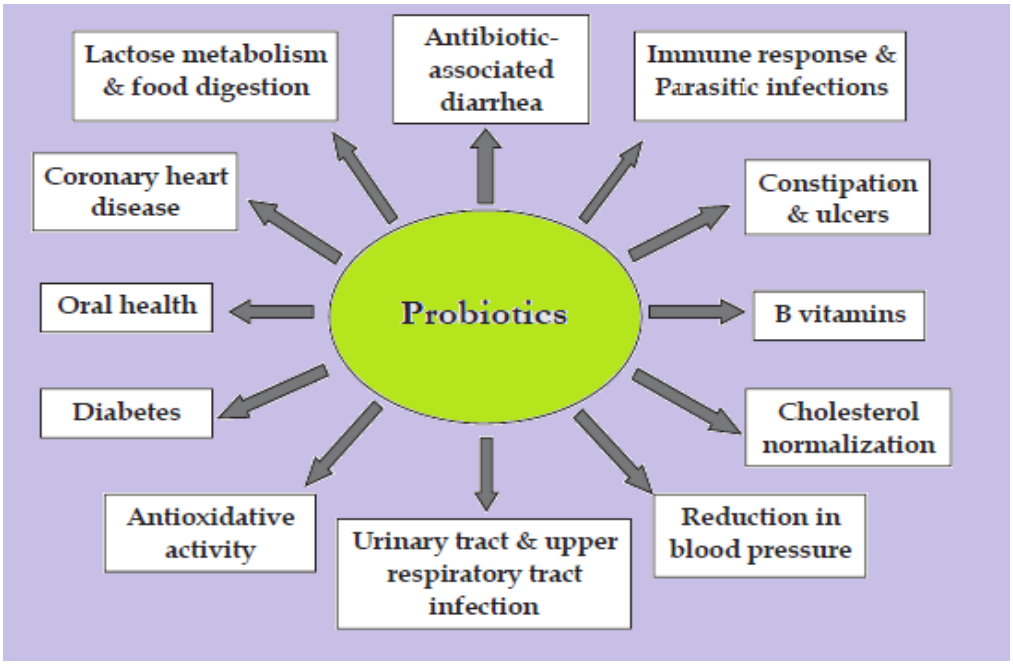

Fig.1.23. Beneficial effects of Probiotics (Nagpal et al., 2012).

Health benefits	Proposed mechanisms involved
Resistance to enteric pathogens	Antagonism activity Adjuvant effect increasing antibody production Systemic immune effect Colonization resistance Limiting access of enteric pathogens (pH, bacteriocins/defensins, antimicrobial peptides, lactic acid production, and toxic oxygen metabolites)
Aid in lactose digestion	Bacterial lactase acts on lactose in the small intestine
Small bowel bacterial overgrowth	Lactobacilli influence the activity of overgrowth flora, decreasing toxic metabolite production Normalization of a small bowel microbial community Antibacterial characteristics
Immune system modulation	Strengthening of nonspecific and antigen-specific defense against infection and tumors Adjuvant effect in antigen-specific immune responses Regulating/influencing Th1/Th2 cells, production of anti-inflammatory cytokines Decreased release of toxic N-metabolites
Anticolon cancer effect	Antimutagenic activity Detoxification of carcinogenic metabolites Alteration in pro-cancerous enzymatic activity of colonic microorganisms Stimulation of immune function Influence on bile salt concentration
Decreased detoxification/ excretion of toxic microbial metabolites	Increased bifidobacterial cell counts and shift from a preferable proteinto carbohydrate-metabolizing microbial community, less toxic and for putrefactive metabolites, improvements of hepatic encephalopathy after the administration of bifidobacteria and lactulose
Allergy	Prevention of antigen translocation into blood stream Prevent excessive immunologic responses to increased amount of antigen

	stimulation of the gut
Blood lipids, heart disease	Assimilation of cholesterol by bacterial cell Alteration in the activity of BSH enzyme Antioxidative effect
Antihypertensive effect	Bacterial peptidase action on milk protein results in antihypertensive tripeptides Cell wall components act as ACE inhibitors
Urogenital Infections	Adhesion to urinary and vaginal tract cells Competitive exclusion Inhibitor production (H2O2, biosurfactants)
Infection caused by Helicobacter pylori	Competitive colonization Inhibition of growth and adhesion to mucosal cells, decrease in gastric H. pylori concentration
Hepatic encephalopathy	Competitive exclusion or inhibition of ureaseproducing gut flora
Neutralization of dietary carcinogens	Production of butyric acid neutralizes the activity of dietary carcinogens
NEC (necrotic inflammation of the distal small intestine)	Decrease in TLRs and signaling molecules and increase in negative regulations Reduction in the IL-8 response
Rotaviral gastroenteritis	Increased IgA response to the virus
Inflammatory bowel diseases, type I diabetes	Enhancement of mucosal barrier function
Crohn's disease	Reduction in proinflammatory cytokines including TNFa, reduction in the number of CD4 cells as well as TNFa expression among intraepithelial lymphocytes
Caries gingivitis	Reduction in gingivitis by L. reuteri, affects on streptococcus mutants, colonization of the teeth surface by lactobacilli Less carries after the ingestion of living or oral vaccination with heat-killed lactobacilli
Enhanced nutrient value	Vitamin and cofactor production

Table 1.4. Major health benefits of probiotics (Nagpal et al., 2012).

Probiotic Strain	Formulation	Brand Name (Manufacturer)	Evidence-Based Efficacy
Single-strain probiotics			
***Bifidobacterium animalis* subsp *lactis* DN-173010**	Yogurt	Activia (Danone)	Constipation
***B.animalis* subsp *lactis* Bb-12**	Capsules, powder in sticks,fermented milk	BB-12 (Chr Hansen)	Eczema
***Bifidobacterium infantis* 35624**	Drink, capsules	Align (Procter & Gamble)	IBS
Clostridium butyricum 588	Tablets, drink	MIYA-BM (Miyarisan Pharm)	AAD *Helicobacter pylori* infection
***Enterococcus faecium* SF 68**	Powder, sachets	Bioflorin (Cerbios-Pharma)	Acute adult diarrhea
***Escherichia coli* Nissle 1917**	Capsules	Mutaflor (Ardeypharm)	No trends
***Lactobacillus acidophilus* Lb**	Sachets, capsules	Lacteol (PUMC Pharm)	Acute pediatric diarrhea
Lactobacillus casei* subsp *Shirota	Fermented milk	Yakult (Yakult)	Constipation, *H. pylori* infection
***L. casei* DN-114001**	Fermented drink, yogurt	Actimel, DanActive (Danone)	AAD, prevention of pediatric diarrhea, respiratory infections
***L. rhamnosus* Lcr35**	Vaginal capsules	Gynophilus	BV
***Lactobacillus johnsonii* La1**	Milk	NC1 (Nestle)	*H. pylori*infections
***Lactobacillus plantarum* 299v (DSM9843)**	Fermented oat gruel in fruit drink, capsules	ProViva (Probi) Darolac-IBS (Araisto)	IBS, CDI
***Lactobacillus reuteri* DSM 17938**	Capsules, yogurt	Protectis (BioGaia)	Acute pediatric diarrhea, cholesterol

