Monitoring of the alteration of haematological, biochemical, oxidative stress indicators, and changes in intestinal microflora at different altitude.

Aims of study: The present study was designed to investigate the alteration of haematological, biochemical, oxidative stress indicators, and changes in intestinal microflora at different altitude.

Experiment No 3.1.- Experiment in rat model

Materials and methods:

The study was conducted with adult, male albino rats of Wistar strain (Supplied from Ghosh animal, Animal foods and animal cages Supplier, Kolkata-54) having the body weight of 110±12 g. They were acclimatized in the laboratory condition for 2 weeks' priories to conducting experiment. Animals were selected randomly and divided into four groups having six animals each (four sets of each group). Group C served as control and was kept at normal room air (normoxia). Group HA-I, HA-II and HA-III was exposed to different barometric pressure. Exposure was carried out in a decompression chamber (Instrumentation India, India) for 28 days following the protocol of Maity *et al* (2012). To search out the alteration of haematological, biochemical, and oxidative stress indicators sample were collected at 7 days' interval. The intestinal samples were collected at 3 days' interval to study the changes in gut microflora. Details experimental procedure is discussed in chapter 2.

Result and discussion:

Physiological Index & Haematological Parameters- After 28 days of the experiment, the body weight was increased in group C, HA-I and decreased in the group HA-II, HA-III in comparison with their initial body weight (Table 3.1.1). In HA-I animals, the

percentage of elevation in body weight was dramatically less than the group C. On the other hand, it was decreased in the group HA-II and group HA-III, due to hypobaric induced oxidative stress. Though the weight of kidney and liver in the group HA-I, HA-II and HA-III were also slightly decreased (Table 3.1.2). This alteration was occurred due to higher metabolic rate, different energy output, loss of body water and several endocrine factors. The reasons for these changes were mentioned in the previous work done by Benso *et al* (Benso *et al.,* 2007) and Wall et al (Wall *et al.,* 2009). It has been revealed that at HA the digestive efficacy and nutrients absorption was lowered (Nakanishi *et al.,* 1995) in addition to the loss of an individual. Primarily, increased in satiety (anorexia) for a meal results in a decrease of food consumption, which can be partly compensated by an increase in meal frequency (Westerterp 2001; Diniz *et al.,* 2005; Bailey *et al.,* 2015). In such stress, hypoxia induced anorexia leads to negative energy and nitrogen imbalance and causes the loss of fat masses of the body. It resulted in significant ($p<0.05$) reduction of body mass index (BMI) of the experimental animals in HA groups during 28 days of hypoxia.

Total RBC, total WBC and haemoglobin (Hb) level were significantly increased in the blood of HA-I, HA-II and HA-III animal groups in comparison to group C. But the expansion of RBC, WBC, and Hb in HA-II and HA-III group is much higher with respect to group HA-I (Table 3.1.3). At hypobaric hypoxic condition, the partial pressure of oxygen (PO_2) was decreased which causes the excessive secretion of erythropoietin to carry out cellular function by increasing blood RBC and Hb (Steven *et al.,* 2000). So, it was revealed that there is a great capacity for physiological adjustments in respect to reduced oxygen pressure in the experimental animals.

Table 3.1.1. Changes in body weight of C (14.7 psi) and HA (11.8 psi; 9.3 psi; 7.3 psi) group on 7, 14, 21, 28th day

Exposure duration (in days)	Different groups	Initial body weight (g)	Final body weight (g)	Body weight change (g/day)	Elevation/reduction in body growth (g%)	Body length (cm)	Body mass index (g/cm2)
7	C	133.75±2.27[a]	149.25±4.3[b]	2.21±0.12	+ 11.62±3.88	12.75±0.52	0.92±0.07
	HA-I	132.83±2.06[a]	144.75±4.81[b]	1.70±0.19	+ 8.99±4.13	12.95±0.45	0.86±0.04
	HA-II	132.75±2.26[a]	119.25±4.6[b]	1.92±0.06	- 10.14±3.71	12.41±0.41	0.77±0.03
	HA-III	133.41±3.08[a]	117.08±4.01[b]	2.33±0.27	-12.19±4.02	14.09±0.46	0.59±0.03
14	C	133.75±2.27[a]	155.33±5.78[b]	1.56±0.21	+ 17.70±5.79	13.25±0.27	0.88±0.06
	HA-I	132.83±2.06[a]	141.41±2.05[b]	0.43±0.08	+ 7.91±1.88	13.29±0.33	0.80±0.80
	HA-II	132.75±2.26[a]	123.66±2.13[b]	0.49±0.13	- 5.25±2.72	12.64±0.20	0.77±0.03
	HA-III	133.41±3.08[a]	117.08±4.01[b]	1.047±0.17	- 11.07±3.85	13.75±0.38	0.61±0.03
21	C	133.75±2.27[a]	164.33±4.41[b]	1.35±0.18	+ 20.93±4.17	13.83±0.40	0.86±0.05
	HA-I	132.83±2.06[a]	143.08±5.4[a]	0.40±0.27	+ 6.31±3.63	13.32±0.37	0.80±0.06
	HA-II	132.75±2.26[a]	126.5±2.88[a]	0.21±0.21	- 3.42±3.91	12.95±0.45	0.75±0.07
	HA-III	133.41±3.08[a]	110.58±4.09[b]	0.65±0.12	- 10.98±3.43	13.04±0.51	0.65±0.04
28	C	133.75±2.27[a]	169.33±4.63[b]	1.25±0.09	+ 26.22±3.73	14.16±0.40	0.84±0.06
	HA-I	132.83±2.06[a]	140.91±3.69[b]	0.23±0.18	+ 4.89±2.11	13.49±0.42	0.77±0.05
	HA-II	132.75±2.26[a]	129.5±2.88[a]	0.13±0.35	- 2.75±1.78	12.95±0.45	0.77±0.06
	HA-III	133.41±3.08[a]	118.25±3.98[b]	0.31±0.37	- 6.87±3.40	12.5±0.54	0.75±0.05

*Values are expressed as mean±SD (n = 6). ANOVA followed by multiple two-tail t-test. The different superscripts (a, b) in a specific row differ from each other significantly (P <0.05).

Table 3.1.2. Kidney and liver somatic index of C (14.7 psi) and different HA (11.8 psi; 9.3 psi; 7.3 psi) exposure group on 7, 14, 21, 28th day.

Exposure duration (in days)	Different groups	Kidney somatic index	Liver somatic index
7	C	0.81±0.01[a]	2.28±0.07[a]
	HA-I	0.80±0.01[a]	2.26±0.04[a]
	HA-II	0.76±0.03[a]	2.21±0.08[a]
	HA-III	0.71±0.02[b]	2.02±0.12[a]
14	C	0.81±0.01[a]	2.28±0.07[a]
	HA-I	0.80±0.02[a]	2.27±0.02[a]
	HA-II	0.75±0.01[b]	2.20±0.06[a]
	HA-III	0.70±0.03[c]	1.98±0.10[b]
21	C	0.81±0.01[a]	2.28±0.07[a]
	HA-I	0.81±0.01[a]	2.29±0.01[a]
	HA-II	0.76±0.02[b]	2.21±0.05[b]
	HA-III	0.68±0.02[c]	1.94±0.08[c]
28	C	0.81±0.01[a]	2.28±0.07[a]
	HA-I	0.83±0.03[a]	2.30±0.02[a]
	HA-II	0.77±0.01[b]	2.23±0.04[a]
	HA-III	0.68 ±0.03[c]	1.92±0.15[b]

* Values are expressed as mean±SD (n = 6). ANOVA followed by multiple two-tail t-test. The different superscripts (a, b, c) in a specific column differ from each other significantly (P <0.05).

Table 3.1.3. Effect of different atmospheric pressure on hematological parameters (RBC, WBC & haemoglobin) of Control (C; 14.7 psi) and HA (11.8 psi; 9.3 psi; 7.3 psi) group on 1, 7, 14, 21, 28th day

Parameter	Groups	Day 1	Day 7	Day 14	Day 21	Day 28
RBC	**C**	6.52±0.43[a]	6.63±0.34[a]	6.65±0.68[a]	6.59±0.38[a]	6.85±0.66[a]
[cumm×1000000]	**HA-I**	7.98±0.64[a]	8.76±0.50[b]	8.80±0.49[b]	8.93±0.63[b]	9.26±0.27[b]
	HA-II	8.65±0.99[b]	10.48±0.61[b]	10.98±0.43[c]	11.31±0.78[c]	11.65±0.80[c]
	HA-III	10.98±0.43[c]	12.15±0.96[c]	12.29±0.70[d]	13.01±0.31[d]	13.15±0.26[d]
WBC/cumm×1000	**C**	7.29±0.51[a]	7.41±0.50[a]	7.46±0.50[a]	7.47±0.38[a]	7.62±0.31[a]
	HA-I	7.79±0.65[a]	7.96±0.23[a]	8.57±0.72[a]	8.91±0.82[b]	9.07±0.67[b]
	HA-II	11.10±0.92[b]	13.44±1.29[b]	13.60±1.0[b]	13.77±0.96[c]	13.92±1.07[c]
	HA-III	12.44±1.31[b]	14.10±0.67[b]	15.10±0.44[c]	15.37±0.59[d]	15.50±0.61[d]
Hb gm%	**C**	8.17±0.57[a]	8.39±0.60[a]	8.50±0.44[a]	8.60±0.23[a]	8.67±0.62[a]
	HA-I	9.32±0.18[b]	10.35±0.65[b]	10.42±0.36[b]	10.52±0.38[b]	10.54±0.52[b]
	HA-II	11.96±0.84[c]	12.96±1.40[c]	13.80±0.60[c]	13.63±0.77[c]	13.77±0.60[c]
	HA-III	11.94±1.36[c]	13.77±0.39[c]	13.89±0.37[c]	13.87±0.41[c]	14.00±0.38[d]

*RBC= Red Blood Cell; WBC= White Blood Cell.

Data are expressed as Mean±SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, and d) in a specific column differ from each other significantly (P< 0.05).

Changes of blood electrolytes and uremia profile-

This experiment revealed that there was a significant ($p<0.05$) low plasma sodium level and a significant ($p<0.05$) high plasma potassium level in group HA-I, HA-II, and HA-III animals as compared to C group (Table 3.1.4) and this alteration was higher first 7th day of the experiment. Plasma chlorine level was also increased but no significant changes were observed among the groups. So due to oxidative stress condition, imbalance of plasma sodium, potassium and chlorine were observed which may due to excess accumulation of waste products in the body. The renal clearance was not properly maintained as a result of electrolytes imbalance (Roy *et al.*, 2013).

In addition to changes of electrolytes, blood urea and creatinine level were significantly increased in group HA-I, HA-II and HA-III animals (hypobaric-hypoxic exposure animals) compared to group C. However, the increase of urea, creatinine was significantly ($p<0.05$) higher in HA-II and HA-III in comparing with the group HA-I (Table 3.1.4). Increased in the level of blood urea and creatinine were considered to be nephrotoxic biochemical markers (Pathan *et al.*, 2013). In renal disease, the blood urea accumulates because the rate of blood urea production exceeds than the rate of clearance. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown (Vanholder and Smet, 1999). Thus blood urea and creatinine may be the renal marker in hypoxic condition.

Table 3.1.4. Changes of blood electrolytes and uremia profile of rats at different altitude.

