General Introduction: Impact of high altitude, hypoxia and probiotics on human health

1.1 Historical perspective on high altitude complications

Oxygen is essential to the aerobic organism. It acts as a final electron acceptor in an electron transport chain and produces energy in terms of ATP. The produced energy helps to maintain the metabolic activities of the cells and overall physiological system. Less supply of oxygen leads to energy deficiency and induce struggle responses. The less oxygen supply to a physiological system known as hypoxia or hypoxemia. Hypobaric hypoxia is an environmental condition at high altitude (HA). Physiological difficulties arise when an individual ascends from sea level to HA. The collective physiological struggle responses at HA are known as acute mountain sickness (AMS). Historical pieces of evidence described AMS in different literature during a journey at HA (Hackett and Roach 2001). Tseen Hanshoo described about "Great and Little Headache" during the trip in the Silk Route before 2000 years ago (Hackett and Rennie, 2002). Another literature in the same period, a Chinese official warned about jeopardizes during the journey from China to Afghanistan. He said to the travelers that they have to cross the “Little Headache Mountain” and the “Great Headache Mountain” where “men’s bodies became feverish; they lose color and were attacked with headache and vomiting”. Aristotle also (384-322 BC) described the travel experiences on Mount Olympus in Macedonia in his one of the writing as “Also, because the scarcity of the air which was there did not fill them with breath, they were not able to survive there unless they applied moist sponges to their noses”. These negative effects had also been recorded by Plutarch’s writings during the crossing of Alexander army into India in 326 BC (Hackett and Rennie, 2002; Paralikar and Paralikar, 2010).

1.2 Altitude air pressure and oxygen availability

The hypoxia at HA is environmental stress arises due to exponential fall of barometric pressure. For example, at sea level, the barometric pressure is about 14.68 psi that decreases to 13.12 psi when an individual ascends to 3281 ft of HA (Shaov and Wan 2005; Paula and Niebauer 2012; Hackett and Roach, 2001; Feriche *et al.*, 2014; Turner *et al.*, 2015).

The relation of air pressure with HA has been expressed in Box 1 by the following equation.

BOX 1. Partial pressure of air and altitude

$p = 101325\ (1 - 2.25577\ 10^{-5}\ h)^{5.25588}$ **(1)**

Where p = air pressure (Pa) h = altitude above sea level (m)

According to the Ideal Gas Law, the partial pressure of a gas in the air mixture is determined as below (Box 2).

BOX 2. Partial air component gas and altitude

$P = Fi \times B,$ **(2)**

Where Fi is the mole fraction of the particular gas component in the air mixture and B is the ambient barometric pressure. Thus, at sea level, the partial pressure of O_2, PO_2= 20.93% × 760 mmHg = 159 mmHg.

Hence, exponential fall of barometric pressure drops the partial pressure of O_2 with increase of HA although the availability of O_2 (20%) is same in the air. The exponential fall of barometric pressure at different HA and its relation to ambient oxygen pressure and its partial pressure has shown in **Table 1.1.**

Table 1.1. Relation of altitude with barometric pressure, ambient PO_2 and PIO_2

Altitude		Pressure			Ambient PO_2 (mm Hg)	PIO_2 (mm Hg)
m	ft	mm Hg	kPa	psi		
0	0	759.6	100	14.68	159.1	149.1
1000	3281	678.7	90	13.12	141.2	132.2
2000	6562	604.5	81	11.68	124.9	116.7
3000	9843	536.9	72	10.38	110.1	102.5
4000	13,123	475.4	63	9.19	96.9	89.7
5000	16,404	419.7	56	8.11	84.8	78.0
6000	19,685	369.4	49	7.14	79.1	67.5
7000	22,966	324.2	43	6.26	67.8	58.0
8000	26,247	283.7	38	5.48	59.3	49.5
8850	29,035	252.7	34	4.88	52.9	43.1

The partial pressure of oxygen (PO_2) in ambient air and inspired air (PIO_2) falls exponentially as a function of increasing altitude and falling barometric pressure.
Adapted from Hackett PH, Roach RC. High-altitude medicine. In: Auerbach PS, editor. Wilderness medicine. Philadelphia: Mosby; 2001. p. 2–43.

1.3 High altitude and pathophysiology

Mountains area is one-fifth of the earth's surface in which 38 million people live permanently at HA 2400 m and 100 million people travel to extreme HA in a year worldwide. Though these environments are harmful to their physiological system, many people like military personnel, sportspersons, pilgrimages and tourists face these extreme conditions for their duties and adventures. During ascend from sea level, the partial pressure of the oxygen dropped exponentially and human start to face the complications on and above 6,000 feet (ft) (Davis *et al.*, 2011). The very HA (12,000 ft to 18,000 ft) causes severe illnesses and above 18,000 ft regarded as the death zone. Although the relative percentage of oxygen (20%) is unchanged in ambient air at HA decreases of oxygen partial pressure, that means the number of O_2 molecules decreases per breath. This

sub-optimal O_2 bio-availability termed as “hypobaric hypoxia” initiates serious effects into the cell which spread throughout the system of an individual (Figure 1.1).