***L. rhamnosus* GG (ATCC 53013)**	Yogurt, capsules	Culturelle (Amerifit Brands) Vifit (Valio)	Acute pediatric diarrhea, AAD
***Saccharomyces boulardii* CNCM I-745 (lyo)**	Capsules	Florastor, Codex, UltraLevure (Biocodex)	AAD, CDI, acute adult and pediatric diarrhea, TD, *H. pylori*infections
Mixtures of probiotic strains			
***L.acidophilus* CL1285 + *L. casei* Lbc80r + *L. rhamnosus*CLR2**	Fermented drink, capsules	Bio K+ (BioK+ Intl)	AAD, CDI
***Lactobacillus helveticus* R0052 (CNCM I-1722) + *L. rhamnosus* R0011 (CNCM I-1720)**	Capsules, sachets	Lacidofil (Lallemand) A'Biotica (Institut Rosell)	*H. pylori* infection, AAD
L. helveticus (bulgaricus)* 4962 + *L. acidophilus	Capsules	Lactinex (BD Diagnostics)	Acute adult diarrhea
***L. reuteri* DSM17938 + *L. reuteri*PTA5289**	Lozenges, powder, capsules	Prodentis (BioGaia)	Dental infections
L. acidophilus +B. animalis* subsp *lactis	Yogurt	AB Yogurt	Improves normal flora
L. acidophilus +Bifidobacterium bifidum	Capsules	Infloran Berna (Intituo Sieroterapico)	Respiratory tract infections
L.acidophilus subsp gasseri+ Bifidobacterium infantis	Capsules	Linex (Sandoz)	AAD
***Bacillus clausii* (4 strains: O/C, N/R84, T84, Sin8)**	Capsules, spores in vial	Enterogermina (Sanofi-Aventis)	Antidiarrheal
Bifidobacterium breve, Bifidobacterium longum, B. infantis, L. acidophilus, L. plantarum, L. casei, L. bulgaricus, Streptococcus thermophilus	Sachets	VSL#3 (Sigma-Tau Pharm Inc)	IBS UC

Table 1.5. Important probiotic products in market (McFarland, 2015).

Probiotics have also been engineered to facilitate an approach that would be more targeted for the prevention and treatment of certain diseases (Sola-Oladokun et al., 2017).

Recently, genetically modified probiotics have been developed for use in versatile delivery vehicles including vaccine delivery, drug administration, and immunomodulation (**Fig. 1.24**).

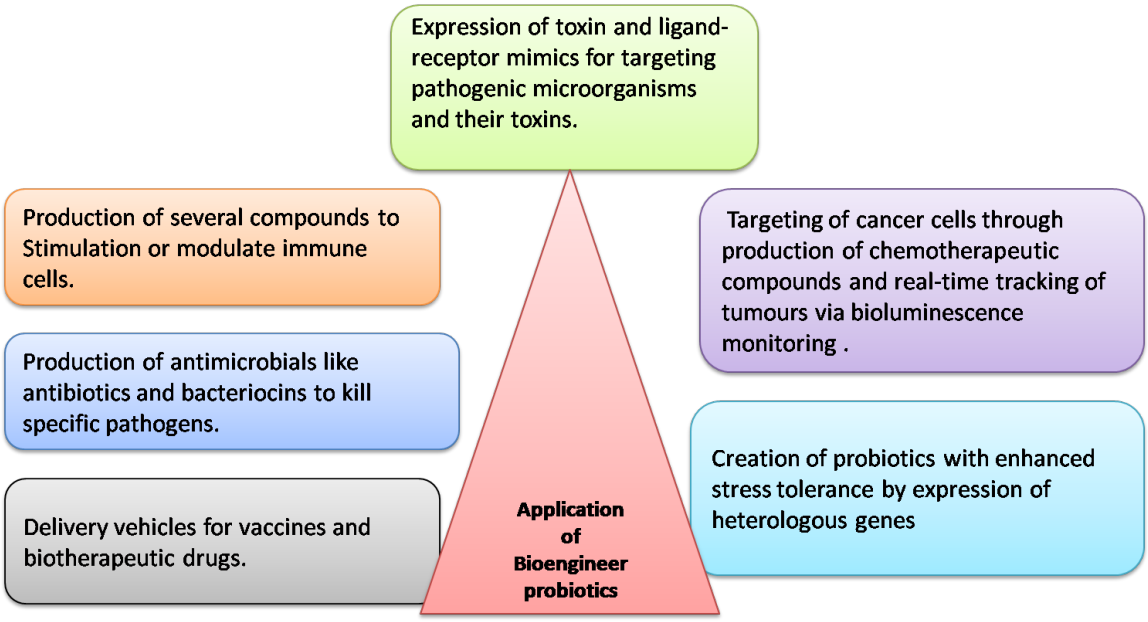

Fig. 1.24. Overview of applications of bioengineered probiotics (Sola-Oladokun et al., 2017).

1.10.5. Prebiotics and SCFAs (Short chain fatty acids)

"Prebiotics are defined as non-digestible food products which beneficially improve the host health by enhancing the growth of gut microflora in colon" (Gibson et al., 2010). They are also defined as "A Prebiotic is the fermented food which induces specific changes in composition and activity of gut microflora to confer beneficial health effects on host" (Gibson et al., 2010). For a food product to be classified as a prebiotic, it should have following characteristics: neither absorbed nor hydrolysed in the upper gastrointestinal tract, acts as substrate for growth of beneficial bacteria in colon, could alter the colonic microflora towards healthier composition, should induce systemic beneficial health effects to the host. Prebiotics such as fructans, galacto-oligosaccharide (GOS), lactulose, xylose-oligosaccharide (XOS), some peptides, proteins, certain lipids (both ethers and esters), gluconic acid are resistant to digestive juices within intestine, therefore they reach to colon where they undergo fermentation by commensal bacteria and utilize them for their growth. Inulin uptake has been reported to increases number of

Roseburia species, *Eubacterium* and *Ruminococcus* (Cummings et al., 1991). These food products can also be called "colonic foods" (**Table 1.6**). Others Compounds like hemicellulose, pectins, resistant starch, and non-starch polysaccharides (plant cell wall polysaccharides and gums) are considered as colonic food but they don't fulfil the criteria for prebiotics (Delzenne *et al*., 1994), hence stimulate the growth of potentially harmful bacteria in colon along with beneficial bacterial species.