Parameter	Groups	Day 1	Day 7	Day 14	Day 21	Day 28
Sodium(mM/lit)	C	156.71±3.80^{a}	160.05±4.25^{a}	162.71±2.04^{a}	161.88±1.74^{a}	162.38±1.80^{a}
	HA-I	148.21±2.41^{b}	137.05±3.95^{b}	141.38±0.67^{b}	143.71±2.67^{b}	144.38±2.05^{b}
	HA-II	146.71±1.86^{b}	133.71±2.67^{b}	131.38±0.67^{c}	134.71±1.87^{c}	138.55±1.89^{c}
	HA-III	145.88±1.64^{b}	132.88±2.17^{b}	131.38±0.97^{c}	130.05±1.10^{d}	129.55±1.16^{d}
Potassium(mM/lit)	C	6.27±0.51^{a}	6.26±0.82^{a}	6.3±0.07^{a}	6.31±0.10^{a}	6.30±0.10^{a}
	HA-I	6.27±0.51^{a}	6.32±0.73^{a}	6.44±0.23^{a}	6.42±0.16^{a}	6.45±0.17^{a}
	HA-II	6.61±0.42^{a}	7.16±0.56^{a}	7.49±0.18^{b}	7.54±0.17^{b}	7.51±0.18^{b}
	HA-III	6.77±0.41^{a}	8.22±0.66^{b}	8.36±0.77^{c}	8.39±0.74^{c}	8.41±0.73^{c}
chlorine(mM/lit)	C	104.51±3.26^{a}	104.35±3.72^{a}	105.35±2.26^{a}	106.35±2.24^{a}	106.18±3.54^{a}
	HA-I	107.18±2.91^{a}	112.68±1.27^{b}	114.35±4.91^{b}	114.51±1.36^{b}	112.68±1.27^{b}
	HA-II	107.18±2.98^{a}	113.97±2.84^{b}	114.68±2.86^{b}	116.51±2.21^{b}	116.85±1.15^{c}
	HA-III	110.88±1.85^{a}	113.3±2.88^{b}	113.15±2.15^{b}	113.65±2.02^{b}	113.48±1.89^{b}
Urea(mg/dl)	C	21.05±0.71^{a}	21.24±0.68^{a}	21.25±0.81^{a}	21.39±0.53^{a}	21.47±0.51^{a}
	HA-I	26.39±1.45^{b}	27.39±1.89^{b}	27.56±1.82^{b}	27.55±1.80^{b}	28.22±1.60^{b}
	HA-II	28.06±1.22^{b}	35.16±3.81^{c}	37.66±2.51^{c}	39.91±2.22^{c}	39.66±4.81^{c}
	HA-III	37.16±0.86^{c}	41.16±1.62^{d}	42.32±0.76^{d}	43.82±1.10^{d}	44.16±0.99^{d}
Creatinine(mg/dl)	C	0.52±0.08^{a}	0.53±0.07^{a}	0.54±0.09^{a}	0.53±0.06^{a}	0.54±0.05^{a}
	HA-I	0.54±0.02^{a}	0.59±0.03^{b}	0.62±0.08^{a}	0.64±0.11^{a}	0.64±0.09^{a}
	HA-II	0.84±0.07^{b}	0.86±0.08^{c}	0.87±0.09^{b}	0.88±0.05^{b}	0.89±0.04^{b}
	HA-III	0.86±0.06^{b}	1.02±0.12^{c}	1.16±0.14^{c}	1.19±0.13^{c}	1.17±0.10^{c}

*Estimation of uremia profile of blood of experimental male albino rats at three different high altitudes. Data are expressed as mean±SE (n = 6). ANOVA followed by multiple two-tail t-test (Dunkan's multiple test) and data with different superscripts (a, b, c, and d) in a specific column differ from each other significantly ($P< 0.05$).

Antioxidant enzymes profile -

Hypobaric-hypoxic related oxidative stress markers in blood, kidney and liver tissue were studied. After exposure of low atmospheric pressure (group HA-I, HA-II and HA-III animals), the antioxidant enzymes activity (CAT, SOD) in blood plasma, kidney and liver were decreased significantly in all experimental groups as compared to group C.

Table 3.1.5. Changes of uremia profile and oxidative stress markers of rats at different altitude.

Parameters	Groups			
	C	HA-I	HA-II	HA-III
Blood CAT (mM/mL/min)	0.79±0.02[a]	0.47± 0.02[b]	0.41±0.04[b]	0.32±0.03[d]
Kidney CAT (mM/mL/min)	0.59±0.07[a]	0.35±0.04[b]	0.28±0.04[c]	0.27±0.03[c]
Liver CAT (mM/mL/min)	0.69±0.03[a]	0.50±0.02[b]	0.36±0.03[c]	0.26±0.03[d]
Blood SOD (mM/mL/min	1.09±0.06[a]	0.80±0.08[b]	0.52±0.08[c]	0.39±0.05[d]
Kidney SOD (mM/mg/min)	1.9±0.47[a]	0.99±0.19[b]	0.94±0.35[b]	0.53±0.22[c]
Liver SOD (mM/mg/min)	0.93±0.17[a]	0.76±0.08[b]	0.49±0.08[c]	0.37±0.04[d]
Blood MDA (nM/mL)	31.18±1.70[a]	47.71±2.32[b]	55.9±1.31[c]	62.2±1.92[d]
Kidney MDA (nM/mL)	50.61±2.13[a]	62.94±2.20[b]	89.05±1.84[c]	87.34±2.18[c]
Liver MDA (nM/mL)	41.18±1.95[a]	53.38±0.74[b]	66.44±1.02[c]	69.03±3.68[c]

*Estimation of uremia profile and oxidative effect in blood, kidney and liver of experimental male albino rats at three different high altitudes. Data are expressed as mean±SE (n = 6). ANOVA followed by multiple two-tail t-test (Dunkan's multiple test) and data with different superscripts (a, b, c, d and e) in a specific horizontal row differ from each other significantly with respect to control ($P<0.05$).

After 28 days of hypoxic exposure, the activities of those enzymes in the above mentioned tissues decreased significantly in Group HA-II and HA-III. The rate of reduction of CAT and SOD were more in kidney and blood of the group HA-III after 28 days (Table 3.1.5). During kidney injury, superoxide radicals generate at the site of damage which modulates SOD and CAT (Pande and Flora, 2002). MDA is an indicator of the degree of lipid peroxidation (Abraham, 2005). Significant increase in MDA levels was observed in plasma, kidney and liver tissue of rats exposed with hypoxic condition (HA-II and HA-III)

compared to group C (Table 3.1.5). This was described that the free radical mediated chain reaction causes the damage of the cell membranes (Zhang *et al.*, 2011).

DNA fragmentation assay-

DNA fragmentation analyses were performed for additional confirmation of hypobaric induced renal injury. Hypobaric induced random fragmentation of genomic DNA along with the subsequent formation of a DNA smear on agarose gel without ladder formation. DNA fragmentation assay suggested that hypobaric hypoxic environment induced renal cell damage through the necrotic pathway with apoptosis (Fig. 3.1.1). Necrosis of kidney tissue is a key marker for detection of histopatho chemical changes.

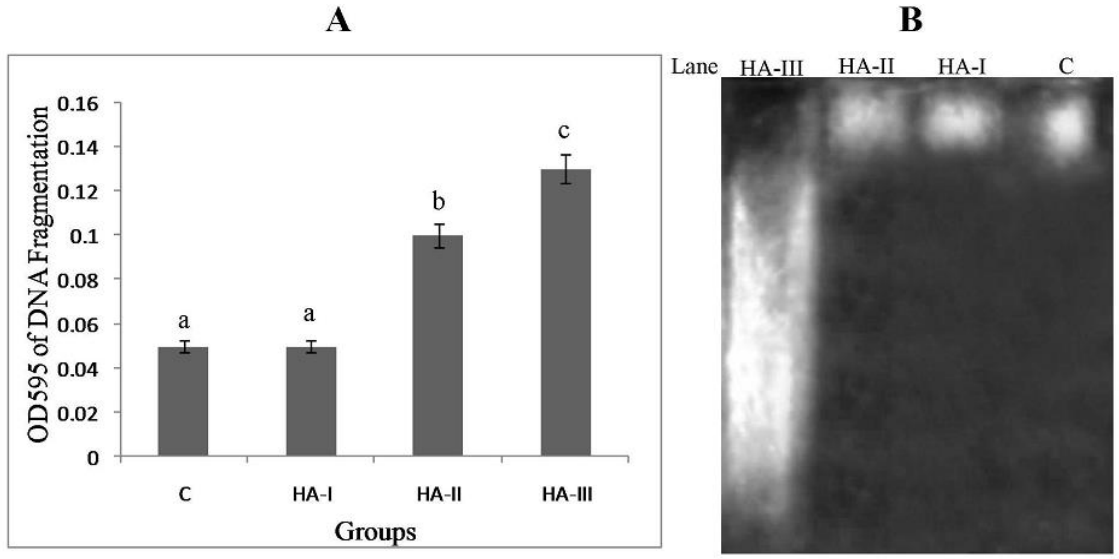


Fig. 3.1.1. DNA fragmentation assay

A. Effect of hypoxia on the extent of DNA, (C) Normal control rats, HA-I (6000 feet/1829 m), HA-II (12000 feet/3657 m) and HA-III (18000 feet/5486 m) experimental rats. Each column represents mean±SE (n = 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($P < 0.05$). B. DNA isolated from experimental kidney tissues was loaded on 1% (w/v) agarose gels. (C) Normal control rats, HA-I, HA-II and HA-III high altitude exposure treated kidney DNA.

Analysis of faecal sample-

The population of total aerobes was 6.13 log_{10}CFU/g in the faecal sample in group C (without exposure to animals). But it was reduced drastically in HA-II and HA-III groups ($p<0.05$) by 4.31 and 2.68 with final GDI of –1.48 and −2.08 (log_{10}C/log10HA-28) respectively. The quantity of total anaerobes increased [(9.1 to 11.7 (log_{10}CFU/g)] with GDI of +1.28. The ratio (ratio of log_{10}CFU/g) of total aerobe and the anaerobic population was altered from 1:1.48 (control) to 1:2.67 (HA-II) and 1:1.48 (control) to 1:4.36 (HA-III) respectively after 28 days of hypobaric-hypoxic exposure. Though ratio (ratio of log_{10}CFU/g) of total aerobe and the anaerobic population was not altered significantly in HA-I group. The *E. coli* content was expended with positive GDI in hypoxic condition. The quantity of strict anaerobes like *Bacteroides* sp. and *Bifidobacterium* sp. were increased with GDI of +1.09; +1.27; +1.43 and +1.05; +1.18; 1.28 after the twenty-eight days of exposure at HA-I, HA-II and HA-III respectively. Normally, they were present (at base level) in fecal sample at a ratio of 1:1.03 (ratio of log_{10}CFU/g) that changed to 1:1.08 (HA-I), 1:1.17 (HA-II) and 1:1.51 (HA-III) respectively after seven days of hypoxic stress (Fig. 3.1.2).

Total lactic acid bacterial population and the selected pathogen like *Salmonella* sp. was also increased. It causes higher anaerobic state of intestinal epithelia and alterations of GI mucosal microenvironment were the major limiting factors for such group (Maity *et al.*, 2012).