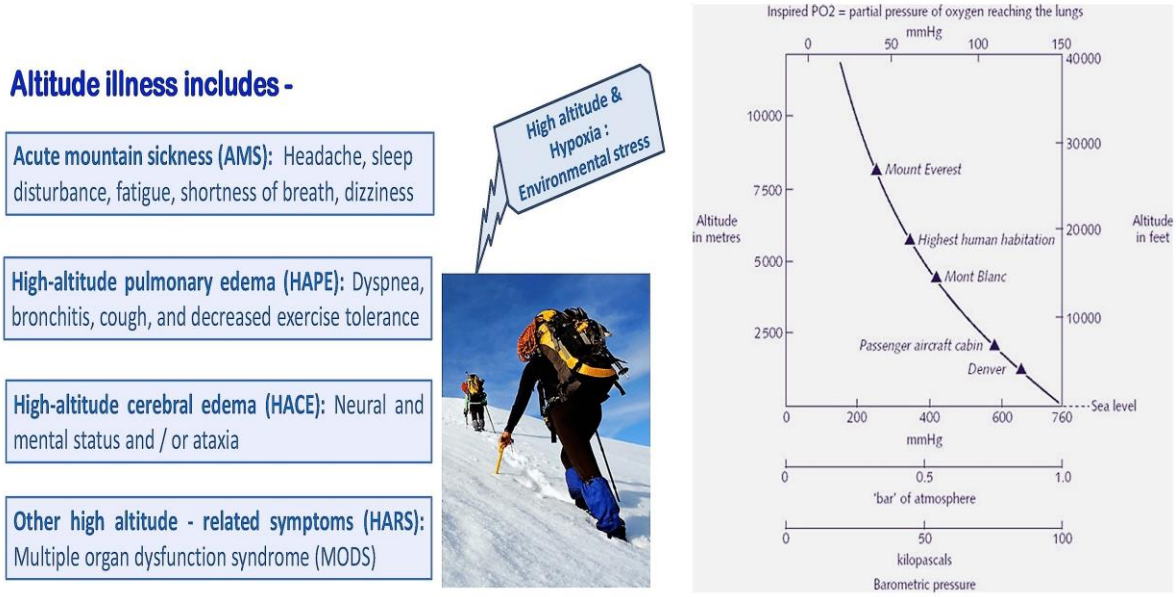

Figure 1.1. Hypoxia & pathophysiology

The molecular and physiological struggle responses for compensation of O_2 termed as ‘acclimatization.’ The categories of HA and its physiological effects that arise during acclimatization has been shown in Table 1.2. However, in biological system acclimatization results in adverse outcomes known as HA induced pathophysiology (Barry and Pollard, 2003; Gallagher and Hackett, 2004; Grocott *et al.*, 2007; Basnyat, 2014). The medical conditions due to physiological struggle response have been described in Box 3. Apart from such problems, an individual suffers from gastrointestinal complications due to changes in gut microflora.

In such situation individuals may suffer from several pathophysiological disorders including changes of the body weight, haematological parameters, gastrointestinal disorders collectively called as altitude-related sickness (ARS) (Shaov and Wan 2005; Paula and Niebauer 2012). It is well-known that the healthy human gastrointestinal (GI) tract is vast and diverse. The microbes consisting of nearly 100 trillion bacterial species

form a complex ecosystem. Generally, these bacteria are very active and play a significant role in human health and diseases (Hao and Lee 2004).

Table 1.2. Categories of high altitude and their physiologic effects

Moderate high altitude 1500 to 3500 m (4921–11,483 ft)	High-altitude illness common with abrupt ascent to above 2500 m (8202 ft) Decreased exercise performance and increased ventilation Only minor impairment in arterial oxygen saturation (SaO_2); arterial PO_2 (PaO_2), 55 to 75mm Hg
High altitude 3500 to 5500 m (11,483–18,045 ft)	Most common range for severe high-altitude illness Abrupt ascent may be dangerous; requires a period of acclimatization SaO_2, 75% to 85%; PaO_2, 40 to 60 mm Hg Extreme hypoxia may occur during sleep, exercise, and high-altitude illness
Extreme altitude 5500 to 8850 m (18,045–29,035 ft)	Progressive deterioration of physiologic function eventually outstrips acclimatization Above the highest permanent human habitation Abrupt ascent almost always precipitates severe high-altitude illness Severe hypoxia and hypocapnia; SaO_2, 58% to 75%; PaO_2, 28 to 40 mm Hg

[Data from Hackett PH, Roach RC. High-altitude medicine. In: Auerbach PS, editor. Wilderness medicine. Philadelphia: Mosby; 2001. p. 2–43.]

Box 3. Terminology of medical conditions at high altitude

High-altitude illness

High-altitude cerebral edema (HACE), High-altitude pulmonary edema (HAPE), Acute mountain sickness (AMS).

Altitude-related medical problems

High-altitude illness (AMS, HACE, HAPE), Peripheral edema, High-altitude headache, High-altitude pharyngitis and bronchitis, High-altitude retinopathy, Periodic breathing, Chronic mountain sickness, Decreased birth weight, Sub acute mountain sickness, Ultraviolet keratitis, Hypothermia and frostbite.

High altitude related gastrointestinal problems

Epigastric discomfort, epigastralgia, anorexia, high altitude flatus expulsion (HAFE), heart burn, dyspepsia, nausea, severe acidity, vomiting, constipation, infectious diarrhea, haematemesis, piles and peptic ulcers.

Altitude-exacerbated conditions

Several congenital and valvular heart diseases, Symptomatic coronary artery disease, Primary and secondary pulmonary hypertension, Obstructive pulmonary disease,poorly compensated congestive heart failure and chronic, Sickle cell disease and Urinary retention from benign prostatic hypertrophy, High-risk pregnancy, and Radial keratotomy.

Any responses of the host inside the state of an exacting range of exogenous (stress, temperature, drugs, cancer, etc.) and endogenous (inflammatory bowel diseases, peristalsis disorders) factors can change the GI microenvironment. The changes of gut microenvironment disturb microbial ecology, which ultimately as a whole affect the function of the physiological system (Rhee *et al.*, 2009). A disproportion of GI microbial population may induce GI problems (Ley *et al.*, 2012). At HA, gastrointestinal disorders are common for soldiers, veterans, athletes, and travelers, which is less explored.

1.4 Haematological responses

During acclimatization, less O_2 saturation in the blood changes the haematological parameters and activates O_2-sensitive transcription factor Hypoxia Inducible Factor 1 alpha (HIF-1α). The expression of the HIF-1α and its associated genes are undetectable in normoxic condition (Vogt *et al.,* 2001). Activation of HIF initially stimulates erythropoietin (EPO) production from kidney which triggers in red blood cell production from bone marrow. As a result, progressive increase in the total haemoglobin (Hb) mass driven by an expansion of red blood cells (RBC) are observed during acclimatization in hypoxia (Boutellier *et al.,* 1990; Stromme and Ingjer, 1994). Subsequently, an increase of RBC increased the peripheral resistances, blood viscosity, haematocrit (Hct) and Hb (Berglund, 1992). Moreover, 2,3-diphosphoglycerate (2,3 DPG) produced by RBC reduced in prolonged hypoxic condition. The lower level of 2,3 DPG in RBC facilitate the O_2 delivery to the tissue to counteract the tissue hypoxia. So, hematological parameters are important clinical markers for the analysis of hypoxic conditions of an individual at HA (Devasena, 2017). On the other hand, the hypoxia increases the count of White Blood Corpuscle (WBC) and recruit neutrophil for inducing inflammation in the neural and gastrointestinal system.

1.5 Kidney functions and hypoxia

The kidney is the main organ that helps to excrete a variety of metabolic waste products through urine. It regulates acid-base balance, electrolytes, extracellular fluids and blood pressure. In the kidney, afferent glomerular arterioles arise from the interlobular arteries and divide dichotomously to form glomerular capillaries. The glomerular capillaries merge together at the vascular pole that form the efferent arterioles. The efferent arterioles go into the peritubular capillary plexus, which surrounds renal tubules to provide oxygen and nutrients. Generally, the circulatory system supplies 20% of cardiac output to kidney.