Carbohydrates	**Colonic foods**	**Prebiotic**
Resistant starch	Yes	No
Non starch polysaccharides		
Plant cell wall polysaccharides	Yes	No
Hemicelluloses	Yes	No
Pectins	Yes	No
Gums	Yes	No
Non digestiable oligosaccharides		
Fructooligosaccharides	Yes	Yes
Galactooligosaccharides	Yes	Yes
Soybean oligosaccharides	Yes	?

Table 1.6. Classification of certain carbohydrates as colonic food prebiotics (Delzenne et al., 1994).

In colon, prebiotics undergo bacterial fermentation to produce short-chain fatty acids (SCFAs) such as butyrate, propionate and acetate, which mediate their function by Ffar receptors and AMPK. SCFAs have various beneficial effects like activation of fatty acid oxidation and inhibition of *de novo* lipid synthesis and lipolysis, ultimately helps in decreasing the body weight and free fatty acids levels in plasma (Lin et al., 2012). SCFAs increases the PGC-1α and uncoupling protein (UCP-1) levels, thereby increases thermogenesis and fatty acid oxidation in brown adipose tissue. SCFAs had been reported to increase leptin expression and reduce cholesterol plasma concentration. For instance, propionate decreases cholesterol synthesis by effecting the enzymes which are the rate determining steps of cholesterol synthesis like hepatic 3-hydroxy-3-methylglutaryl-CoA synthase and 3-hydroxy-3-methylglutaryl-CoA reductase. Furthermore, butyrate acts as energy sources for the enterocytes as well as maintains colonic health (**Fig. 1.25**).

Fig. 1.25. Several cellular function which affected by butyrate produced in colon (Bordin et al., 2004).

SCFAs determines mucous thickness, decrease permeability by increasing tight junction proteins expression, influence gut motility and satiety (Bordin et al., 2004). Butyrate acts as an anti-inflammatory molecule (Luhrs et al., 2002) through suppressing the activation by inhibiting the HDAC (Place et al., 2005). NF-kB acts as transcription factor for activating pro-inflammatory cytokines, inflammatory enzymes such as iNOS, cyclo-oxygenase-2, adhesion molecules, acute phase proteins and immune receptors and its suppression by butyrate leads to decrease in levels of proinflammatory cytokines, myloperoxidase, cyclo-oxygenase-2 and adhesion molecules. Butyrate also exhibits anti-inflammatory effects by inhibition of interferon-c production and further signaling as well as upregulation of peroxisome proliferator-activated receptor-ϒ (PPARϒ) (Schwab et al., 2007) which is a transcription factor mediates anti-inflammatory effects (Dubuquoy et al., 2006). Acetate, propionate and butyrate have found to decrease the LPS-stimulated TNFα release from neutrophils, suppress NF-ϰB reporter activity as well as downregulation of immune-related gene expression like iNOS and cytokines such as IL-1a, IL-6 (Tedelind et al., 2007).

SCFAs affects the liver metabolism by reducing intrahepatocellular lipid levels, hepatic cholesterol synthesis, liver triglyceride and cholesterol content and hepatic glucose production (**Fig. 1.26**) (Byrne et al., 2015). Butyrate being the preferred energy source for colonocytes, hence majority get rapidly utilized at the epithelium while majority of propionate and acetate produced in the gut drains into the portal vein. Liver uptake of acetate was not significant, therefore hepatic changes associated with SCFA are largely because of propionate. Propionate acts as a gluconeogenic substrate and reported

to inhibit the utilisation of acetate for lipid and cholesterol synthesis. Additionally, SCFAs may have an indirect effect on hepatic metabolism by inducing L-cells to secrete gut hormone GLP-1 which results into free fatty acid accumulation in the liver and reduce hepatic steatosis. In enterocytes, butyrate has been found to activate the expression of genes involved in intestinal gluconeogenesis while propionate in itself acts as a substrate for intestinal gluconeogenesis. Acetate and propionate have also been reported to inhibit adipose tissue lipolysis via FFA2 resulting into decrease in plasma free fatty acid concentrations.

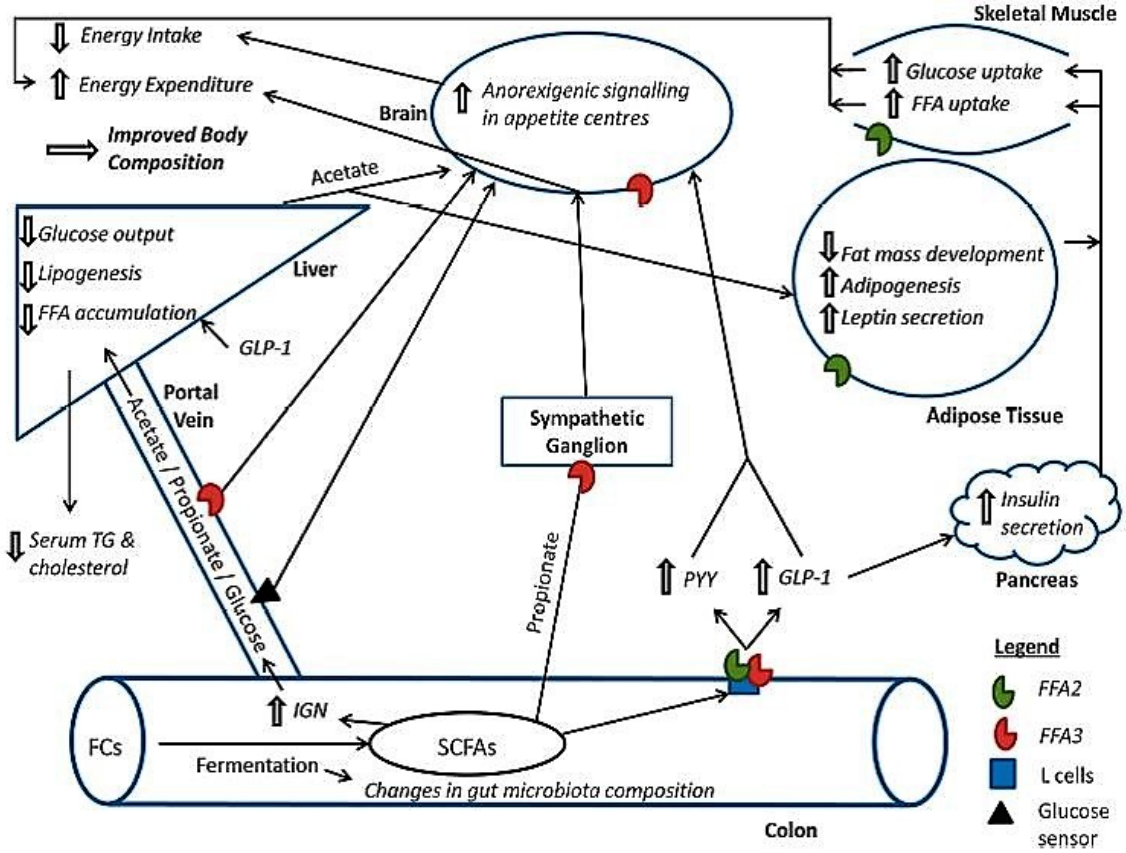

Fig. 1.26. Beneficial effects of colonic SCFA production. FCs, fermentable carbohydrates; FFA, free fatty acids; FFA2, free fatty acid receptor 2; FFA3, free fatty acid receptor 3; GLP-1, glucagon like peptide-1; IGN, intestinal gluconeogenesis; PYY, peptide YY (Byrne et al., 2015).