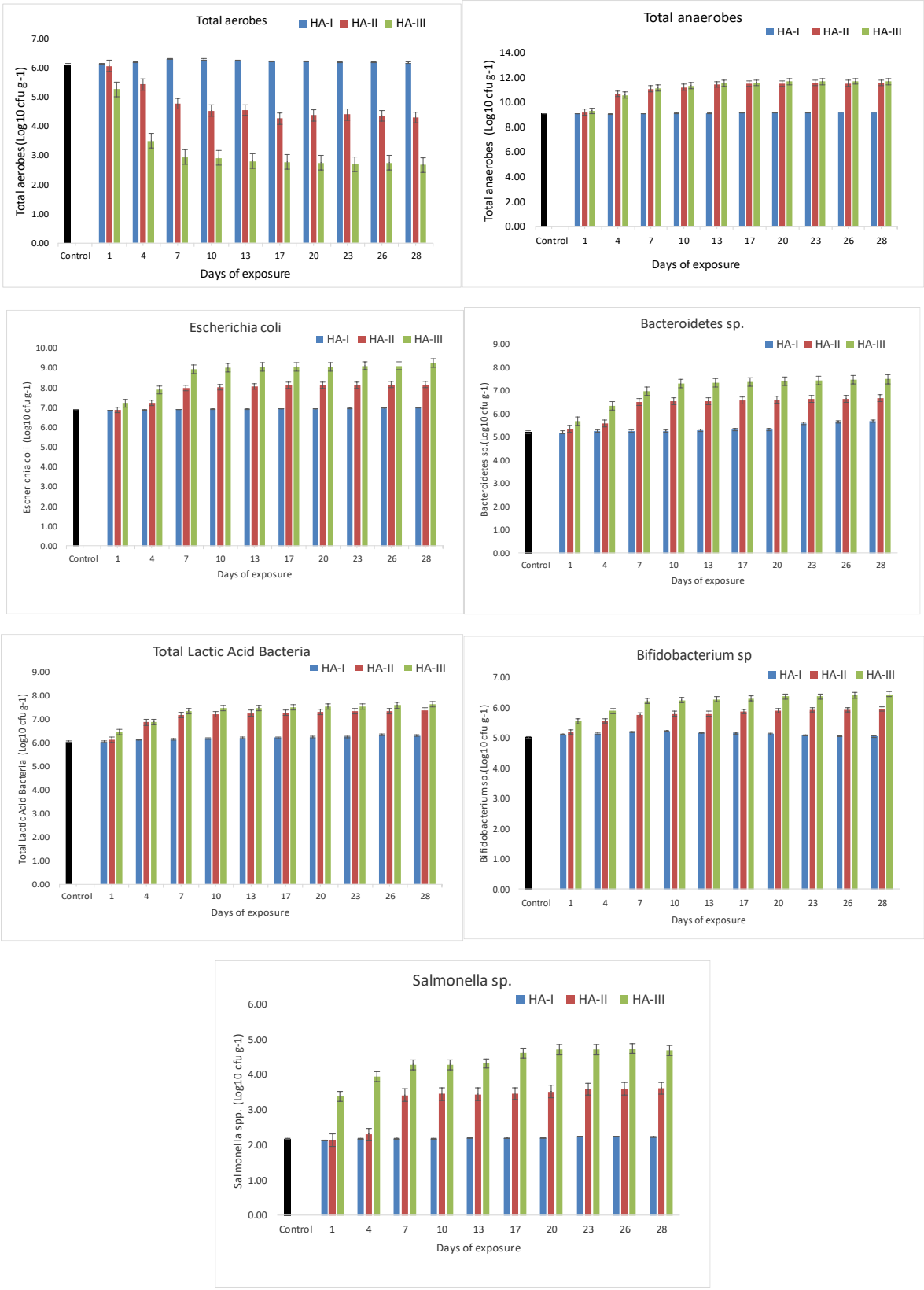
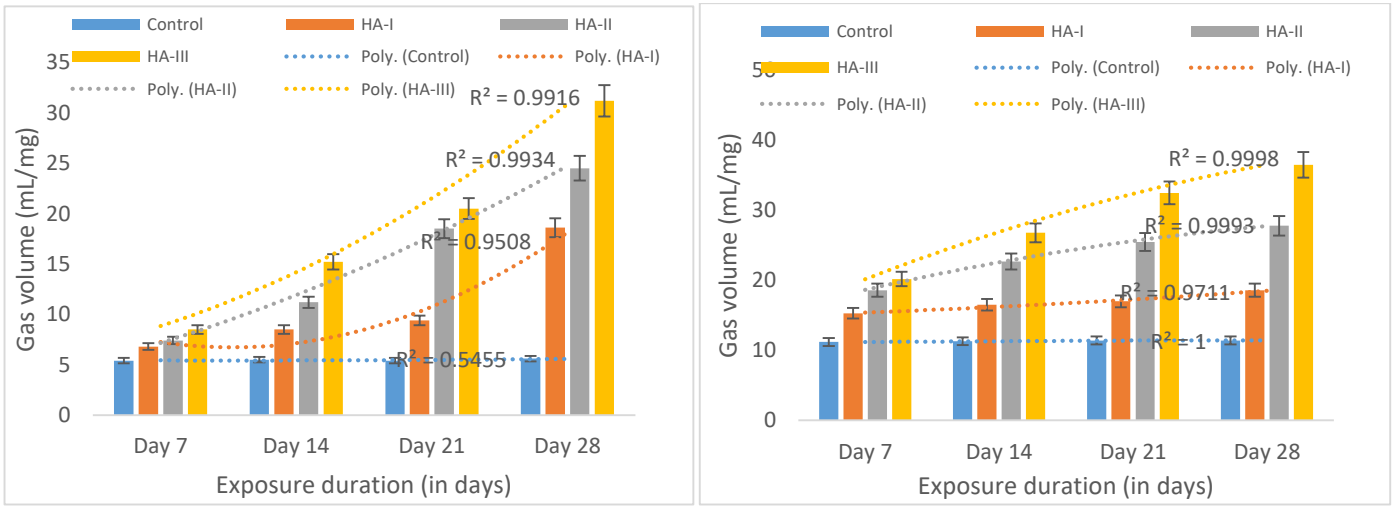


Fig. 3.1.2. Alteration of some indicator bacterial populations in the fecal sample of rat after 28 days of hypobaric hypoxia at different altitude exposure.

It was well known that the *E. coli* population was generally 10^2 times higher than total aerobes in the faces. Total aerobes, facultative anaerobes (*E. coli*) and total anaerobes present in the ratio of $4.36:1:4.03\times10^5$. But this may differ within the species and even between individuals in the same species (Maity *et al.*, 2009). At a lower level of oxygen, this ratio was changed to $1:2.94\times104:2.16\times10^7$. *E. coli* cell proliferation (10^6) was higher as it possessed an elaborate genetic regulatory network for oxygen sensing (Holy and Chmelar, 2012). The study revealed that 6-h immobilization stress initiates the increase of the concentration of *E. coli* in the proximal sections (the duodenum and the jejunum) of the digestive tract (Gritsenko *et al.*, 2000). This rapid expansion of *E. coli* population may inspire the progress of other strict anaerobes like *Bacteroidetes* sp. *Bifidobacterium* sp. and *Lactobacillus* sp. and pathogenic *Salmonella* sp. (Gombosov *et al.*, 2000). But it was not eventually decrease the growth of *Bacteroidetes* sp. and lactic acid bacteria which were lower than other anaerobes.



A **B**

Fig. 3.1.3. Gas formation ability by the composite bacterial populations in rat small intestine (A) and large intestine (B) of control and different HA groups on 7th, 14th, 21th and 28th day.

Metabolic activity of microbial consortium-

Fermentation of dietary polysaccharides produces different gases like carbon dioxide, methane and hydrogen (Pimentel *et al.*, 2013). From the present study, in the small intestine, the capability of gas-formation by composite microflora in C group was 5.4 mL/g that increased to 18.6 mL/g (HA-I), 24.5 mL/g (HA-II) and 31.2 mL/g (HA-III) (Fig. 3.1.3A). In case of the large intestine, microflora can produce 11.2 mL/g in C group which is found to increase up to 18.6 mL/g, 26.8 mL/g and 36.5 mL/g after 28 days of hypobaric hypoxic stress in different HA group (Fig. 3.1.3B).

Table 3.1.6. Changes in the enzyme profiles (luminal enzymes activities) on different days of acclimatization

(HA-I)						
	Hypobaric hypoxic exposure duration (in days)					**Increase/ decrease (%)**
Enzyme activity	**0**	**1**	**2**	**4**	**7**	
α-amylase	210.22±3.45^{a}	233.34±4.18^{b}	242.22±4.34^{b}	251.44±3.05^{b}	254.57±6.21^{b}	21.01
Proteinase	5.81±0.06^{a}	5.75±0.05^{a}	6.45 ±0.02^{b}	7.82 ±0.08^{c}	7.10 ±0.1^{d}	22.2
β-glucuronidase	3.45 ±0.05^{a}	3.94 ±0.03^{b}	4.63 ±0.03^{c}	6.84 ±0.02^{d}	6.15 ±0.06^{d}	77.8
Alkaline phosphatase	5.23 ±0.02^{a}	6.32 ±0.02^{b}	8.27 ±0.05^{c}	8.57 ±0.03^{d}	9.18 ±0.2^{e}	75.52
(HA-II)						
α-amylase	258.22±4.75^{a}	263.34±6.12^{a}	292.82±7.54^{b}	311.44±5.25^{c}	354.57±6.78^{d}	37.73
Proteinase	6.41±0.02^{a}	5.65±0.10^{b}	8.45 ±0.12^{c}	8.85 ±0.06^{d}	9.18±0.21^{e}	43.21
β-glucuronidase	4.35 ±0.05^{a}	3.62 ±0.04^{b}	4.68 ±0.03^{c}	6.64 ±0.04^{d}	7.05 ±0.03^{e}	62.06
Alkaline phosphatase	6.13 ±0.05^{a}	7.42 ±0.07^{b}	7.87 ±0.05^{c}	8.50 ±0.12^{d}	9.18 ±0.18^{e}	49.75
(HA-III)						
α-amylase	250.22±11.5^{a}	332±5.45^{b}	372.62±8.14^{c}	391.74±10.5^{d}	414.57±7.88^{e}	65.68
Proteinase	5.72±0.12^{a}	5.25±0.04^{b}	7.45 ±0.12^{c}	9.05 ±0.16^{d}	9.25±0.01^{d}	61.71
β-glucuronidase	3.65 ±0.03^{a}	3.72 ±0.06^{a}	4.68 ±0.04^{b}	5.64 ±0.05^{c}	6.05 ±0.02^{d}	65.75
Alkaline phosphatase	4.73 ±0.15^{a}	4.92 ±0.08^{a}	5.87 ±0.04^{b}	7.10 ±0.10^{c}	7.38 ±0.08^{d}	56.02

*Data are expressed as Mean±SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, and d) in a specific row differ from each other significantly ($P< 0.05$).