However, oxygen tensions in renal tissues are comparatively low (Brezis *et al.,* 1984; Schurek *et al.,* 1990). In hypoxia, less oxygen supply somewhat sensitizes kidney for production of EPO for the adjustment of oxygen demand in renal tissue. In chronic hypoxia, acid-base balance, neuroendocrine reflexes, hemodynamics and fluid balance hindered the kidney functions that may lead to injury.

Diuresis and natriuresis occur with exposure to hypoxic challenges that arise within the first hour to 1-2 days. The diuresis is characterized by increased sodium, potassium, and bicarbonate excretion increased urinary pH and a fall in ammonium (Brenner *et al.,* 1982). Although total volume and solute output rise in parallel; however, urinary osmolality is not altered significantly (Risdon *et al.,* 1968). In general, adaptation to chronic hypoxia leads to negative fluid balance and maladaptation results in fluid retention in the interstitial space of a tissue.

1.6 Gut microbiome and kidney disease

In hypoxia, imbalance of bacterial population known as "dysbiosis". The dysbiotic microflora is a source of endotoxin that can initiate an inflammatory response in the host. Furthermore, protein fermentation by gut microbiota generates different toxic metabolites like p-cresol and indoxyl sulphate (Macfarlane *et al.,* 1986). Disintegration of gut barrier results in oxidative stress which allows the translocation of endotoxin and bacterial metabolites throughout the host's circulation and leads to uremic toxicity, inflammation, progression of chronic kidney disease (CKD). Several targeted interventions which aim to re-establish the intestinal symbiosis either by neutralizing bacterial endotoxins or adsorbing gut-derived uremic toxins (Vitetta *et al.,* 2012; Hide *et al.,* 1996). Translocation of endotoxin from the gut into the systemic circulation indirectly induce the inflammation of kidney. Targeting the gut microbiome to restore symbiosis can be an alternative strategy in reducing inflammation and uremic toxins in systemic circulation.

1.7 GI complications at HA and indigenous gut microflora

Trillions of microbes on the mucosal surface of human gut break down the indigestible foods and thereby provide us energy from the undigested food. Intestinal flora can also perform several beneficial functions like maturation of intestinal physiology, immune system, and inhibit the colonization of harmful bacteria, etc. The atmospheric air pressure is an effective modulator and can breakdown the overall intestinal microbial ecology has been studied (Maity *et al.*, 2009; Maity *et al.*, 2013). HA exposure supply less oxygen in mucosal layer of the gut. The change of oxygen concentration is sensed by indigenous microflora and alter their metabolic process. Besides, metabolic network and correlation among the microbes get hampered in altered situation. Simultaneously, activation of vagal nerve and lower in gut motility provide the opportunity to the ingenuous microbes to spend more time in the lumen. Higher retention time of microbes in lumen and enlargement of anaerobic microbes produce more acids and gas. Production of gas also known as high altitude flatus formation create a distension and initiate a pain sensation that is experienced by sojourners. Generally, visitor to HA face such gastric complications above 1,493 m that may severe above 5,486-6,096 m (Anand *et al.*, 2006; Adak and Khan, 2019). Gastrointestinal disorders that include indigestion, acid formation, flatulence, vomiting, anorexia, diarrhoea, etc. (Clark *et al.*,2007).

1.8 Microbial distribution throughout GI tract

The human gastrointestinal microflora forms a complex ecosystem consists of approximately 300-500 bacterial species. The number of bacteria in gut is about 10 times higher than that of eukaryotic cells in the human body. In the healthy host, gut bacteria start to colonize in gut soon after birth thereafter a stable ecology established after 3 years of age.

1.8.1. Microflora in upper gastrointestinal tract

The GI tract starts from oral cavity to anus. The number of taxa and diversity in oral cavity varied according to sampling location. Firmicutes (*Streptococcus, Veillonellaceae, Granulicatella*), Proteobacteria (*Neisseria, Haemophilus*), Actinobacteria (*Corynebacterium, Rothia, Actinomyces*), Bacteroidetes (*Prevotella, Capnocytophaga, Porphymonas*) and Fusobacteria seem to dominate oral microbiota in both adults and children (Aas *et al.,* 2005; Zaura *et al.,* 2009; Ling *et al.,*2010).

1.8.2. Microbiota in stomach

Previously, stomach was thought to be sterile due to short retention time and higher antimicrobial effects of gastric acid (Pei *et al.,* 2004). In 1984 Marshall and Warren discovered colonisation of *Helicobacter pylori* on the gastric mucosa. It may present as a natural habitant of gastric mucosa in healthy individual (Bik *et al.,* 2006). Now, *H. pylori* has been explored as a the aetiology of gastritis.

1.8.3. Microbiota in small intestine (SI)

The small intestine, consists of duodenum, jejunum and ileum. The main functions of these is segment are digestion and absorption of nutrients. In the duodenum part of SI, bile, pancreatic secretions, low pH and lower transit time, impedes bacterial density. In contrast, distal part of intestine favour the bacterial colonization of bacteria in term of diversity and population density. Microbial population density of duodenum is 10^1-10^3 CFU/ml, whereas jejunum and ileum conquered by approximately 10^2-10^6 CFU/ml. Gram-positive aerobes or facultative anaerobes reflecting the major bacterial flora whereas coliforms rarely exceed 10^3 colony-forming units per gram (CFU/g) (Figure 1.2). The ileum proximate to large intestine consist of major six phyla *viz.* Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Verrucomicrobia and Actinobacteria. The jejunum microbiota, dominated by *Streptococcus* is

found to be significantly different to the ecosystem observed from distal ileum upto rectum. This portion is also dominated by *Bacteroidetes* and *Clostridium* cluster XIV and IV (Collins *et al.*, 1994, Wang *et al.*, 2005). Analysis of SI luminal content revealed *Lactobacilli, Streptococci, Enterococci* and γ-Proteobacteria are prevalent in the jejunum of human (Kerr *et al.*, 2015). The bacterial diversity in jejunum and proximal of ileum mainly dominated by aerobic species whereas anaerobes are predominant in distal part of ileum and colon. Bacterial colony counts may be as high as 10^9-10^{12} CFU/mL **(Figure 1.2)** in the terminal ileum immediately proximal to the ileocecal valve, with a predominance of gram-negative organisms and anaerobes.