Gluconic acid can be considered as prebiotics, as it is remain unabsorbed in intestine and reaches to colon to be utilized by *Bifidobacteria* and *Lactobacilli* species to produce acetate and lactate which is further utilized by *M. elsdenii* to produce butyric acid production (Tsukahara et al., 2002). Piglets fed on gluconic acid increased the butyric acid content in faeces as well as proportion of *Bifidobacteria* and *M. elsdenii* in colon

with increase in SCFAs levels accounts for 26% of total SCFA and linear increase in acetic & butyric to propionic acid ratio with increasing gluconic acid intake (Tsukahara et al., 2002; Biagi et al., 2006). Experimental results have shown increase in SCFA levels on gluconic acid administration.

1.10.6. Synbiotics

An ideal synbiotic consist of single or multiple strain of probiotics and a mixture of prebiotics, they provide the substrate to the probiotic organism to be utilized by fermentation thereby enhancing their growth (Farnworth, 2001). This eventually accelerate the growth and survival of desired probiotics as well as of autochthonous strains of intestinal tract (Fotiadis et al., 2008). In the gastrointestinal tract by activating the metabolism of health-promoting bacteria synbiotics improve the colonisation and survival of live microbial dietary supplements, consequently helps in preventing various diseases like cancer and atopic dermatitis (Gibson et al.,1995) (**Fig. 1.27**).

1.11. Gut microbiota: Novel detoxification strategy for heavy metals

1.11.1. Bioremediation by bacteria in environment

A unique process to remove metals from contaminated sites involves the interaction of bacterial species with metals. Heavy metals cannot be degraded and metabolized, therefore microorganisms have evolved strategies to either transform them into less toxic forms or binds them intra or extracellularly or by actively transporting them out from the cell, thus inhibiting their harmful interactions with host cell (White and Gadd, 1998). These resistance mechanisms are mainly encoded within plasmid but in some cases their genes are inserted inside chromosome such as Cd efflux in *Bacillus*, mercury resistance in *Bacillus* and As efflux in *E. coli*.

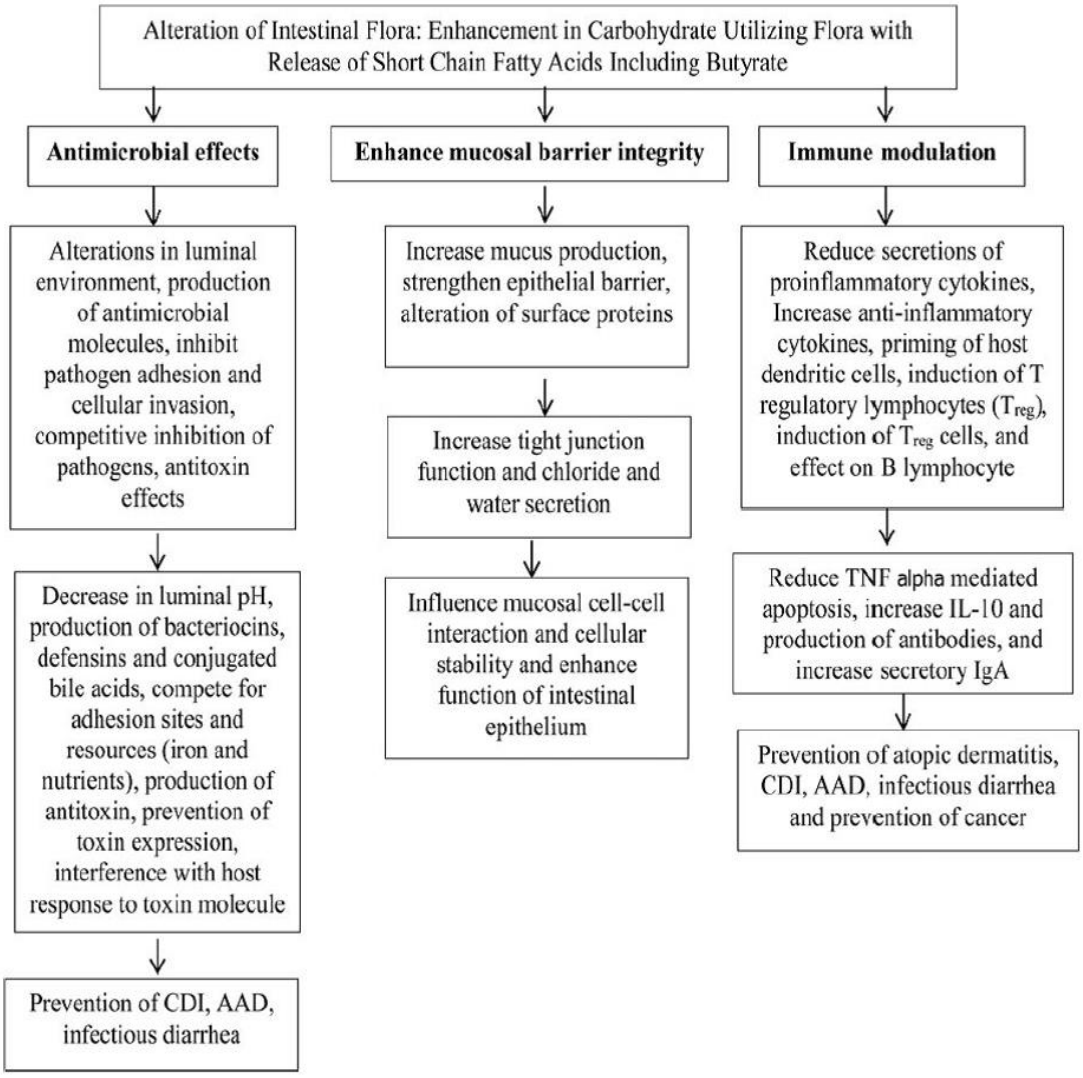

Fig.1.27. Biologic effects and mechanisms of action of prebiotics, probiotics, and synbiotics (Patel et al., 2015).