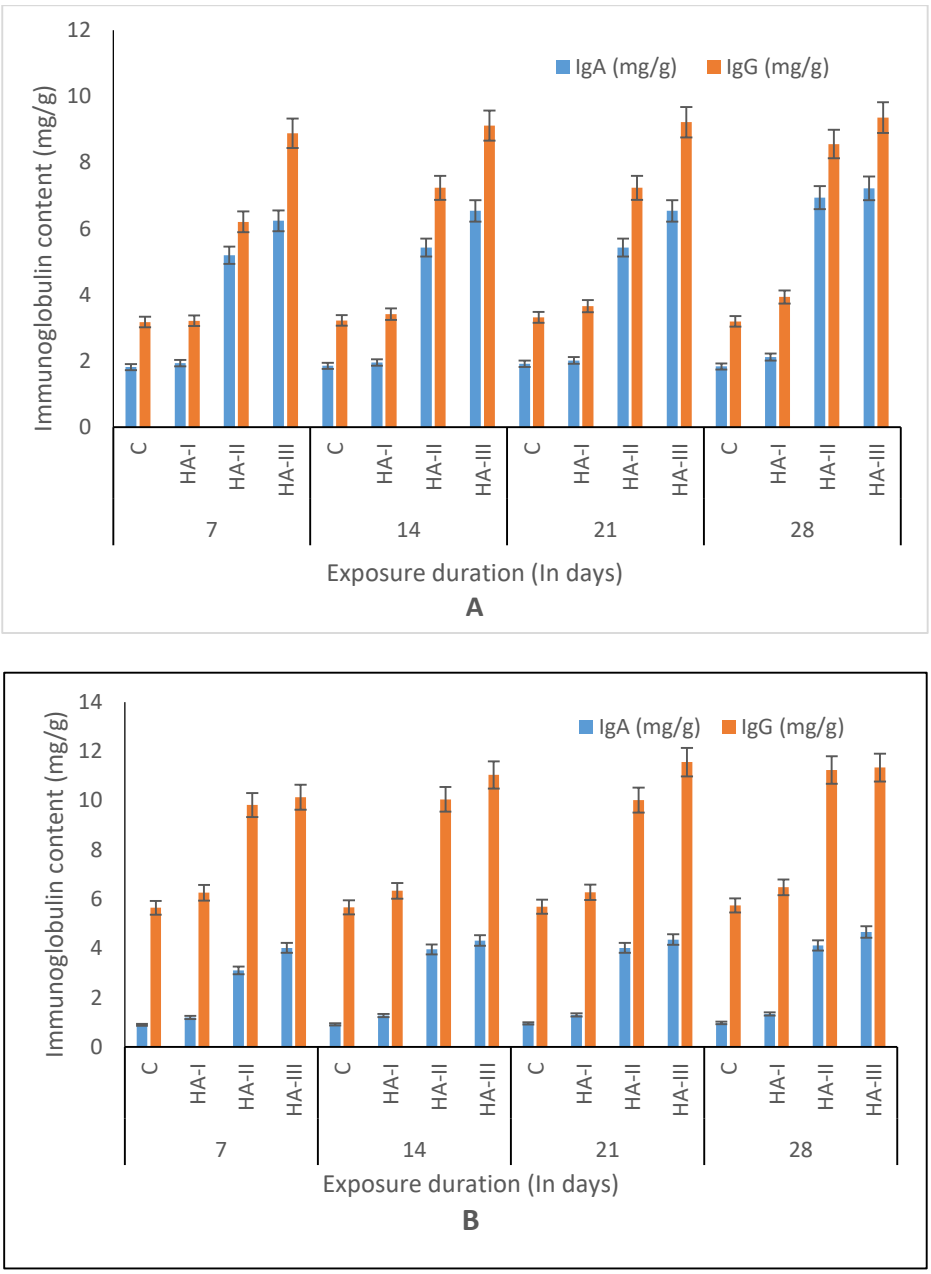


Fig. 3.1.4. In albino rat model, the level of IgG and IgA in small intestinal (A) and large intestinal (B) luminal content of Control (C; 14.7 psi) and HA (11.8 psi; 9.3 psi; 7.3 psi) groups at different days (7, 14 21 and 28th day) of hypobaric hypoxic stress

Visual observation indicated mild colonic bleeding with irregular dystrophy inflammation sign on the small intestine and shrunken cecum was observed after 28 days in HA-II and HA-III groups. The inflammation of both small intestine and cecum indicated the higher gas formation in the lumen that may create a pressure to the gut wall. The microbial

enzyme activity was also used for the evidence of the alternation of GI micro-environment during HA. The base levels of alkaline phosphatase, proteinase, β-glucuronidase and α-amylase activity increased respectively (%) in their specific activity after seven days of acclimatization (Table 3.1.6). The alkaline phosphatase activity removed the phosphate from glutamine of the lipid moiety to reduce the LPS toxicity and create a less toxic situation (Bates *et al.,* 2006). The α-amylase activity digests the undigested polysaccharides to salvage energy and facilitated the acid accumulation in the colon (Gloster *et al.,* 2005).

Evaluation of different immunoglobulins-

In small intestinal luminal content, the immunoglobulin, IgA and IgG levels were 1.82 and 3.18 mg/g in C group that progressively increased HA-I, HA-II and HA-III to 2.12; 6.94; 7.22 and 3.94; 8.56; 9.36 mg/g respectively (Fig. 3.1.4A). The IgA and IgG level in the large intestinal content of C group was 0.9 and 5.65 mg/g. These level were progressively increased HA-I, HA-II and HA-III to 1.34; 4.12; 5.67 and 6.48; 11.24; 11.34 mg/g respectively after 28 days of hypobaric hypoxic adaptation (Fig. 3.1.4B). This showed that hypoxia induced inflammatory response is principal to the activation of the mucosal and lymphoid related immune system resulted in higher level of IgA and IgG secretion as a protective measure. The same fashion of immune responses was also noted in the human GI tract during acclimatization at HA for 7 days at Leh (~3,505 m) (Samanta *et al.*, 2018).

Histological assessment-

Histological assessments of kidney segments of control and experimental animals are presented in Fig. 3.1.5. Kidney sections of hypobaric hypoxic unexposed animals (group C) and exposed group HA-I showed well organized kidney tissue (Fig. 3.1.5A & Fig. 3.1.5B) respectively. On the other hand, kidney sections of hypobaric hypoxic exposed animal of group HA-II and group HA-III showed the moderate and massive glomerular

and renal tubular damage by inflamed and necrotic epithelial cells which is shown in Fig. 3.1.5C & Fig. 3.1.5D respectively.

This happens due to low level of oxygen in the air, improper exchange of gases in the lungs (ventilation, diffusion and perfusion), not enough haemoglobin for oxygen transfer and improper function of the cardiovascular system (Eckardt *et al.*, 2005). Researchers revealed that these causes not only critical situations in cardiopulmonary conditions but also in the regulation of the microenvironment of every cell (Brenner *et al.*, 1982).

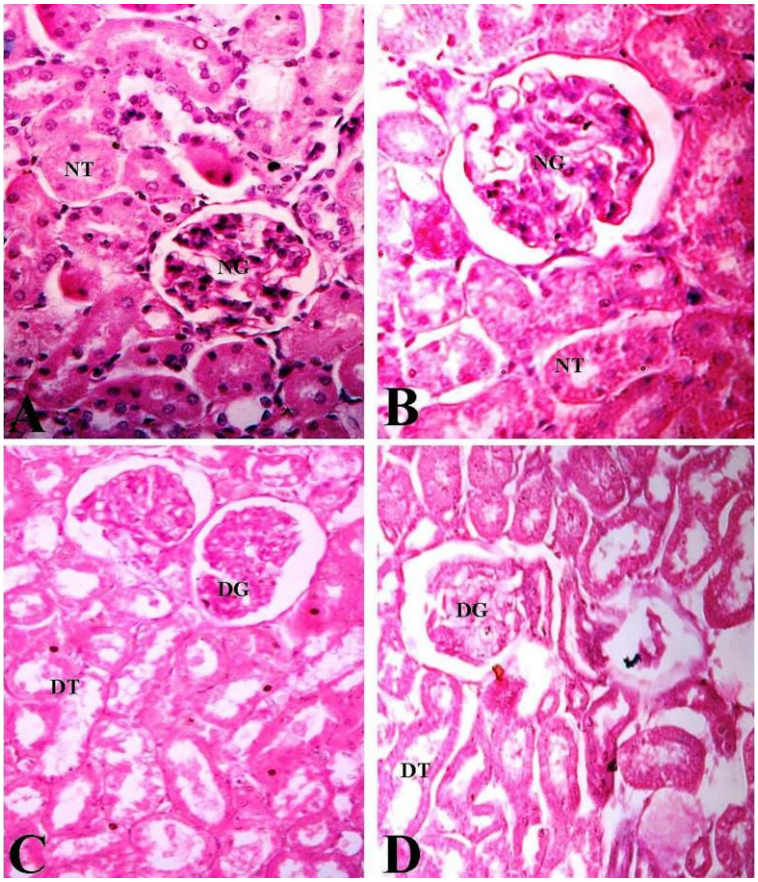


Fig. 3.1.5. Representation of light microscopy of kidney tissue section Hematoxylin-eosin stain X 100. A: Histology of kidney tissue in group C (Control), B: Histology of kidney tissue in group HA-I (6000 feet/1829 m), C: Histology of kidney tissue in group HA-II (12000 feet/3657 m), D: Histology of kidney tissue in group HA-III (18000 feet/5486 m) NG: Normal glomerulus, NT: Normal renal tubules with intact well organized cellular boundary that are not affected due to the lower atmospheric pressure, DG: Damaged glomeruli and DT: Damaged tubules are dilated with loss of cellular boundary

During renal filtration, most tubular segments have a very inadequate ability for anaerobic energy generation, thus reliant on oxygen to continue active trans tubular reabsorption of solutes particularly sodium ions. Erythropoietin (EPO), responsible for hematopoiesis and creates a signal from renal cortex of the kidney. The synthesis or induction of EPO in peritubular fibroblasts of the renal cortex of kidney is regulated by a negative feedback loop response to renal tissue hypoxia (Meinders *et al.,* 2011; Painschab *et al.,* 2015). Hence, the limitations of oxygen supply in renal tissue render the susceptibility of kidney hypoxia, may lead to the pathogenesis of chronic renal injury.

Conclusion:

The lower atmospheric pressure at high altitude have an adverse effect that can do the following functions

1. Reduce body weight during acclimatization.
2. Decrease organ weight including kidney & liver at HA-II and HA-III.
3. Increase the concentration of RBC, Hb and WBC.
4. Imbalanced the electrolytes and increase the uremic toxins like urea and creatinine.
5. Lower expression of antimicrobial enzymes induces the oxidative damage.
6. Increase of uremic toxins induces the nephrotoxicity and the damage the renal tubule.
7. Hypoxia causes for the imbalance of microbial population density and metabolic activity, which induce acid and gas formation.
8. Increase of immunoglobulin (IgG & IgA) helps to protect the hypoxia induce microbial dysbiosis during acclimatization.

Experiment No 3.2- Experiment in human subject

Materials and methods:

Twelve young healthy Indian army male soldiers (base level residents) within the age group of 25 – 30 years were selected for this study. The body mass index (BMI) of them was approximately 24.55 kg/m^2 (±0.84). They all were healthy, not under the treatment of any medication, and they were not suffering from any bacterial or viral infections. They consumed army-specific homogenous diets throughout the experiment. The sea level (i.e. Base line or '0' day) study was also carried out at Delhi (barometric pressure 740 mm Hg). After recording the physiological parameters and collecting the samples, the subjects were flown to an altitude of 3,505 m at Leh (barometric pressure 483 mm Hg) in the Western Himalayas. The subjects arrived at a 3500 m altitude in the morning and the day of arrival was taken as day one at 3500 m.

The fecal samples (~10 g) were collected in a pre-sterilized spatula–container at Delhi, and these were considered as the 'Base Line' (or '0' day sample) samples. Thereafter, the samples were collected on the first, fourth, and the seventh days at Leh, Jammu and Kashmir, India (~ 3500 m) during acclimatization. The samples were transported in a sterilized carrier solution containing peptone, 10%(w/v); glycerol, 5%(v/v). pH was adjusted to 7.0±0.2, and the samples were stored at 4 °C until the analysis.

Blood samples were collected from subjects using 21-Gauge needles (21G) mounted on a 5-mL syringe (Hindustan Syringes and Medical Devices Ltd, Faridabad, India) into heparin-coated sample bottles for the analysis. Samples were collected on '0' (base line), during the first, fourth, and seventh day periods.

The collected data are presented as the arithmetic mean of three replicas (mean±SE). The variations in microbial count hematological parameters were examined by one-way

ANOVA. The alteration in the bacterial quantity was tested by Bonferroni for post hoc testing. Significant variation was accepted at the level of 5 %, i.e. $p<0.05$.