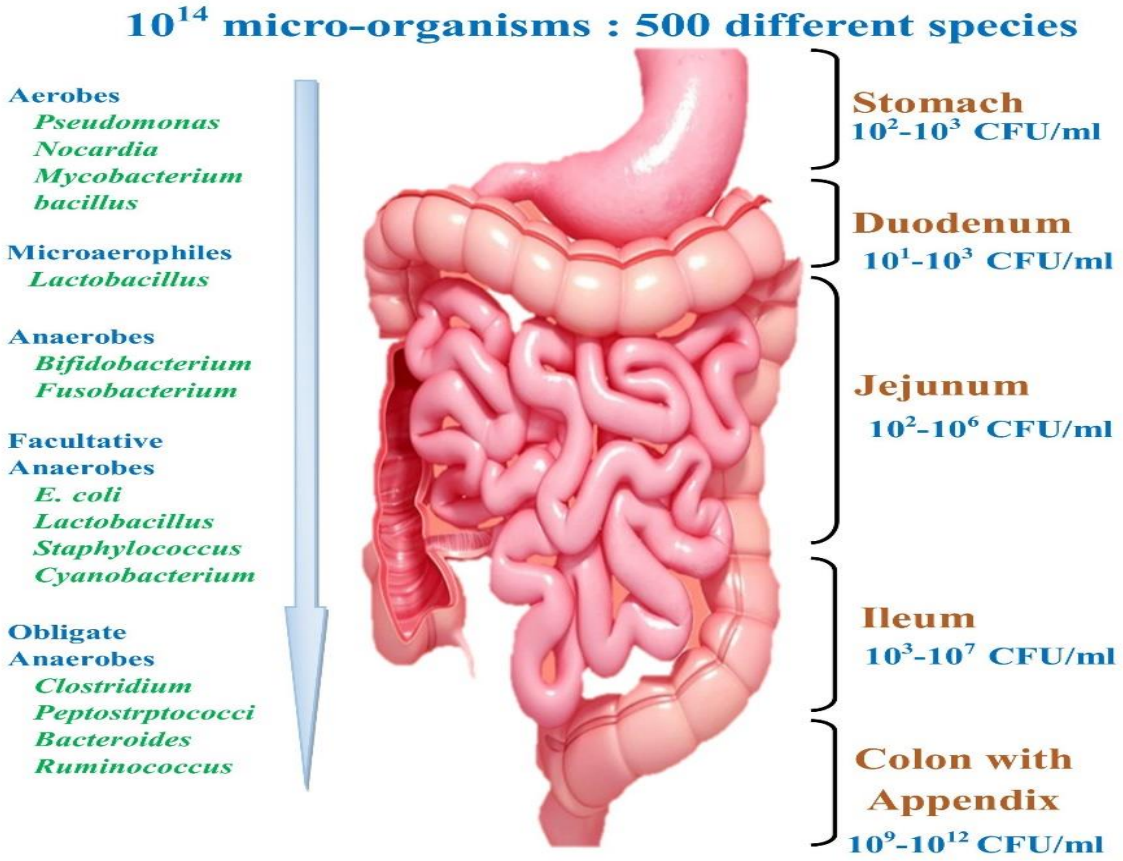

Figure 1.2. Distribution of different bacterial population along with the GI tract and its population density bacteria at various points in the adult human intestine and arrow indicated increases in anaerobes concentration rather to aerobes toward the distal part of GI tract.

1.8.4. The large intestinal microbiota

Large intestine of human function helps in absorption water and it is the major site for microbial fermentation. The slower transit rate and rich nutritional environment enhances the bacterial density at about 10^{12} cells/g in the luminal content (Figure 1.2) (Guarner and Malagelada, 2003). Firmicutes and Bacteroidetes phyla of bacteria predominate in the large intestine. Firmicutes mainly consists of *Clostridium* cluster XIV and *Bacteroides* (Eckburg *et al.*, 2005, Walker *et al.*, 2011). In healthy humans Proteobacteria have a minor contribution to large intestinal ecosystem. In colon anaerobic bacteria was outnumbered the aerobic bacteria by a factor of 100-1000:1.

1.9 Functional aspects of gut flora

The gut microbiota mainly performs three essential functions *viz.* protective, structural, and metabolic. The metabolic function of gut microbiota helps to produce vitamins, amino acids, and bile acid biotransformation etc. Bile acid biotransformation and cholesterol metabolism are mediated by microbial associated enzymes, have implications for cholesterol and glucose metabolism (Lefebvre *et al.*, 2009). Microbiome provides biochemical pathways for the fermentation of non-digestible fibre and endogenous mucus. Digestion of non-digestible fibre produce short chain fatty acids (SCFAs) and gases (Wong *et al.*, 2006). The major SCFAs are acetate, butyrate, and propionate mainly absorbed by colonocytes enhancing the absorption of salts and water. Butyrate is a preferred energy source for epithelial cells, and is almost entirely utilized by the colonic epithelium (Hamer *et al.*, 2008; Layden *et al.*, 2013; Pedersen, 2015). Butyrate is also involved in the modulation of immune system and epigenetic effect in regulation and expression of genes (Yadav *et al.*, 2013). Acetate transported to liver and it is used as a substrate for cholesterol biosynthesis. In contrast, propionate supplementation in the diet was shown to reduce cholesterol levels *in vivo*. Clinical trials yet to be confirmed in case

of these observations. Apart from SCFAs, other microbial metabolites including lactate, ethanol, succinate, formate, valerate, caproate, isobutyrate, 2-methyl-butyrate, and isovalerate also have an important role in our physiological system.

Gut microbiota perform protective function in which it prevents the colonization of pathogens. Experimental evidences on germ free mice showed the underdeveloped lymphatic systems, with fewer Peyer's patches and lymphoid follicles. Moreover, fewer intestinal dendritic cells and less B cell development was observed in conventional mice support the role of indigenous microflora in development of immune functions (Broderick, 2015). The component of indigenous microflora like capsular polysaccharide A of *Bacteroides fragilis* help to development of regulatory T cells that directly regulate the inflammation of the gut (Hooper and Macpherson, 2010; Artis and Spits, 2015; Round and Mazmanian, 2010). Commensal organisms prevent pathogens by competing for attachment sites and nutrients. The commensals also activate the innate immune system in production of antimicrobial peptides including β-defensins and cathelicidin that indirectly control the pathogens (Kleessen and Blaut, 2005). Host innate immune system can also recognize the pathogen associated molecular patterns like lipopolysaccharides, peptidoglycans, bacterial CpG-DNA motifs and simultaneously trigger the adaptive immune response (Agren *et al.*, 2006). The intestinal mucosa a part of the innate immune system activated by getting signal through the pattern recognition receptors, such as toll-like receptors. The cumulative effects lead to the production and release of protective peptides, cytokines, chemokines, and enhance phagocytes. Therefore, commensal bacteria of the gastrointestinal tract play active roles in the development and homeostasis of the immune responses (Hooper *et al.*, 2002).