1.11.2. Human tolerance to metals

Human gastrointestinal (GI) microbiota consist of largest microbial community and accommodate two orders of more genes than found in human genome (Reid, 2010); thus, generates immense genetic and enzymatic diversity. Gut microbiota can regulate digestion by producing enzymes for metabolic breakdown of components of diet (Martens et al., 2011; Serino et al., 2012). Hence it can be predicted that microbes comes in contact with metals through contaminated food and water and protects the host by adsorption (Turroni et al., 2009). Upon higher consumption of metals, lower levels of microbiota were observed in clinical samples (Fierens et al., 2007; Zubero et al., 2010)

and only 40 to 60% of ingested metals are absorbed across the intestinal barrier into the body (Mahaffey et al., 2004).

1.11.3. Gut microbiota: Mechanisms Of Action

Metals can bind with bacterial cells through precipitation, complex formation with nitrogen and oxygen ligands and ion exchange reactions with peptidoglycan and teichoic acid (Vrieze et al., 2010). The *Firmicutes* represent the major phylum of microbes within colon and mainly composed of Gram-positive species such as *Bacillus*, *Clostridium*, and also *Lactobacillus*. They are considered to be good metal absorbers due to high content of peptidoglycan and teichoic acid in their cell walls while Gram-negative bacterial are poor metal absorbers due to lower amount of these components in cell membrane. Therefore, gastrointestinal tract comprises of large bacterial populations have potential to bind and sequester metals that enter into body through contaminated food and water.

Detoxification is the phenomena of removing harmful agents from body while detoxication is the mechanism of preventing entry of toxicants inside the body (Jin et al., 2009). Gut microbiota utilize detoxication mechanism by binding metals on their surface to prevent their entry inside the body. Chemolithotrophic bacteria that use inorganic sources of energy such as metals are used in environmental remediation but are not suitable for use in human application. Soil bacteria can also acts as opportunistic or obligate pathogens inside the human body and free forms of metals such as iron are also limiting for growth (Schryvers et al., 1999).

1.11.4. Sequestering heavy metals by probiotic bacteria

Fermented milk products or food supplements most often consist of probiotic microorganisms. *Lactobacilli* is widely used probiotic food supplement and had also been reported to function to reduce metal toxicity in humans (Sinha et al., 2011). It has resistance mechanisms involving efficient binding and sequestering of heavy metals to their cell surface and then further removal by subsequent defecation (**Fig. 1.28**).

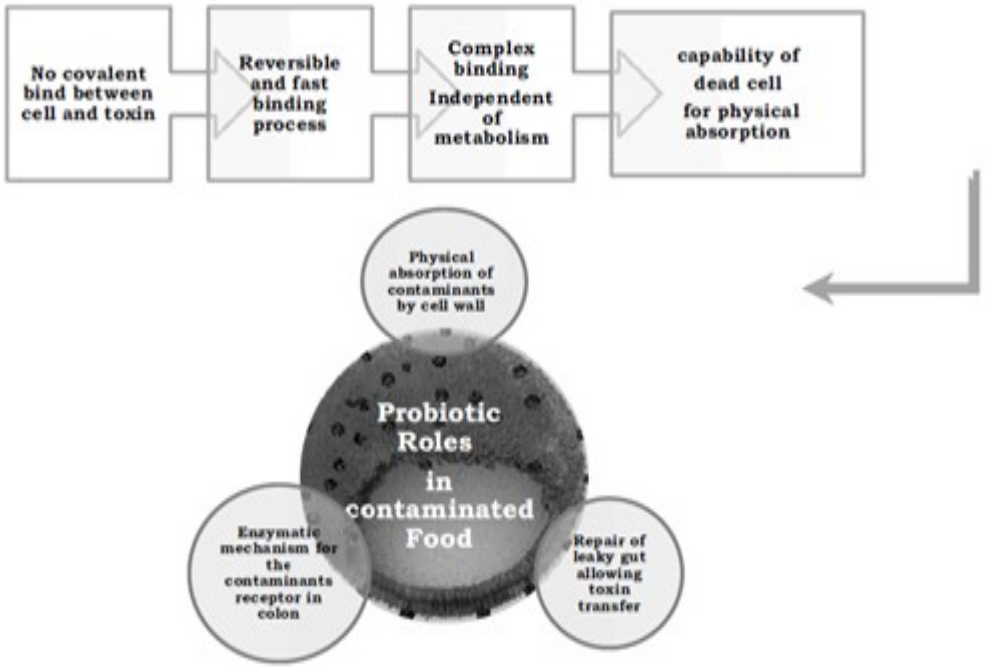

Fig. 1.28. Characteristics of Probiotics for reducing contaminant toxicity (Zoghi et al., 2014).

1.11.5. Probiotics against Arsenic toxicity

Adsorption of heavy metals on surface occurs due to attraction between the net negative bacterial cell and the positively charged metal, but unlike the other heavy metals As posseses an anionic negatively charged, thus cannot be adsorbed on bacterial cell surface. Several attempts have been made to overcome the charge issue of As by methylating bacterial surface to neutralize the negative charge on surface to enhance attraction between positively charged amino groups on the cell wall and negatively charged metals (Halttunen et al. 2007a). The most-abundant ionic groups that give net negative charge to *Lactobacillus* are carboxylic and phosphate groups while the peptidoglycan layer and surface proteins such as S-layer proteins contributes to net positive charge. Studies conducted on probiotic strains like *Lactobacillus acidophilus* and *Lactobacillus crispatus* DSM20584 showed the production of S-layer proteins which is responsible for its protective effect against As. *Lactobacilli* containing yogurt observed to remove As, thus found to be of immense use in countries like India and Bangladesh.

1.11.6. Probiotics against Lead and Cadmium toxicity.

Cationic metals such as Pb and Cd are easily adsorbed on negatively charged cell membranes of *Lactobacillus* and *Bifidobacterium* species (Halttunen et al., 2007b).

Maximum binding abilities of *Lactobacillus rhamnosus* LC-705 and *Propionibacterium freudenreichii* reported after 1 h of exposure of Pb and Cd varies with pH as in *B. subtilis* and *E. coli*. (Ibrahim et al., 2006). Exopolysaccharides produced by Gram-positive *B. subtilis*, *Lactobacillus rhamnosus* GG and some *Bifidobacterium longum* strains are known to contain anionic charged groups such as carboxyl, hydroxyl, and phosphate, which are capable of binding cationic metals such as Cd and Pb. In *Lactobacillus kefir* strains CIDCA 8348 and JCM 5818, the precipitation of metals were seen in the cell S-layer as well as changes in the S-layer protein secondary structure were observed using electron microscopy and Fourier transform infrared spectroscopy (FTIR) (Gerbino et al., 2011). Other strains such as *Enterococcus faecium* EF031 and probiotic *E. faecium* M74 could also sequester heavy metals. 11 high tolerant strains were isolated from the widespread study conducted on 53 different lactic acid bacteria with the ability to bind Cd and Pb.