Result and discussion:

A large number of aerobic bacteria were present in the fecal samples at normobaric conditions. But it was reduced significantly ($p<0.05$) after the seventh day of acclimatization at Leh. The quantity of total anaerobes was 9.10 (log_{10}CFU/g) on the 0^{th} day at base line (Delhi) and increased significantly to 11.15 (log_{10}CFU/g) after the 7^{th} days of acclimatization. The ratio of total anaerobe and aerobic bacteria was 10^3 in Dehli; which has increased to 10^7 on the seventh day at Leh. The *E. coli* content was 6.9 (log_{10}CFU/g) at the base level, it was expended after the seventh days' acclimatization of the hypoxic condition and the changes of the above population which were significant ($p<0.05$).

Table 3.2.1: Alteration of microbial population in human feces (Army Personnel - AP) on different days during acclimatization at high altitude (3500m).

Microbial parameters	Hypobaric hypoxic exposure duration (in days)			
	'0' (Base Line)	**1**	**4**	**7**
Total aerobes	6.13 ± 0.554^a	6.30 ± 0.561^a	4.77 ± 0.548^b	3.94 ± 0.560^c
Total anaerobes	9.1 ± 0.581^b	9.10 ± 0.583^b	11.10 ± 0.584^a	11.15 ± 0.585^a
Escherichia coli	6.9 ± 0.482^c	6.97 ± 0.480^c	7.98 ± 0.483^b	8.95 ± 0.481^a
Bacteroidetes sp.	7.03 ± 0.275^c	7.20 ± 0.271^c	7.78 ± 0.700^b	8.23 ± 0.277^a
Total Lactic Acid Bacteria	6.3 ± 0.312^b	6.34 ± 0.310^b	7.35 ± 0.313^a	7.45 ± 0.314^a
Bifidobacterium sp.	5.21 ± 0.450^b	5.38 ± 0.451^b	6.54 ± 0.453^a	7.08 ± 0.452^a
Salmonella sp.	2.26 ± 0.474^c	2.33 ± 0.470^c	3.52 ± 0.471^b	4.21 ± 0.472^a

**Microbial population density was expressed (mean of* log_{10}CFU/g±*SD). Letters (a, b, c) in superscript form in the row are significantly different at p<0.05.*

The quantity of strict anaerobic such as *Bacteroides* sp. and *Bifidobacterium* sp. was increased after the 7^{th} day (Table 3.2.1). The total lactic acid bacterial population was

increased insignificantly ($p>0.05$) at the 7th day of acclimatization. Selected pathogen particularly *Salmonella* sp. was increased at the 7th day of acclimatization than the base line population.

Table 3.2.2: Changes physiological parameter on different days of acclimatization to 3500m.

Changes of physical parameter				
	Base Line	**HA Day 1**	**HA Day 4**	**HA Day 7**
Body Weight (kg)	69.5±2.58	69±2.67	66±2.69	66±2.65
Heart Rate (pulse/min)	64.83±3.49	81.5±7.46	84.33±6.20	81.66±6.41
SPO_2 (%)	98.83±0.23	91.66±1.07	97.16±0.43	97.83±0.92
Temperature (ºF)	98.41±0.21	96.3±0.80	96.96±0.62	97.06±0.63

** Data are expressed as Mean±SD.*

The alteration of urea and creatinine levels were significant in group AP, it was noticed that during acclimatization (AP group) urea and creatinine levels were increased gradually and on the 7th day of hypoxic exposure they returned to the initial base line values **(Table 3.2.3).**

Table 3.2.3: Changes of blood parameter of Army Personnel (AP) on different days of acclimatization and its alteration after 7th day at 3500m.

Blood parameters	**Groups**	**Hypobaric hypoxic exposure duration (in days)**			
		'0' (Base Line)	**1**	**4**	**7**
Hb (g/dl)	AP	$14.16±0.52^{c}$	$16.1±0.31^{a}$	$15.76±0.48^{b}$	$15.84±0.52^{b}$
Hematocrit (Hct)	AP	$50.8±1.93^{a}$	$51.4±1.71^{a}$	$48.4±1.19^{b}$	$48±1.41^{b}$
Urea (mg/dl)	AP	$33.16±1.43^{c}$	$37.83±3.37^{b}$	$43±3.22^{a}$	$34.5±3.21^{c}$
Creatinine(mg/dl)	AP	$0.83±0.05^{c}$	$0.95±0.08^{a}$	$1.06±0.08^{a}$	$0.87±0.04^{b}$

**Data are expressed as Mean±SD. Letters (a, b, c) in superscript form in the row are significantly different each other at $p<0.05$.*

The body weight of the experiment group of the army personnel (AP) was decreased at the end of 7th day of acclimatization as compared to their initial body weight; though it was

not changed significantly ($p<0.05$) **(Table 3.2.2).** Initially the body temperature and SPO_2 was decreased during the acclimatization which was further increased towards the normal level. The heart rate **(Table 3.2.2),** level of haemoglobin (Hb) and hematocrit (HCT) were found to be significantly increased during the acclimatization **(Table 3.2.3).**

The microbial enzyme activity was also used for the evidence of the alternation of GI micro-environment during HA. The activity of alkaline phosphatase, proteinase, β-glucuronidase and α-amylase was increased respectively after 7th day of acclimatization **(Table 3.2.4).**

Table 3.2.4: Changes in the enzyme profiles on different days of acclimatization

Enzyme activity	Hypobaric hypoxic exposure duration (Day)				
	'0' (Base Line)	**1**	**2**	**4**	**7**
α-amylase	200.22±4.45^{d}	210.34±4.38^{c}	222.22±4.11^{b}	241.44±5.05^{a}	244.57±4.21^{a}
Proteinase	3.83±0.06^{d}	4.05±0.05^{c}	4.47 ±0.02^{b}	5.82 ±0.08^{a}	5.90 ±0.1^{a}
β-glucuronidase	5.75 ±0.06^{e}	6.04 ±0.04^{d}	6.63 ±0.03^{c}	7.82 ±0.02^{b}	8.05 ±0.05^{a}
Alkaline phosphatase	4.43 ±0.01^{e}	5.12 ±0.02^{d}	6.17 ±0.02^{c}	6.67 ±0.04^{b}	7.18 ±0.1^{a}

* Data are expressed as Mean±SD. Letters (a, b, c, d, e) in a row are significantly different each other at $p<0.05$.

The population of microbes present in the microenvironment of the GI tract performs several important and essential activities in the host body. Little disturbances in the balanced gastrointestinal environment result in the alteration of the whole ecosystem with significant physiological changes (Rhee *et al.,* 2009). Acute exposure to high altitude, the individual suffers from AMS due to the decreased level of inspired PO_2 at the time of traveling from sea level. Acclimatization to high altitude decreases the tissue oxygen delivery, which causes microcirculatory dysfunctions and cellular dyslexia including

indigestion, acid gas formation, bowel motility, and permeability. These dyslexia in the gastrointestinal tract (GI) mucosa leads to metabolic dysfunctions that finally exerts a deleterious effect on the largest number of GI symbionts.

The results of this study showed that the total aerobes of the fecal samples decreased significantly during acclimatization of army personnel at high altitude, and the total anaerobes increased after 7^{th} days of HA acclimatization. This is likely to be related in respect to the higher anaerobic state of intestinal epithelia and the alteration of GI mucosal microenvironment which was the major factor causing the modulation of specific bacterial subpopulations.

It has been established that the *E. coli* population was generally 10^6 times higher than the total aerobes in faeces. The total aerobes, facultative anaerobes (*E. coli*) and total anaerobes are present in the ratio of $4.36:1:4.03\times10^5$ in faeces, but this may vary within the species and even between individuals in the same species (Maity *et al.,* 2009). At a lower level of oxygen, this ratio was changed to $1:2.94\times10^4:2.16\times10^7$ and the proliferation of *E. coli* was higher (10^6), as it possessed elaborate genetic regulatory network for sensing oxygen (Holy *et al.,* 2012). It has been shown that immobilization for six hours, induces the increase of the concentration of *E. coli* in the proximal sections (the duodenum and the jejunum) of the digestive tract (Gritsenko *et al.,* 2000). This rapid expansion of *E. coli* population may encourage the growth of other strict anaerobes (*Bacteroidetes* sp. *Bifidobacterium* sp. *and Lactobacillus* sp.) and pathogen (*Salmonella* sp.) in anaerobic respiration (Gombosov *et al.,* 2011). But it is not clear, why the *Bacteroidetes* sp. and lactic acid bacteria were lower rather than other anaerobes. The growth of Gram-negative bacteria can cause a serious burden in the gut lumen due to poisoning with the bacterial lipopolysaccharides.

The loss of body weight at hypobaric hypoxic conditions has been described in several studies (Benso *et al.,* 2007). In the present study, the final body weight of the army personnel (AP) was not changed significantly during the 7^{th} day period of time in the experiment, but there was a tendency to weight loss (Table 3.2.2) which may be attributed by higher metabolic rate, different energy output, and the loss of body water as well (Wall *et al.,* 2009). Initially, the body temperature and SPO_2 were decreased, and at the 7^{th} day of acclimatization these were increased due to the lower oxygen concentration in the air. In such conditions, it has been reported that the heart rate was found to be altered significantly due to the activity of the autonomic nervous system (Bhaumik *et al.,* 2013).

The level of haemoglobin and HCT were increased during acclimatization to HA. Literature has revealed that hypoxia causes the excessive secretion of erythropoietin (EPO) which results in increase of blood RBC and Hb levels (Wickler *et al.,* 2000; Mizuno *et al.,* 2008) in order to compensate the reduced blood oxygen contents. In the current study, blood Hb and HCT were found to be increased (Table 3.2.2) as described by Mairbaurl in the year of 2013. It was known several decades ago that erythrocytes are produced from the successive maturation of different erythroid progenitors which are responsive to erythropoietin (EPO). The rise in HCT and haemoglobin contents suggests that HA increases the EPO production (EPO is a glycoprotein hormone produced by the kidneys and secreted into the plasma), producing then haematological changes, (Gouttebargea *et al.,* 2012).

The alkaline phosphatase activity removed the phosphate from glutamine of the lipid moiety to reduce the LPS toxicity and create a less toxic situation (Bates *et al.,* 2006). The α-amylase activity digests the undigested polysaccharides to salvage energy and facilitated acid accumulation in the colon (Gloster *et al.,* 2008).

Conclusion:

From the experiment, it concluded that at lower pressure of atmospheric oxygen in high altitude is responsible to lessen the blood oxygen level and results in the distressed physiological buffering system of the body which consequently do the following changes

1. Body weight, SPO_2 and body temperature decrease during acclimatization at HA. At lower level of oxygen, heart rate increase to maintain an adequate supply of O_2 to tissues or cells.
2. Increase the concentration of RBC, Hb and WBC.
3. Microbial population density and metabolic activity have altered, which ultimately effects on the normal gut flora inducing the growth of pathogenic bacteria and its associated enzymes.

Analysis of effectiveness of the potential probiotic supplementation for health improvement at hypobaric condition.

Aims of study: The present study is designed to measure the effect of probiotic supplements for the improvement of health at the hypobaric condition. In this study health parameters like haematological, biochemical, oxidative stress indicators were measured along with the study on kidney and liver toxicity at different altitude.

Experiment No 3.3- Biochemical analysis of health parameters after probiotic supplementation

Materials and methods:

Animals were selected randomly and divided into four groups having six animals each (four sets of each group). Group NC served as control and were exposed to normal room air (normoxia). Group HA-I, HA-II and HA-III was exposed to different barometric pressures. Different commercial probiotics were administrated orally and groups were labeled as CP-1 (VSL#3); CP-2 (TruBiotics); CP-3 (Yogut); CP-4 (Propolis Plus). Exposure was carried out in a decompression chamber (Instrumentation India, India) for 7 days.