1.10 Redox biology of the gastrointestinal epithelia

The antioxidant machinery of intestinal epithelia including enzymatic and non-enzymatic antioxidant system regulate the adverse effects of oxidant. A gradient oxygen saturation profile exists along with anatomy of the intestine as well as from mouth to anal. The intestinal mucosa layer is influenced by the in blood flow and O_2 supply (Colgan and Taylor, 2010). The enzymatic systems in mitochondrial, cytosolic and extra-cellular part help to convert reactive superoxide to less powerful hydrogen peroxide. After the actions of SOD, produced H_2O_2 is converted into water catalyzed by catalase (CAT). Like SOD and CAT activities, reduced glutathione (GSH) and oxidized glutathione (GSSG) pool both in small and intestinal epithelia is very crucial to maintain the intracellular redox pool. In hypoxic stress, superoxide dismutase (SOD) reduced both in small and large intestinal epithelia during acclimatization at HA (Robin, 1982; Loiacono and Shapiro, 2010). Cells are continuously threatened by reactive oxygen or nitrogen species (ROS/RNS), which are generated during oxygen mediated stress. The reactive metabolites can hamper the inter-convertible reduced and oxidized forms glutathione, i.e., GSH/GSSG, Cys/CySS, or reduced and oxidized thioredoxin Trx/TrxSS of cellular redox systems in epithelial cells. The redox potential (Eh) of intracellular GSH/GSSG varies between −260mV to −240mV at proliferation, −220mV to −200mV at differentiation, and −170mV to −150mV at apoptosis (Jones, 2002). Besides, redox gradient of GIT favours the establishment of a defined ecological niche of aerobes, facultative anaerobes and anaerobes to prevent colonization of pathogens, supports intestinal nutrition and regulates the mucosal immune system. But the redox environment of the GI epithelia is primarily depending upon by the action of different enzymes and more importantly on NADPH oxidase 2 concentration. In physiological hypoxia, the imbalance of enzymatic or non-enzymatic redox may cause of negative association of anaerobes with GI

epithelia and modulate the mucosal metabolic and immune system. Loss of epithelial barrier function and rapid influx of unrestricted luminal antigens underlies the pathology of gut during hypobaric hypoxic stress (Tian *et al.*,2007, Fernandez-Blanco *et al.*, 2015).

1.11 Probiotics

Probiotics are known as live microbial food ingredients that have a beneficial effect on human health (Salminen *et al.*, 1998). The idea of probiotics developed at the beginning of 20th century. Elie Metchnikoff (1905), a Russian scientist proposed that lactic acid bacteria (LAB) has health beneficial role by "intestinal autointoxication" and the subsequent aging could be suppressed by modifying the gut microbiota. He believed that when consumed, probiotics can positively influence the microflora of the colon, decreasing toxic microbial activities. During the First World War, *E. coli* Nissle 1917 strain was isolated by a physician named Alfred Nissle, from a healthy combatant of army suffering from infectious diarrhoea and has since been used as a commercial probiotic (Nissle., 1993). In 1953 the term "probiotic" defined to denote all organic and inorganic food complexes for the improvement of health as a supplement (Kollath, 1953). Lilly and Stillwell (1965) proposed probiotics are to be "microorganisms promoting the growth of other microorganisms". Later, the concept of probiotics considered as 'live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition'. This means ingestion of live and high numbers of organism showed health beneficial effects. Presently, definition of probiotic was given by World Health Organization (WHO), according to which probiotics are "live microorganisms which, when administered in adequate amounts confer health benefit to the host" (WHO., 2001). Till date Lactic Acid Bacteria (LAB), such as *Lactobacillus* sp., *Enterococcus* sp. and *Bifidobacterium* sp. were found to be more effective to confer health beneficial effects. Among lactic acid bacteria, *Lactobacillus* has concerned a lot of attention

for their potential probiotic effects in human health. Usually *Lactobacillus* comprised of 100 known species, particularly those belong to beneficial and non-pathogenic genera.

1.12 Probiotic and health benefit

Now a day, health awareness created demand and expanding market of probiotic due to their beneficial effects. For example, it can antagonize pathogenic bacteria by reducing luminal pH, adjusting the balance of intestinal flora, reducing of blood cholesterol, inhibiting and reducing the risk of tumors and cancer, stimulating the immune system, stimulation of vitamin C production, and enhancement of digestion (Gilliland, 1999; Leroy and Vuyst, 2004). Probiotic bacteria decrease the luminal pH, as has been demonstrated in patients with ulcerative colitis (UC) following ingestion of the commercial probiotic preparation VSL#3 (Venturi *et al.*,1998). In a fatal mouse Shiga toxin-producing *E. coli* O157:H7 infection model, the probiotic *B. breve* produced a high concentration of acetic acid, consequently lowering the luminal pH. This pH reduction was associated with increased animal survival (Asahara *et al.,* 2004). Production of antimicrobial compounds, termed as bacteriocins, produced by probiotic bacteria is also likely to contribute to their beneficial activity (Savignac *et al.,* 2016). The inhibitory activity of these bacteriocins varies. Some are active against a much wider range of Gram-positive and Gram-negative bacteria as well as yeasts and molds (Nemcova *et al.,* 1997). For example, the probiotic *L. salivarius* UCC118 produces a peptide against pathogens such as *Bacillus, Staphylococcus*, *Enterococcus, Listeria,* and *Salmonella* species (Flynn *et al.,* 2002). Another probiotics of *Lactobacillus* species active against *Helicobacter pylori* infection of gastric mucosa due to release of bacteriocins and thereby protects the adherence of this pathogen on epithelial cells. Probiotics can reduce the epithelial injury during exposure to *E. coli* O157:H7 and *E. coli* O127:H6. The pre-treatment of intestinal (T84) cells with lactic acid-producing bacteria was reported to

reduce the ability of pathogenic *E. coli* to inject its virulence factors into the cells or breaching of the intracellular tight junctions (Sherman *et al.*, 2005; Cho *et al.*, 2015). Besides, probiotics were also reported to protect diarrhea caused by rotavirus and lactose intolerance, inflammatory bowel diseases, constipation, and controlling of blood pressure, immune health, and food allergies (Figure 1.3).