1.11.7. Designer Probiotics

Probiotic can show variable valuable traits but their effects are highly specific to certain strains (Kumar et al., 2016; Singh et al., 2016). Genetic engineering had made it possible to design strains with multiple beneficial effects. Since the past two decades rigorous work had been done in the field of probiotic genetic engineering for applications in human health. Engineered probiotics can be used as drug delivery systems to target specifically the mucosal sites, thereby, avoiding the side effects associated with systemic administration of the therapeutic agents. Administration of recombinant L. lactis strain sAGX0085 and *E. coli* Nissle can target tumours, therefore, could be used in tumour diagnosis and therapy. Probiotics designed to produce antimicrobial peptides produced by commensal bacteria could be used against drug-resistant pathogens. To prevent obesity probiotics could be designed to produce certain therapeutic factors such as leptin that could increase the satiety as well as sensitivity to adipose mediated negative feedback signals. In mice fed on high fat diet, obesity was reduced by orally administering *E. coli* expressing acylphosphatidylethanolamines (NAPE) for 8 weeks in drinking water. Probiotics could also be exploited to offer protection against bacterial vaginosis, sexually transmitted diseases such as human papilloma virus and HIV. Several other beneficial effects of designer probiotics are summarized in **Table 1.7**.

Microbial species (origin)	Modification induced	Model	Inferences/remarks
Bacillus subtilis subtilis	Expression of *Helicobacter pylori* urease B protein on *B. subtilis* spore coat protein CotC as fusion reporter	Mice	Prolonged colonization of recombinant *B. subtilis* in GI tract of mice, significant (84%) reduction in *H. pylori* load in the stomach, indicating that orally administered urease B-producing spores being immunogenic could provide protection against *H. pylori* infection
Bacillus subtilis	*B. subtilis* expressing human IL-1receptor antagonist (IL-1RA)	Rat and rabbit	Expression of intact and active IL-10 IL-1RA protein,mucosal administration of recombinant *B. subtilis* released cytoplasmic recombinant protein withbiological activity *in vivo* that prevented endotoxin induced shock and death
***Escherichia coli* Nissle 1917**	Expression of CAI-1 in *E. coli* Nissle 1917, termed as (Nissle-cqsA)	Infant mice	Pretreatment for 8 h with Nissle-cqsA increased survival of mice against *Vibrio cholerae*. The strategy was suggested to be an inexpensive approach to use bioengineered commensal bacteria to prevent humans from invading bacterial pathogens
***Lactobacillus jejuni* 1153 (Human vaginal strain)**	Surface-anchored two domain CD4 (2D, CD4) linked to a peptidoglycan in the cell wall of *L. jejuni* 1153	*In vitro* modeling	Uniform expression of recombinant protein on *Lactobacillus* cell surface. The recombinant protein adopted a native functional conformation
***Lactobacillus jensenii* (Human vaginal strain)**	Secretion of 2D, CD4 proteins, anti-CD4 recognizing onformation dependent antibody, and bound HIV-1 gp120	HeLa cells	Inhibition of HIV-1 entry into target cells in a dosedependent manner.The study represents an important step toward development of engineered commensalbacteria within vaginal microbiota to inhibit heterosexual transmission of HIV
***Lactobacillus jensenii* (Human vaginal strain)**	Expression of anti-HIV chemokine RANTES and C1C5 RANTES	CD(+) T cells and macrophages	Inhibition of HIV in CD4+ cells and macrophages by both the variants
Lactobacillus jensenii	Expression of potent HIV-inhibitor cyanivirin-N (CV-N), inhibition of	Mice	Successful colonization of vaginal epithelium by the engineered strains administered to mice in

1153	CCR5-HIV (BaL), infectivity *in vitro* with 50% inhibitory concentration of 0.3 nM		estrus phase. The study was reported to be an expensive and durable approach to prevent HIV infection in women
***Lactobacillus jensenii* (Human vaginal strain)**	Expression of HIV1-entry inhibitor, modified cyanovirin-N (mCV-N) in *L. jensenii* (LB-mCV-N)	Rhesus macaque model SHIVSF 162P3	Detection of higher IL-1RA, lower load of Simian HIV,indicating the potential of engineered LB-mCV-N as a safer microbiocide
***Lactobacillus jensenii* 1153 (Human vaginal strain)**	Expression of HIV-entry inhibitor modified cyanovirin N (mCV-N)	Human cervical	Expression of mCV-N with anti-HIV activity conserved in epithet lial cell lines, expression of higher immunomodulatory potential by recombinant *L.jensenii* activity compared with control strains of *L. jensenii* 1153. Recombinant *L. jensii* 1153 wererecommended for clinical trials in humans
Lactococcus lactis	Expression of Der p2 in *L. lactis* in different cell components(extracellular, intracellular and cell wall)	Mouse model	Oral pretreatment of mice with live recombinant *L.lactis* prevented the development of allergen-induced airway inflammation by induction of specific mucosal immune tolerance
***L. lactis* (food grade strain)**	Expression of cytokine IL-27 in *L. lactis* (LL-IL-27)	Mouse model	LL-IL-27-mediated protection of mice from T-cell transfer-induced enterocolitis and death, mucosal delivery of LL-IL-27 was proposed to be an effective and safer therapy for IBD
***L. lactis* NZ9000 (food grade strain)**	Expression of IGF-1 (rtmIGF-1)	–	Functional and stable expression of rtmIGF-1 in *L. lactis*,recombinant *L. lactis* could act as host for producing rtmIGF-1, and delivery system for IGF-1
L. lactis	A thymidine-dependent recombinant strain expressing mature human IL-10	Monocyt ederived DCs	Reduced symptoms of Crohn's disease, ease ofbiological containment; anti-inflammatory effects mediated by programming DCs to induce suppression of Th cells
***L. lactis* sAGX0085**	Expression of human Trefoil factor (hTFF-1)	Hamster model	Improved repair of gut epithelial damage likely to occur during chemotherapy or radiotherapy-induced mucositis in cancer patients

Table 1.7. Applications of designer probiotics in humans (Kumar et al., 2016).