Result and discussion:

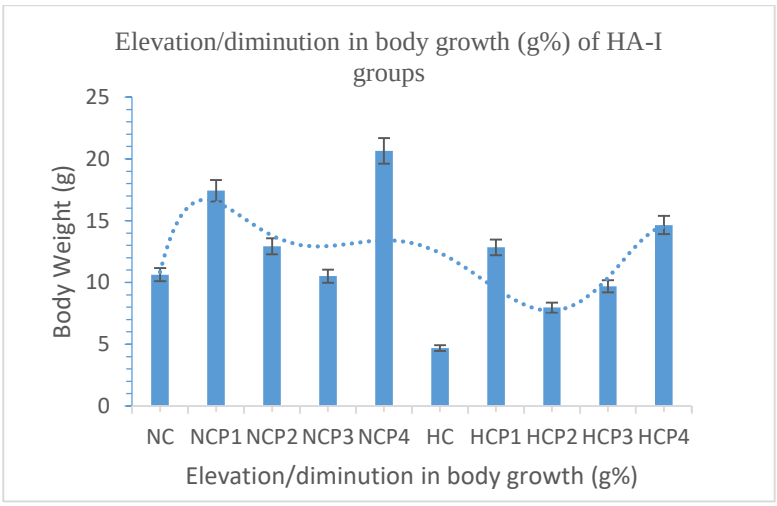
Physiological Parameter-Body weight was increased at the end of the experiment in NC, NCP1, NCP2, NCP3 and NCP4 groups compared to their initial body weight whereas the body weight of HA-II and HA-III group was decreased significantly (-7.03%; -13.73% respectively). It was observed that, though body weight of HA-I is higher than initial body weight but the growth rate was lower compare to NC and probiotic treated group (Table-3.3.1).

Table 3.3.1:Changes in body weight, kidney and liver somatic index of NC (14.7 psi), HA (11.8 psi; 9.3 psi; 7.3 psi) and probiotic treated group on 7th day of experiments.

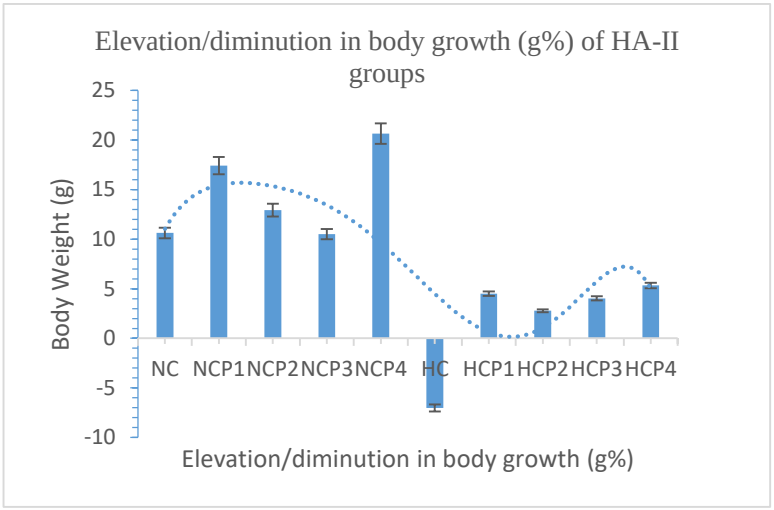
Different altitude with respective air pressure	Different groups	Initial body weight (g)	Final body weight (g)	Elevation/ diminution in body growth (g%)	Kidney somatic index	Liver somatic index
Positive control group Sea level (<500 m); Barometric pressure: 14.7 psi	NC	108.1±1.24	119.6±1.16	10.63	0.81±0.01[a]	2.28±0.07[a]
	NCP-1	106.1±3.52	124.6±4.16	17.43	0.83±0.02[a]	2.32±0.04[a]
	NCP-2	112.1±2.52	126.6±3.41	12.93	0.82±0.01[a]	2.33±0.03[a]
	NCP-3	114.1±5.12	126.1±2.26	10.51	0.84±0.01[a]	2.42±0.03[a]
	NCP-4	104.1±4.51	125.6±3.95	20.65	0.78±0.03[a]	2.12±0.02[b]
HA control (HA-I: 11.8 psi; HA-II: 9.3 psi; HA-III: 7.3psi)	HA-I	110.5±3.87	115.7±3.27	4.70	0.81±0.03[a]	2.29±0.02[a]
	HA-II	102.4±4.12	95.2±3.34	-7.03	0.76±0.03[a]	2.21±0.08[a]
	HA-III	108.8±1.87	94.6±1.89	-13.05	0.71 ± 0.02[b]	2.02±0.11[c]
HA-I + Probiotic	HACP-1	107.4±3.51	121.2±2.61	12.84	0.83±0.01[a]	2.32±0.08[a]
	HACP-2	110.4±2.51	119.2±1.65	7.97	0.83±0.02[a]	2.30±0.02[a]
	HACP-3	111.4±4.34	122.2±2.15	9.69	0.82±0.03[a]	2.31±0.03[a]
	HACP-4	109.2±2.51	125.2±4.61	14.65	0.82±0.02[a]	2.35±0.11[a]
HA-II + Probiotic	HACP-1	106.4±1.12	111.2±4.18	4.51	0.80±0.02[a]	2.32±0.01[a]
	HACP-2	107.2±1.52	110.2±2.15	2.78	0.81±0.03[a]	2.31±0.03[a]
	HACP-3	116.5±4.24	121.2±3.56	4.03	0.80±0.02[a]	2.33±0.07[a]
	HACP-4	112.4±2.98	118.2±4.18	5.33	0.83±0.04[a]	2.34±0.02[a]
HA-III + Probiotic	HACP-1	110.2±1.67	112.6±2.12	2.17	0.72±0.03[b]	2.26±0.10[a]
	HACP-2	111.5±1.60	108.6±1.12	-2.51	0.71±0.01[b]	2.25±0.11[a]
	HACP-3	112.2±2.65	105.6±1.98	-5.88	0.74±0.02[b]	2.17±0.04[b]
	HACP-4	116.2±5.67	111.6±2.10	-3.95	0.73±0.01[b]	2.22±0.11[a]

*Values as mean±SD within a row followed by superscripts (a, b, c) significantly different at P<0.05.
**CP-1:VSL#3; CP-2:TruBiotics; CP-3:Yogut; CP-4:Propolis Plus.

After administration of the four different probiotics in different four hypoxic treated groups (HACP1; HACP2; HACP3 and HACP4), the percentage of body weight was increased, although it was significantly lower in HA-II and HA-III groups as compared to control group due to hypobaric induced oxidative stress (Fig. 3.3.1).



A



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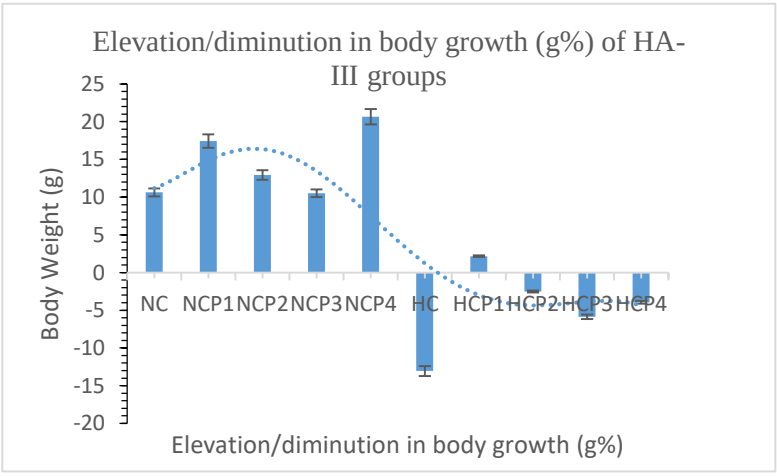


C

Fig. 3.3.1: Elevation/diminution in body growth (g%). (A: HA-I, Moderate altitude exposure; B: HA-II, High altitude exposure; C: HA-III, Extreme high altitude exposure.

From the study it was observed that the weight of kidney and liver in the group HA-I, HA-II and HA-III were also slightly decreased in comparison to probiotic treated group but not changed significantly in group HA-I (Table-3.3.1), which may due to higher metabolic rate, different energy output, loss of body water and several endocrine factors. When prebiotics were supplied to groups the final body weight increased though it was not similar to CP group (17.43%; 12.93%; 10.51%; 20.65% respectively). The definite reason for the increase in body mass is unknown. It may be a beneficial effect of probiotic bacteria which influence the development of gut microflora and had been manipulated to achieve increased feed conversion and pathogen reduction (Shannon *et al.,* 2002), enhanced the digestion of host animals and developed gut efficiency by rising nutrient absorption (Gritsenko *et al.,* 2000).

Haematological Analysis-Total RBC, total WBC and haemoglobin level were significantly increased in the blood of HA-I, HA-II and HA-III animals group, comparison to group NC. But expansion in HA-II and HA-III group is much higher with respect to group HA-I (Table 3.3.2). At hypobaric hypoxic condition partial pressure of oxygen (PO_2) was decreased which is responsible for the excessive secretion of erythropoietin to carry out cellular function by increasing blood RBC and Hb. It was a great ability for physiological adjustments to compensate for this reduced pressure gradient. In our study blood RBC, Hb and WBC were increased which supported the previous work (Mizuno *et al.,* 2008; Steven and Timothy, 2000; Louise *et al.,* 2006). It was noted that RBC, Hb and WBC of probiotics feed groups were also increased significantly ($P< 0.05$) with compared to the control group but lower than HA control group. It may be due to the beneficial immunomodulatory effects of probiotics.

Table 3.3.2: Effect of different atmospheric pressure and probiotics supplementation on haematological parameters (RBC, WBC and haemoglobin) of male rats.

Parameter	Groups	CP-1	CP-2	CP-3	CP-4
RBC [cumm×1000000].	NC	6.65±0.41^{a}	6.65 ±0.41^{a}	6.65 ±0.41^{a}	6.65 ±0.41^{a}
	NCP	6.72±0.23^{a}	6.59±0.34^{a}	6.74±0.24^{a}	6.81±0.34^{a}
	HA-I	8.78±0.31^{b}	8.78 ±0.31^{b}	8.78 ±0.31^{b}	8.78 ±0.31^{b}
	HA-I-CP	8.54±0.45^{b}	7.98±0.52^{b}	7.16±0.23^{a}	7.18±0.56^{a}
	HA-II	10.11±0.24^{c}	10.11 ±0.24^{c}	10.11 ±0.24^{c}	10.11 ±0.24^{c}
	HA-II-CP	9.78±0.32^{c}	9.46±0.21^{d}	9.13±0.16^{d}	8.28±0.17^{b}
	HA-III	12.54±0.18^{d}	12.54 ±0.18^{e}	12.54 ±0.18^{e}	12.54 ±0.18^{d}
	HA-III-CP	10.87±0.26^{e}	10.13±0.45^{c}	10.53±0.76^{c}	9.13±0.29^{b}
WBC/cumm×1000	NC	7.38±0.17^{a}	7.38±0.17^{a}	7.38±0.17^{a}	7.38 ±0.17^{a}
	NCP	7.61±0.27^{a}	7.21±0.17^{a}	7.41±0.38^{a}	7.31±0.41^{a}
	HA-I	7.98±0.34^{a}	7.98±0.34^{a}	7.98±0.34^{a}	7.98±0.34^{a}
	HA-I-CP	7.59±0.52^{a}	7.81±0.34^{a}	7.58±0.27^{a}	7.79±0.28^{a}
	HA-II	13.65±0.11^{b}	13.65±0.11^{b}	13.65±0.11^{b}	13.65±0.11^{b}
	HA-II-CP	8.12±0.76^{a}	8.10±0.56^{a}	8.98±0.76^{c}	8.45±0.11^{a}
	HA-III	14.32±0.63^{c}	14.32±0.63^{b}	14.32±0.63^{b}	14.32±0.63^{b}
	HA-III-CP	9.45±0.14^{d}	12.41±0.15^{c}	13.87±0.09^{b}	11.19±0.26^{c}
Hb gm%	NC	8.34 ±0.12^{a}	8.34±0.12^{a}	8.34±0.12^{a}	8.34±0.12^{a}
	NCP	8.76±0.34^{a}	8.44±0.16^{a}	8.14±0.35^{a}	8.23±0.27^{a}
	HA-I	10.45±0.15^{b}	10.45±0.15^{b}	10.45±0.15^{b}	10.45±0.15^{b}
	HA-I-CP	9.34±0.37^{a}	8.98±0.45^{a}	9.76±0.18^{c}	9.89±0.06^{c}
	HA-II	13.22±0.15^{c}	13.22±0.15^{c}	13.22±0.15^{d}	13.22±0.15^{c}
	HA-II-CP	9.56±0.23^{a}	12.87±0.56^{c}	11.34±0.35^{e}	11.98±0.45^{d}
	HA-III	13.89±0.34^{d}	13.89±0.34^{d}	13.89±0.34^{d}	13.89±0.34^{c}
	HA-III-CP	10.67±0.27^{b}	12.78±0.34^{c}	12.78±0.19^{f}	12.01±0.12^{c}

*Values as mean±SD within a row followed by superscripts (a, b, c, d, e) significantly different at P<0.05.