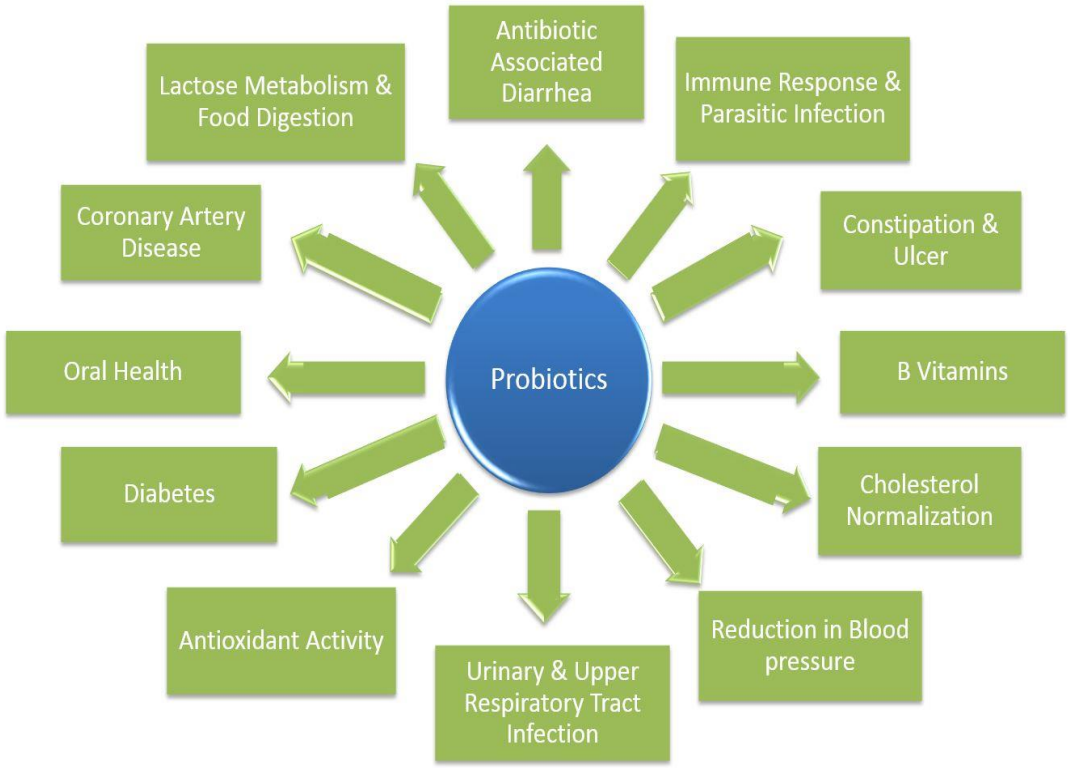

Figure 1.3. Role of probiotics against systemic diseases.

1.13 Global scenario of commercial probiotics

The knowledge in probiotic continues to expand on daily basis. The enlightened attention in 'these magic bugs' has grown significantly during the last few years because of their protective role in the gut to retain the gut healthy and fit. Probiotics have great acceptance amongst individuals searching for alternative and "natural" means to promote intestinal health. The progress of probiotic products in the developed world has been pretty amazing.

According to a new market study report, 'Probiotics Market' (2009-2014), published by Markets and Markets, the global probiotics market is estimated to be worth US $ 31.1 billion by 2015 as compared to Europe and Asia accounting nearly 42% and 30% of the total revenues respectively. In global scenario, Europe contributes major business on probiotic products with an estimated $13.5 billion as recorded in 2014. Whereas, Asia is estimated to be second leading segment with an estimated CAGR value of about 11.2% which has reached to $9.0 billion by the year of 2014. A Frost and Sullivan study estimates that the probiotic ingredient market in US was $450 million in the year of 2010. Probiotic products especially Japanese functional foods share business of $3.23 billion globally. Japan spent $126 per person every year on functional foods and it is higher than other countries including US ($67.9), Europe ($51.2) and Asia ($3.20). Some of the probiotic products available worldwide which is very notable as Yakult in Japan, Life way Kefir in Europe, Activia creamy yoghurt in USA, VSL#3 in India etc. (Indian consumer survey, 2010).

Table 1.3. List of selected commercial probiotics & its specification

Probiotic	Manufacturer	CFU count (bn or CFU/capsule)	No. of strains	Name of strains	Recommended dose	Purpose claimed
Becelac PB	Dr. Reddy's Laboratories Ltd. India	85	4	*S. faecalis, C. butyricum, B. mesentericus, L. sporogenes*	Not mentioned	Digestive system and healthy immune function
VSL#3	Sigma Tau Pharmaceuticals	112.5	8	*Bifidobacterium breve, B longum, B infantis, Lactobacillus acidophilus, L plantarum, L paracasei, L bulgaricus, S thermophilus*	1 capsule daily	Treat and prevent diarrhea, including traveler's diarrhea and rotaviral diarrhea in children and healthy immune function
Nature,s Bunty 10	Nature's Bounty, INC., USA	2×10^{10}CFU/ capsule	10	*L. plantarum* 299v (Lp299v®), *L. bulgaricus* Lb-87, *L. paracasei*DSM 13434, *L. plantarum*DSM 15312, *L. salivarius* Ls-33, *L. brevis* Lbr-35, *L. acidophilus* La-14, *B. lactis* Bl-04, *L. paracasei* Lpc-37, L. casei Lc-11	2 capsules daily	Digestive system and healthy immune function
TruBiotics	Bayer	1.5	2	*L acidophilus, B animalis*	2 capsules daily	Treat diarrhea caused by antibiotics
Yogut	Swiss Garniar Life Sciences	5.0	5	*L acidophilus, L rhamnosus, B bifidus, B longum,B infantis*	Not mentioned	Digestive system and healthy immune function
Propolis Plus	Essential Formulas	21.6	12	*B breve, B infantis, B longum, Enterococcus faecalis, L acidophilus, L brevis, L bulgaricus, L casei, L fermentum, L helveticus, L plantarum,*	2 capsules daily for 21 days	People with chronic infection (bacterial, fungal and viral infection), compromised immunity, helpful for sore throat, conjunctivitis, sinus congestion,