1.12. *Escherichia coli* Nissle as potential probiotic strain

Lactic acid bacteria are most conventionally used non-pathogenic microorganisms with health-promoting characteristics but other strains have also been arising such as *Escherichia coli* (*E. coli* Nissle 1917) and certain strains of yeast ('*Saccharomyces boulardii*') with human application. Alfred Nissle in 1917 detected and isolated *Ec*N and observed its antagonistic activity against few pathogenic enterobacteria. Characteristics that make the *Ec*N suitable candidate to be used as probiotics are as follows : it is a non-pathogenic, does not carry pathogenic adhesion factors, does not produce any enterotoxins or cytotoxins, it is not invasive, not uropathogenic, rapidly killed by non-specific defense factors of blood serum, carries genomic islands (GEIs) integrated into its chromosome coding for several fitness factors such as genes for production of microcins which prevent the growth of other enterobacteria (Sonnenborn and Schulze, 2009) (**Fig. 1. 29**).

The other unique feature of *Ec*N is a special lipopolysaccharide in its outer cell membrane responsible for its immunomodulating properties without showing immunotoxic effects (Behnsen et al., 2013) (**Fig. 1.30**). *Ec*N protects the epithelial barrier and cures the leaky gut phenomena by stimulation of epithelial defensin production and inducing 'sealing effect' on the tight junctions of the enterocytes (Zyrek et al., 2007). *Ec*N metabolism products such as acetic acid promotes colonic motility helpful against constipation. *Ec*N found to be therapeutically effective for ulcerative colitis, chronic constipation, and acute and protracted diarrhea and induce the development of the gut immune system in animal models and human newborns. *Ec*N had also been genetic engineered to enhance its utility in various therapeutic preparations (**Table 1.8**) (Ou et al., 2016). Bioengineered *Ec*N had been developed for diagnostics, drug development, solid tumors diagnosis in clinical studies, activation of multiple pro-drugs in situ for solid tumor treatments.

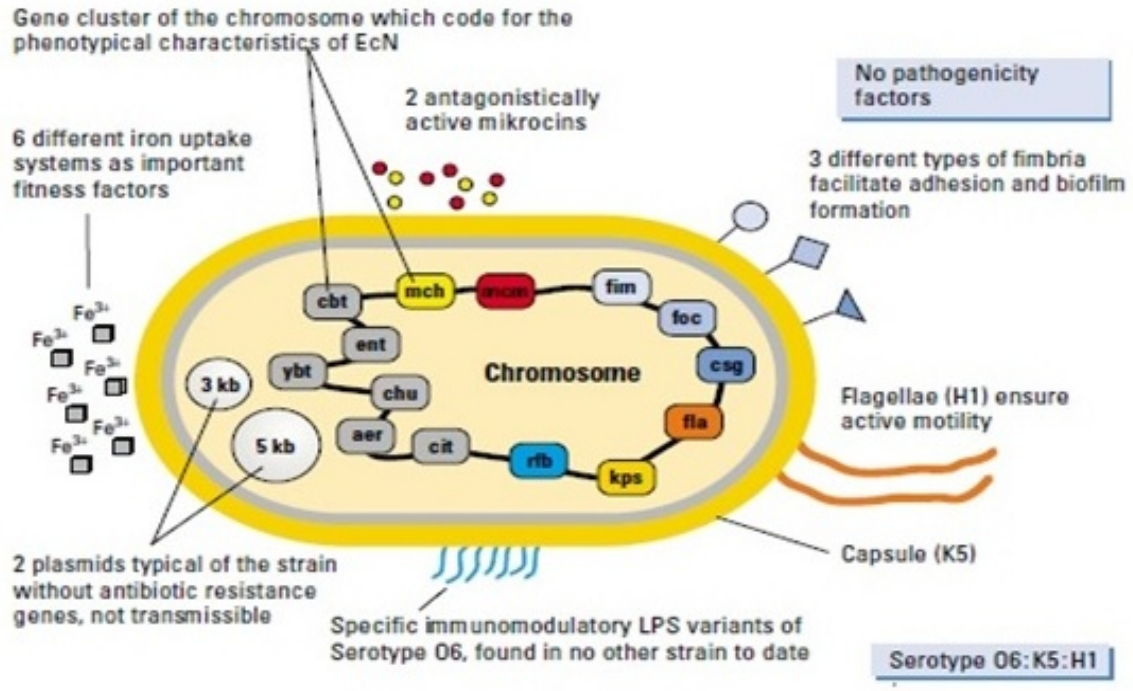

Fig. 1. 29. Phenotypical characteristics of *E. coli* Nissle 1917 and its gene loci on the bacterial chromosome (Schulze et al., 2006)

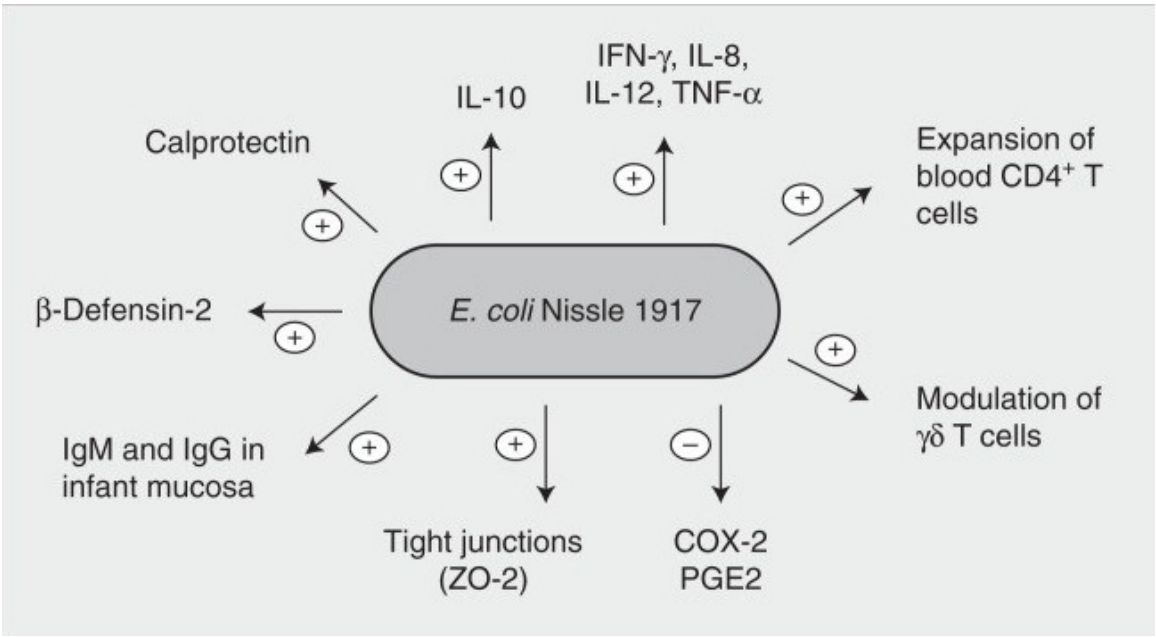

Fig. 1.30. Immune modulation by *E. coli* Nissle 1917 (Behnsen et al., 2013).