Antioxidant stress markers and toxicity study-

The result of ambient oxygen pressure is responsible for oxidative damage in different organs. At HA, reduced tissue oxygen transport is considered for the modifications of cellular energy producing pathways and mitochondrial function. This phenomenon termed as cellular dysoxia (McGinnis *et al.,* 2014). This adjustment alters the O_2 dependent metabolic pathways in generating of ROS and RNS, this leads to oxidative stress in cellular level. One of the best known of this enzyme is SOD. Superoxide is one of the most plentiful ROS formed by the mitochondria, while SOD catalyses the breakdown of superoxide into hydrogen peroxide and water and is consequently a central controller of

ROS levels (Landis and Tower, 2005). Oxidative stress and lipid peroxidation are early events related to the generation of radicals during hypobaric hypoxia. Previous studies have demonstrated that acute exposure to high altitude increases the lipid peroxidation and suppresses the antioxidant defence mechanisms in renal tissues (Samanta *et al.*, 2014). After the exposure of low atmospheric pressure (group HA-II and HA-III animals), the antioxidant enzymatic activity in blood plasma, kidney, liver, large intestine and small intestine were also decreased significantly in all experimental groups in compared to group C (control). At the end of the experiment, the activities of those enzymes in above mentioned tissues were decreased significantly in Group HA-II and HA-III. The rate of alteration was much more in the liver than kidney and blood plasma. In probiotic administrated groups, these alterations were maintained which indicate the advantageous effect of probiotics (Table 3.3.3). However, at hypobaric hypoxia, MDA levels were significantly increased, when compared with normal control rats. On administration of these four commercial probiotics, the levels of MDA decreased significantly when compared with the control group (Fig. 3.3.2). At HA, animals were found to have a decreased level of SOD and CAT activity when compared with the normal control rats. This may due to enhanced lipid peroxidation or inactivation of the antioxidant enzymes. When rats were treated with the probiotics, the activity of SOD and CAT was increased significantly as compared with the HA group ($p<0.05$). The generation of reactive oxygen species appears as an early occurrence which precedes cell damage in hypoxic hepatotoxicity (Manov *et al.*, 2002). In the study, the hypoxia-induced nephrotoxicity was investigated by biochemical measurements and histopathological analyses, which coincide with the observations of Corcoran et al in the year of 1985.

A line of evidence suggests that intracellular glutathione plays an essential role in the detoxification and prevention of toxicity in the liver and kidney (Newton *et al.*, 1996). The

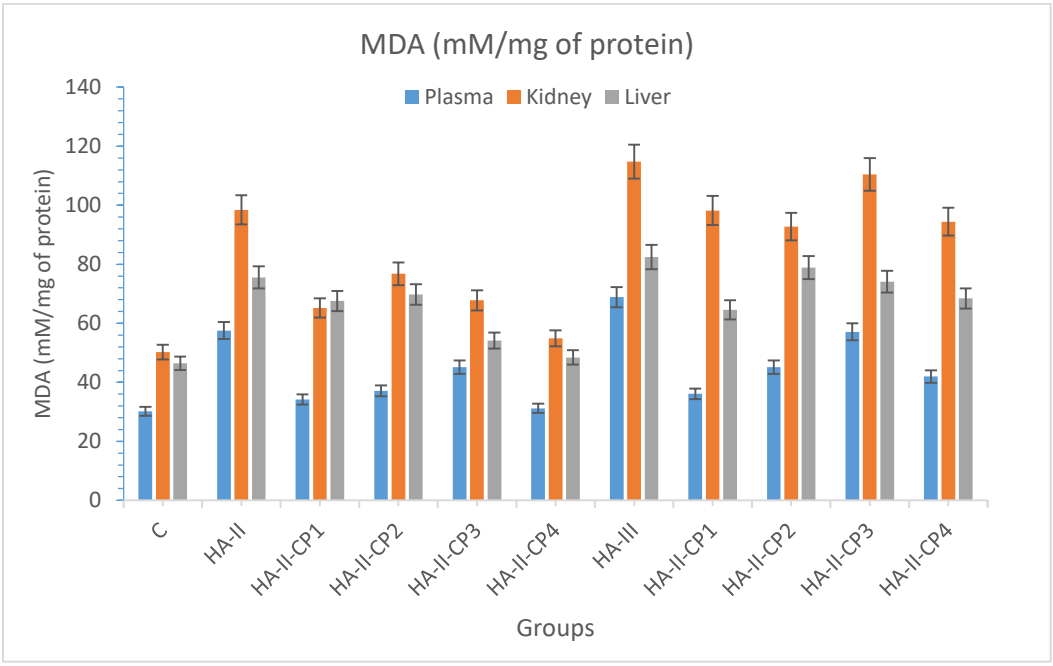
generation of the reactive oxygen species appears as an early event which precedes intracellular glutathione depletion and cell damage in the liver (Manov *et al.*, 2002). Here, hypobaric hypoxia also caused a significant decrease in glutathione content. The administration of probiotics bacteria was found to be helpful for the uplifting of glutathione reduction. ROS can be detoxified by a number of antioxidant enzymes and glutathione contents which protect cells from oxidative damage by scavenging it. Probiotics are maintaining the level of glutathione which may increase the level of GSH enzyme and results in higher resistance of the cell toward oxidative stress. After probiotics treatment, the level of serum AST and ALT in hypobaric induced rats were decreased as compared with the normal group possibly due to the removal of toxic compound by the probiotic bacteria (Fig. 3.3.3).

Table 3.3.3: The activity of catalase and SOD level of the plasma, kidney, liver, large intestine and small intestine epithelia of the Control (C; 14.7 psi) HA (9.3 psi; 7.3 psi) and probiotic treated groups at the 7th day of hypobaric hypoxic stress

Parameter	Catalase (mM/mL/min)					SOD (mM/mg/min)				
Groups	**Plasma**	**Kidney**	**Liver**	**LI* epithelia**	**SI** epithelia**	**Plasma**	**Kidney**	**Liver**	**LI* epithelia**	**SI** epithelia**
C	0.73±0.02^{a}	0.78±0.04^{a}	0.81±0.03^{a}	24.35±0.13^{a}	245.27±2.31^{a}	1.05±0.17^{a}	2.53±0.19^{a}	0.98±0.06^{a}	6.14±0.56^{a}	114.11±1.23^{a}
HA-II	0.41±0.07^{b}	0.36±0.07^{b}	0.24±0.06^{b}	16.21±0.09^{b}	176.89±3.56^{b}	1.02±0.11^{a}	0.94±0.17^{b}	0.45±0.13^{b}	5.32±0.03^{b}	86.76±2.78^{b}
HA-II-CP1	0.68±0.03^{a}	0.72±0.14^{a}	0.76±0.12^{a}	23.17±0.67^{a}	234.12±5.13^{c}	0.98±0.25^{a}	2.35±0.27^{a}	0.96±0.04^{a}	6.15±0.23^{a}	105.61±6.56^{a}
HA-II-CP2	0.64±0.02^{c}	0.76±0.02^{a}	0.65±0.02^{c}	24.63±0.34^{a}	238.54±0.98^{c}	0.89±0.07^{a}	1.13±0.19^{c}	0.95±0.08^{a}	6.12±0.06^{a}	109.13±3.53^{a}
HA-II-CP3	0.56±0.09^{d}	0.68±0.10^{a}	0.69±0.12^{a}	19.98±0.91^{c}	221.44±2.71^{d}	0.94±0.1^{a}	1.34±0.09^{c}	0.89±0.07^{c}	6.02±0.17^{a}	100.38±1.78^{c}
HA-II-CP4	0.65±0.06^{a}	0.81±0.21^{a}	0.83±0.01^{a}	24.78±0.21^{a}	229.32±5.76^{c}	0.95±0.14^{a}	1.27±0.14^{c}	0.92±0.10^{a}	6.09±0.11^{a}	105.71±3.76^{a}
HA-III	0.29±0.08^{e}	0.15±0.11^{c}	0.11±0.02^{c}	10.31±0.45^{d}	96.13±4.16^{e}	0.35±0.04^{b}	0.35±0.32^{d}	0.22±0.13^{d}	4.72±0.18^{c}	80.13±5.26^{b}
HA-II-CP1	0.64±0.02^{c}	0.58±0.11^{d}	0.63±0.21^{a}	19.36±0.03^{e}	210.23±1.16^{f}	1.07±0.3^{a}	2.11±0.12^{e}	0.72±0.11^{e}	5.34±0.20^{b}	90.12±0.06^{d}
HA-II-CP2	0.53±0.23^{d}	0.46±0.03^{e}	0.57±0.10^{c}	22.78±0.23^{a}	112.34±6.78^{g}	0.90±0.04^{a}	1.89±0.14^{e}	0.45±0.12^{b}	5.23±0.07^{b}	83.78±0.56^{b}
HA-II-CP3	0.48±0.18^{d}	0.52±0.15^{e}	0.63±0.11^{c}	14.78±0.17^{f}	118.32±3.76^{g}	0.65±0.14^{c}	1.23±0.10^{c}	0.44±0.06^{b}	5.11±0.11^{b}	92.56±0.89^{d}
HA-II-CP4	0.61±0.07^{a}	0.45±0.13^{e}	0.55±0.09^{d}	16.23±0.11^{b}	196.45±5.56^{f}	0.96±0.12^{a}	1.11±0.06^{c}	0.67±0.14^{e}	5.67±0.15^{b}	83.76±0.18^{b}

***Large intestine epithelia; **Small intestine epithelia**

Values as mean±SD within a column followed by superscripts (a, b, c, d) significantly different at P<0.05.