				Streptococcus thermophilus		colds, influenza, bronchitis, disorders of the ears, periodontal disease, pneumonia, bile infections, gastric ulcer, infection of the urinary tract, intestinal infections and eczema.
Garden of life	Dr. formulated	90	13	*L. acidophilus, L. plantarum, L. rhamnosus, L. casei, Lactobacillus paracasei, L. acidophilus, L. salivarius, L. acidophilus NCFM, L. gasseri, L. brevis, L. bulgaricus,B. bifidum, B. lactis, B. breve, B.infantis*	1 capsule	Not mentioned
Pre Pro HS	Fourrts (India) Laboratories Pvt. Ltd.,India	33	10	*L. acidophilus, L.rhamnosus, L. actobacillus casei, L. plantarum, L. bulgarious, B. longum, B. infantis, B. breve, S. thermophilus*	Not mentioned	Treatment of diarrhea
Renadyl	Kibow Biotech Inc. USA	4.5×10^{10} CFU/ capsule	3	*S. thermophilus*(KB27), *L. acidiphilus*(KB27), *B. longum*(KB31)	1capsule per day	Establishes Kidney Balance Naturally
BIFILAC	Tablets (India) Ltd. India	8.3	4	*S. faecalis, C. butyricum, B. mesentericus, L. sporogenes*	1-2 tablets thrice per day	Treat and prevent diarrhea, including traveler's diarrhea and rotaviral diarrhea in children.

Research on microbiota has encouraged a dramatic increase in the area of scientific, industrial, and public sector as plausible agents for management of health and diseases. Generally, imbalance of gut microbiota in which abundance of bad microbes overweigh the beneficial ones lead to dysbiosis. Probiotic therapy helps to maintain the normal gut microbiota and enhancement of health beneficial effects has led to promising results in a large number of well-designed (clinical) studies. Several experimental works have been performed on intestinal barrier dysfunction and microbial modification in hypobaric hypoxia. But a little work has been done in relation to GI homeostasis and other health markers at hypobaric hypoxic conditions in relation to probiotics. So, the present study emphasized on possible alternative way-out through the use of commercial probiotic to maintain the normal ecosystem of our intestine and other notable health parameters at an altered atmospheric pressure.

HA hypoxia altered the gut microbiota. The alteration of this microbiota creates a pressure to the gut ecological niche results in pathophysiological consequence. Although experimental work addressed the changes of microbiota in relation to high altitude conditions however few experimental works has been performed on the therapeutic attribute to resettle the gut microenvironment and microbiota for maintenance of overall health of an individual. The present study was carried to evaluate the beneficial effect of certain commercially available probiotics on gut microflora as well as health benefit on experimental animals at hypobaric atmosphere.

Thus, on the basis of foregoing research and earlier reports, the following objectives were undertaken in this research programme:

1. Monitoring of the alteration of heamatological, biochemical, oxidative stress indicators, and changes in intestinal microflora at different altitude.
2. Analysis of effectiveness of the potential probiotic supplementation for health improvement at hypobaric condition.
3. Comparative study of intestinal enzymes before and after probiotic supplementation of experimental animals at hypobaric condition.

Ultimate Goal: Ultimate Goal of this study is to evaluate the effect of commercially available probiotics on the management of hypobaric stress and overall improvement of health profiles at hypobaric environment.

The present experimental work was performed on male albino rat as an experimental model animal and they were grouped into hypobaric hypoxic (HA) and normobaric (C) as control. HA complications generally occurred above1,493 m and extreme altitudes above 5,486–6,096 m and people suffer from AMS (Anand *et al.*, 2006; Adak *et al.*,2013). So our study was done on three different barometric pressures (Table 1.4) for the period of maximum 28 days. Healthy, adult, albino Wistar strain rats weighing 110±12 g will be used for the study. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were kept in cage in a temperature-controlled room (24±2 ºC) with 12-12 h dark-light cycle at 60±10% RH. The principle of laboratory animal care of National Institute of Health USA guideline was monitored during the experiment. Exposure will be carried out in a decompression chamber (Instrumentation India, India) maintained at 10±2.5 ºC temperature and 65±10 per cent relative humidity. During stress, different physiological parameters and food intake capacity were monitored and compared with NB group. The outline of the experiment and overall parameters that have been studied was presented schematically in Figure 1.3. To analyze the health profile during the experimental period blood sample was collected and physiological parameter was also recorded. The luminal content of defined small and large intestinal part was collected and diversity of different indicator microbial population and activities of some microbial originated enzymes were enumerated (Table 1.5). The epithelial layer was subjected to evaluate the oxidative stress parameters and inflammatory mediators (Table 1.5). In relation to the oxidative damage and inflammation, GI morphology, kidney and liver was examined by histological staining and electron microscopic methods.

Animal experiments were permitted by Ministry of Environment, Government of India under registration no. 1905/PO/Re/S/2016/CPCSEA issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethic Committee (IAEC) of Department of Nutrition, Raja N. L. Khan Women's College, Midnapore, West Bengal, India. All animals used in these experiments were free from any type of infection.

Table 1.4. Exposure to different barometric pressure

Moderate altitude exposure	High altitude exposure	Extreme high exposure
6000 feet/1829 m	12000 feet/3657 m	18000 feet/5486 m
Barometric pressure: 11.8 psi (Pounds Per Square Inch)	Barometric pressure: 9.3 psi (Pounds Per Square Inch)	Barometric pressure: 7.3 psi (Pounds Per Square Inch)