Application category	Disease/function
Preventive	Diarrhea in piglets and newborn calves
	V. cholera infection
	Yersiniosis
	AIDS
	Influenza
	Lyme disease
	Ocular surface diseases
	Trachoma
	Peanut allergy
Therapeutic	IBD
	CD
	B16 melanoma and 4T1 breast tumors
	Diabetes
	Peanut allergy
Carriers	Invade mammalian cells
	EcN OMV platform
Diagnostics	Liver metastasis
Health care	Human health

Table 1.8. Overview of genetically engineered *Ec*N 1917 for clinical exploration (Ou et al., 2016).

1.12.1. Genetically modified *E. coli* Nissle as therapy against heavy metal toxicity

vgb gene encoding for *Vitreoscilla* haemoglobin (VHb) enhanced the growth and survival of probiotic *E. coli* CFR16 in oxygen limiting conditions of gastrointestinal tract and protected against carbon tetrachloride induced oxidative damage due to its peroxidase activity (Kumar et al., 2014). Hence *vgb* gene was inserted in genome of *Ec*N to enhance its survival within gut and to locate its colonization *gfp* gene was also inserted (Singh et al. 2014; Singh et al. 2015).

Sodium citrate was found to be effective in reducing the Pb levels to normal or near normal in Pb poisioned humans (Kety et al., 1941). Most bacteria, including gut microbiota, do not secrete citric acid. However, high levels of citric acid secretion up to 9 mM was achieved in many bacteria by overexpression of an artificial citrate operon (*csYF-citC*) consisting of NADH insensitive *E. coli cs* Y146F mutant gene along with *S. typhimurium* Na^+ dependent citrate transporter (*citC*) gene (Adhikary et al., 2014; Wagh et al., 2014; Yadav et al., 2014).

Organic acids such as 2-Ketogluconic acid can chelate Cd as well as Pb (Lowry et al., 2010). *E. coli* does not encode PQQ biosynthesis genes in their genome but possess glucose dehydrogenase (GDH) apoprotein (Pedrosa et al., 2011). Incorporation of *pqq* gene clusters in *E. coli* resulted in PQQ biosynthesis and active GDH enzyme to produce gluconic acid which acts as prebiotic (Goosen et al., 1989; Khairnar et al., 2003; Yang et al., 2010). Gluconic acid gets converted into 2-ketogluconic acid in the periplasm by FAD dependent gluconate dehydrogenase (GADH) enzyme encoded by *gad* operon (Toyama et al., 2007 and Yum et al., 1997). *E. cypripedii* ATCC 29267 *gad* operon overexpressed in *E. coli* enabled secretion of high amount of 2-ketogluconic acid (Yum et al., 1997).

*Ec*N was also known to impede the reactive oxygen species and tightens the tight junction (Schumann et al., 2012; Zyrek et al., 2007)). The probiotic *E. coli* CFR 16 expressing *Vitreoscilla* hemoglobin (*vgb*) and *pqq* gene not only acted as an antioxidant but protected against CCl_4 and dimethyl hydrazine induced liver and colon damage and also prevented altered neurotransmitter status (Kumar et al., 2014; Pandey et al., 2014; 2015). Similarly, *Ec*N producing PQQ is found to be more effective than orally given PQQ against alcohol and rotenone induced oxidative stress (Singh et al., 2014; 2015).

arsM gene encoded by certain microorganisms which encodes for an enzyme As (+3) SAM methyltransferase which catalyzes the conversion of inorganic As into non toxic volatile trimethylarsine gas (Qin et al., 2006). This reaction occurred by adding methyl group from SAM (S-adenosyl methionine) converting SAM into SAH and subsequent

formation of methylated arsenicals, which are then excreted out from the microbes. Biotransformation of As into methylated arsenicals and eventually into trimethylarsine gas (non toxic) is considered as detoxification mechanism (Cullen and Bentley, 2005). Heterologous expression of *arsM* from *Rhodopseudomonas palustris* confers As(III) resistance to an arsenic-sensitive strain of *Escherichia coli* by methylating inorganic As to volatile TMA (III) gas (Qin *et al*., 2006). *arsM* has been cloned into *Pseudomonas putida KT2440* for bioremediation of environmental As. *P. putida* expressing *arsM* was more tolerant in As-contaminated soils with 5-fold more resistant to arsenite than the wild type strain (Chen *et al*., 2013). Studies have revealed that methylated species showed lower absorption in Caco-2 cell lines (Calatayud *et al*., 2010), indicating prevention of damage induced by As.

Therefore, genetically modified synbiotic *E. coli* Nissle (*Ec*N) producing PQQ as antioxidant, gluconic acid as prebiotic, citric acid as well as 2-ketogluconic acid as chelator and volatile trimethyarsine gas could be used as therapy against Cd, Pb and As insult. Currently available techniques for the detoxification of heavy metals primarily involves chelation therapy employing use of synthetic chelators which are toxic or bioremediation which is either ineffective or expensive. Hence, millions of microbes residing in gut can be used as reseviour for the constitutive production of remedial factors like antioxidants, chelators and other detoxifying enzymes to overcome heavy metal toxicity. Therefore, first chapter of this thesis is mainly focused over the development of genetically modified *Ec*N producing PQQ and citric acid against Cd induced toxicity. The second, third and fourth chapter deals with analysis of effectiveness of genetically modified *Ec*N producing PQQ and 2-ketogluconic acid against Cd induced toxicity, Pb induced immunotoxicity and Cd-Pb coexposure. The sixth chapter of present work is focused on the development of genetically modified *E. coli* Nissle producing PQQ and ArsM against As induced toxicity.

Thus, based on the above mentioned literature following are the objectives for the thesis work:

Objectives:

- Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing NADH insensitive citrate synthase-sodium dependent citrate transporter (*cs*YF-*cit*C) and pyrroloquinoline quinone (*pqq*) gene cluster in amelioration of cadmium induced toxicity in rats.
- Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing gluconate dehydrogenase (*gad*) and pyrroloquinoline quinone (*pqq*) gene cluster in amelioration of cadmium induced toxicity in rats.
- Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing gluconate dehydrogenase (*gad)* and pyrroloquinoline quinone (*pqq)* gene cluster in amelioration of LPS/GalN induced damage in lead treated rats.
- Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing gluconate dehydrogenase (*gad)* and pyrroloquinoline quinone (*pqq)* gene cluster against long term coexposure of cadmium and lead in rats.
- Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing As(III) S-adenosylmethionine (SAM) methlyltransferase (*arsM*) and pyrroloquinoline quinone (*pqq)* gene cluster in amelioration of arsenic induced toxicity in rats.