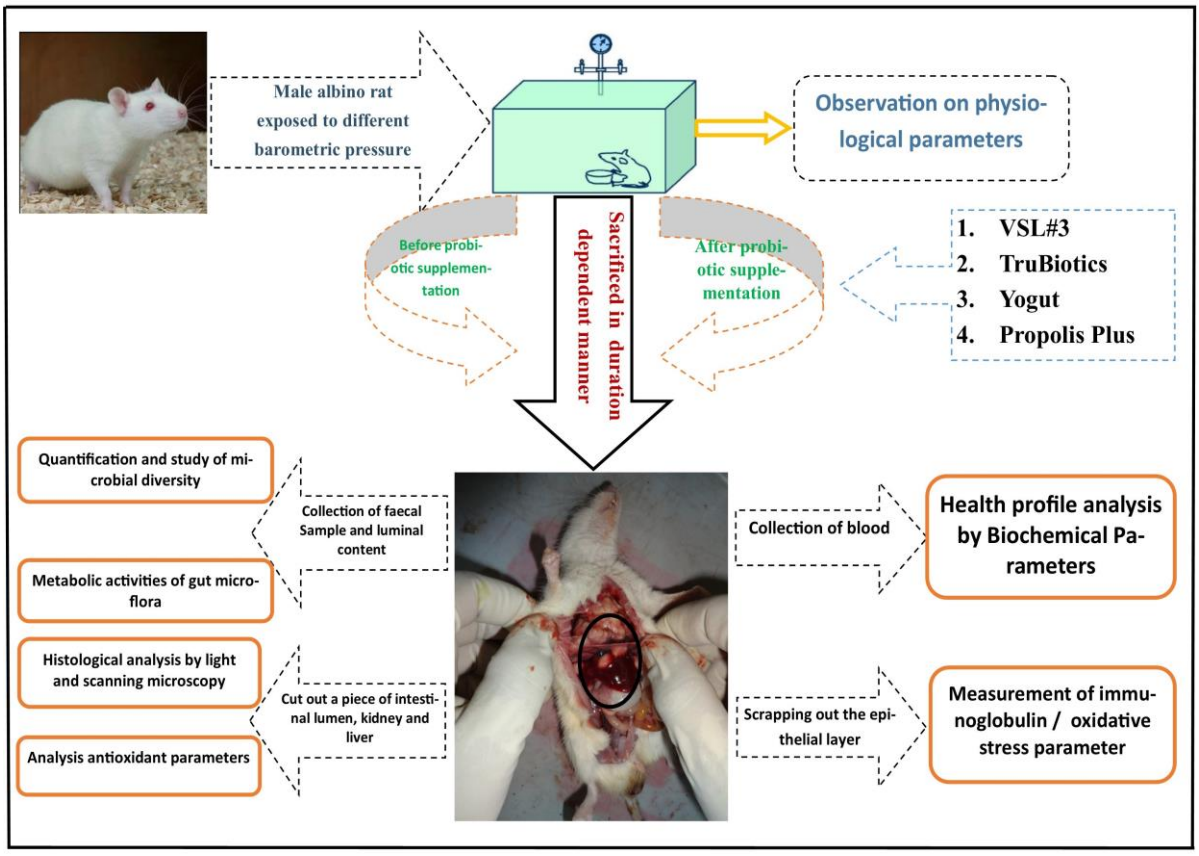

Figure: 1.4. Schematic presentation of experimental procedure

Table 1.5. Different experimental parameters adopted in the present study

Types of the study		Sample type	Parameters adopted	Methods followed	References
Physiological parameters		Model experimental animals	Analysis of Somato-body weight-index	Measurement of food intake capacity and body height and weight	Das *et al.*, 2011; Mandal *et al.*, 2017
Health profile analysis/ Uremic profile	**Heamatological parameter**	Collected blood from the rat	WBC, RBC Count by haemocytometer & Haemoglobin estimation.	Standard biochemical and spectrophotometric method	Arpita *et al.*, 2012; Burtis and Ashwood, 1999
	Lipid profile	Collected blood from the rat	Blood Cholesterol level, Total triglyceride level, Total VLDL level, Total HDL Level	Standard biochemical (kit) and measure by semiauto analyzer	Daniela *et al.*, 2014; Florian et., 2009
	Electrolyte analysis	Collected blood from the rat (plasma)	Quantification of Blood Na^+, K^+ and Cl^-.	Standard biochemical (kit) and measure by electrolyte analyzer	Sunderman, 1959; Pradhan *et al.*, 2013
	Toxicity study	Collected serum from the rat.	Quantification of GOT and GPT in serum	Standard biochemical (kit) and measure by semiauto analyzer	Goel, 1988
	Antioxidant enzymes study	Collected blood, liver, kidney and intestinal epithelia from the rat.	Quantification of SOD, catalase in tissue and plasma sample	Standard biochemical and spectrophotometric method (kit method)	Beers, 1952
	Oxidative stress marker	Collected blood from the rat.	Quantification of MDA	Standard biochemical and spectrophotometric method (kit method)	Lapenna and Cuccurullo, 1993
	Uremic profile	Collected blood, liver, kidney	Urea, Creatinine	Kit methods	Burtis and Ashwood, 1999
Immune toxicity Study	**KIM - 1**	Kidney	Kidney injury marker	Kit methods (ELISA)	Sun *et al.*, 2017
Quantification and diversity of microflora	**Evaluation of gastrointestinal Pathophysiology**	Fecal sample, small and large intestinal luminal content	Total aerobes	Enumerated by standard pour-plate technique in single-strength trypticase soya agar (HiMedia, India)	Wehr and Frank 2004; Maity *et al.*, 2012; Adak *et al.*, 2013
			Total anaerobes	Reduced Wilkins Chalgren agar (supplemented with sodium succinate, hemin, vancomycin, menadione, oleandomycin phosphate	

				polymyxin B and nalidixic acid)	
			Escherichia coli	Mac-Conkey agar media	
			Bacteroidetes sp.	Bacteroides bile esculin agar (supplemented with gentamicin 100 mg/L)	
			Total Lactic Acid Bacteria	De Man, Rogosa and Sharpe agar (MRS)	
			Bifidobacterium sp.	Bifidobacterium, rogosa SL and KF streptococcal agar (HiMedia, India).	
			Salmonella sp.	Brilliant green agar modified (HiMedia, India)	
Metabolic activities of gut	**Gas formation ability of microbial consortium** **Faecal enzymes activity**	Faecal aliquot of small and large intestinal luminal content	α-amylase, Proteinase, β-glucuronidase, Alkaline phosphatase	Standard biochemical and spectrophotometric method	Adak *et al.*, 2013
Inflammatory response	**Immunoglobulin**	Faecal aliquot of small and large intestine	IgG and IgA	Turbidometric method	Paul *et al.*, 2013; Jana *et al.*, 2012
Morphometric Analysis of the Gut	**Structural morphology**	Small and large intestine	study the arrangement of microvilli, deformed villi and the intercellular space between epithelia	Histological staining (Hematoxylin and Eeosine (H&E) stain using Mayer's progressive method), light and scanning microscopy	Adeneye *et al.*, 2008; Lee *et al.*, 2006; Adak *et al.*, 2014
Histology study	**Structural morphology**	Liver and Kidney	Study of renal tubules, glomeruli	Histological staining (Hematoxylin and Eeosine (H&E) stain using Mayer's progressive method), light microscopy	Mani, 2010; Khorsandi and Orazizadeh, 2008
Statistical analysis	Statistical analysis of experimental data by sigmaplot 11.0 (USA) software. One-way ANOVA and the multiple comparisons of all possible pairs will do by Tukey t test (SPSS-10.0). The alteration in bacterial quantity was tested by Bonferroni for post hoc testing. Significant variation was accepted at the level of 5 %, i.e. $p<0.05$.				