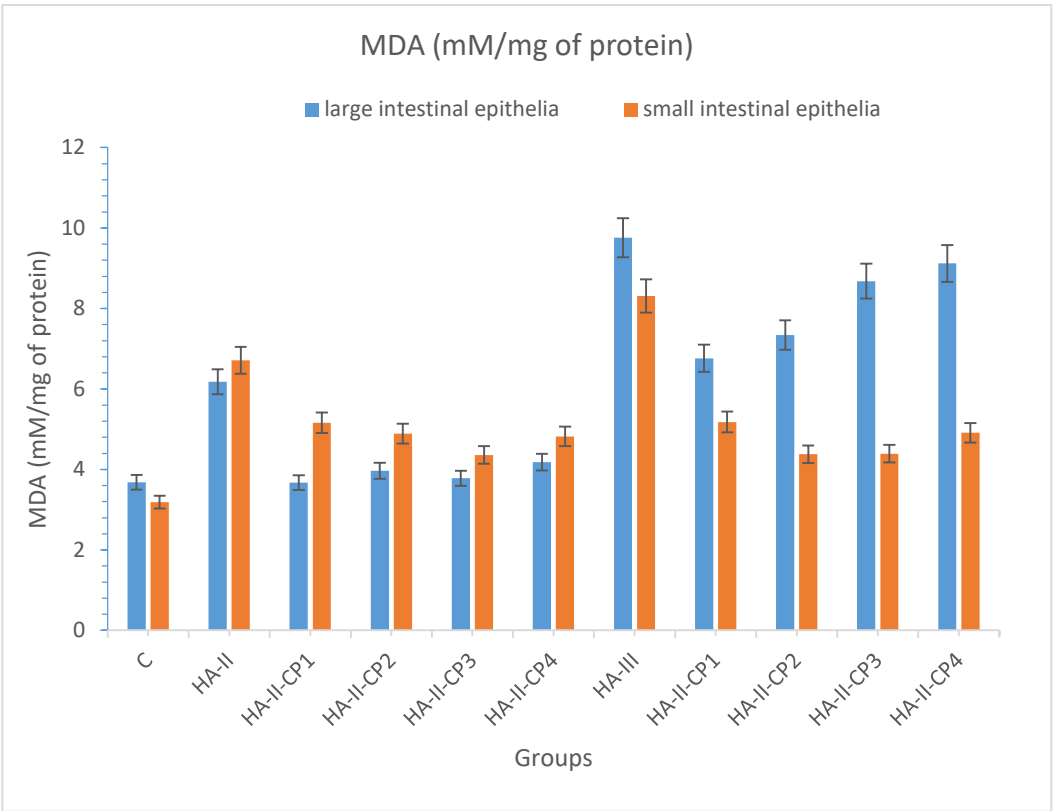


Fig. 3.3.2: The activity of MDA of the plasma, kidney, liver, large intestine and small intestine epithelia of the C and the different HA group at 7th day of hypobaric hypoxic stress

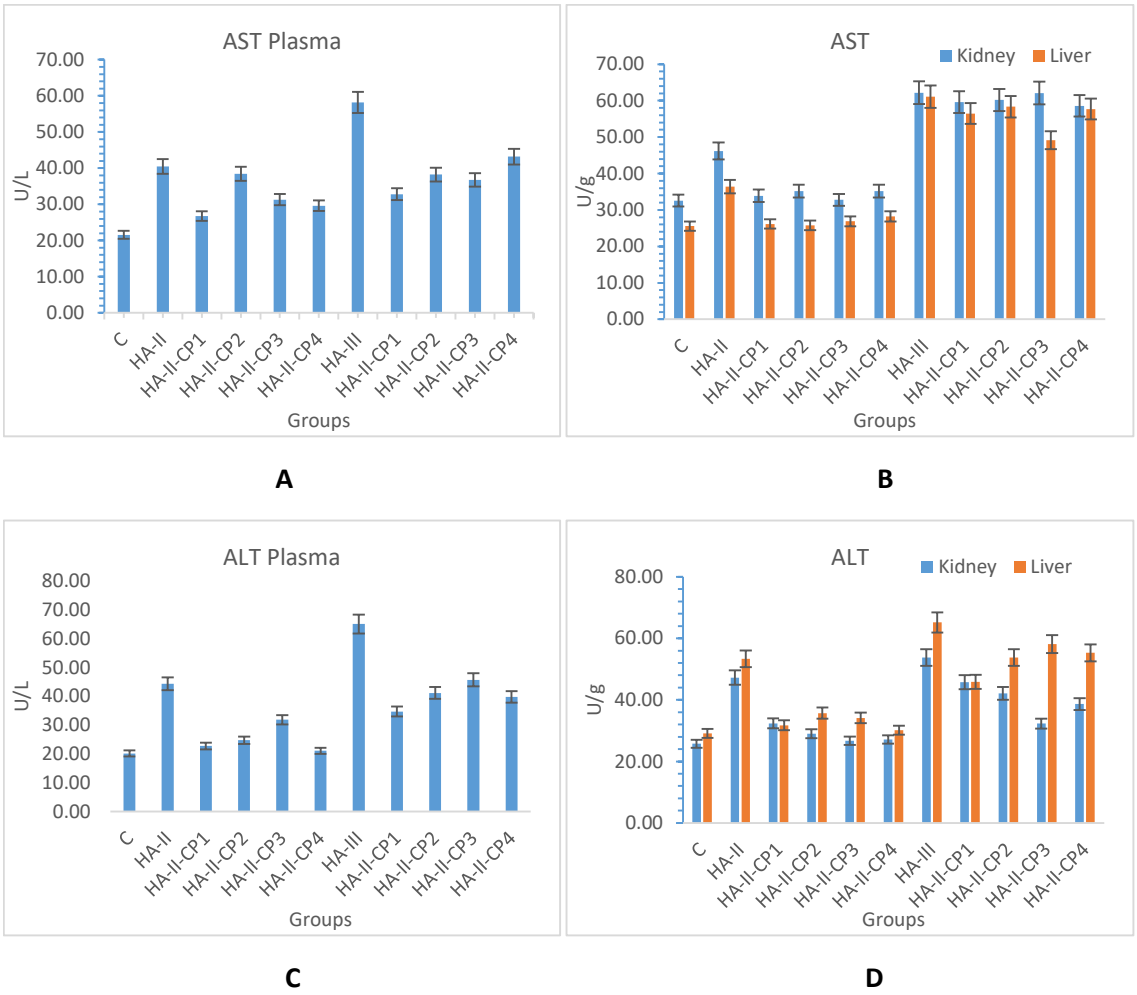


Fig. 3.3.3: The activity of AST and ALT of the plasma, kidney and liver of the C and the different HA group at 7th day of hypobaric hypoxic stress.

A: Plasma AST; B: Kidney & Liver AST
C: Plasma ALT; B: Kidney & Liver ALT

Lipid profile study-

Most of the current information concerning the effect of hypoxia on lipid metabolic pathways point out the changes of metabolism in plasma lipid. The liver plays an important key role in the metabolism of lipid including its biosynthesis, lipoprotein secretion, and reverses the transport of cholesterol. Lipid biosynthesis in the liver is regulated by a family of transcription factors and the sterol regulatory element binding proteins (SREBPs) which include SREBP-1 and SREBP-2 (Shimano, 2001). SREBP-1 preferentially regulates enzymes involved in fatty acid synthesis including stearoyl-CoA desaturase 1(SCD-1). SREBP-2 regulates cholesterol biosynthesis and its uptake, especially through the regulation of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (Bruder *et al.,* 2005; Perry *et al.,* 2007).

In the present study, it was revealed that the blood total cholesterol, LDL and triglycerides (TG) levels were increased with the largest effect as evidenced on 7^{th} day at HA-II and HA-III, whereas HDL level was decreased in blood on 7^{th} day ($p<0.05$), which supported previous studies (Table 3.3.4). Acclimatization to high altitude and its resultant erythropoiesis possibly increases serum cholesterol level. Consequently, the relocation to high altitude may increase the risk of arteriosclerotic cardiovascular disease (Jonathan, 1996). In the probiotic treated group compared with placebo probiotics treatment could significantly reduce the value of TC and increased the value of HDL which is similar to the work of Li *et al.,* in 2004. The TG and LDL level was significantly decreased in the probiotic treated groups as compared to HA control group. Gilliland et al. have shown some strains of *Lactobacillus acidophilus* may decrease cholesterol absorption by enhancing the binding of cholesterol to the intestinal lumen in the year of 1990. Other conceivable cholesterol lowering properties of probiotics are deconjugation of bile by bile salt hydrolysis, binding of cholesterol to cellular surface and co-precipitation of

cholesterol with the deconjugated bile (Gilliland *et al.*, 1985). This study showed no significant improvement in total serum cholesterol, LDL-cholesterol and or HDL-cholesterol after the administration of probiotics in hypobaric hypoxic treated animals.

Table 3.3.4: Blood Cholesterol level, Total triglyceride level, Total VLDL level, Total HDL Level of different hypobaric exposure animals.

Parameter	Groups	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)
Plasma	**C**	54.17±2.11^{a}	36.70±2.10^{a}	14.30±1.10^{a}	55.76±1.09^{a}
	HA-II	73.12±1.23^{b}	24.22±2.42^{b}	21.12±1.56^{b}	65.78±2.56^{b}
	HA-II-CP1	55.56±3.14^{a}	25.88±1.52^{b}	15.76±0.57^{a}	56.76±0.89^{a}
	HA-II-CP2	52.14±3.09^{a}	22.13±0.98^{b}	17.89±1.81^{a}	58.45±1.02^{c}
	HA-II-CP3	48.31±2.15^{c}	19.21±0.56^{c}	15.11±1.21^{a}	58.81±1.43^{c}
	HA-II-CP4	42.78±1.11^{d}	21.56±2.12^{b}	17.18±1.56^{a}	57.92±2.56^{c}
	HA-III	81.56±3.47^{e}	20.18±1.89^{b}	28.76±0.98^{c}	78.29±1.98^{d}
	HA-II-CP1	78.89±1.45^{e}	35.78±1.54^{a}	15.45±0.95^{a}	61.93±2.45^{b}
	HA-II-CP2	76.45±4.51^{b}	26.45±1.27^{b}	27.14±0.89^{c}	67.62±1.76^{b}
	HA-II-CP3	77.34±1.21^{e}	30.78±0.33^{d}	20.12±1.64^{b}	65.69±1.45^{b}
	HA-II-CP4	79.45±2.31^{e}	31.34±0.54^{d}	17.56±1.87^{a}	58.72±2.76^{c}

*Values as mean±SD within a column followed by superscripts (a, b, c, d, e) significantly different at P<0.05. (A) Triglycerides (TG; mg/dl); (B) Total cholesterol, (T-Chol; mg/dl); (C) LDL-cholesterol, (LDL-Chol; mg/dl); (D) HDL-cholesterol (HDL-chol; mg/dl); and (E) VLDL cholesterol (VLDL; mg/dl),

Conclusion:

High altitude hypoxia causes the imbalance of haematological and physiological parameters as well as induce the oxidative stress. The impact of HA was more intense upto seven days of acclimatization at HA. Probiotic from different commercial products were treated along with hypoxic stress upto seven days and the following results are found.

1. Treatment of commercial probiotics maintain the body weight and physiological parameters during seven days of stress.
2. Probiotic treatment helps to resettle the blood RBC, Hb and WBC in contrast to hypoxic stress.

3. Probiotic treatment improved the antioxidant defence mechanism in different organs like liver, kidney and the mucosal layer of both small and large intestines to reduce the oxidative stress.
4. Lipid profile was significantly lowered in different probiotics treated group as compared to HA group.
5. Probiotics were effective against HA-I and HA-II groups however it was not potential in HA-III group which is above 18000 ft.

Experiment No 3.4 - Study of uremic profile of rats at different altitude after supplementation of probiotic.

Materials and methods:

Animals were selected randomly and divided into four groups having six animals each (four sets of each group). Group NC served as control and were exposed to normal room air (normoxia). Group HA-I, HA-II and HA-III were exposed to different barometric pressure. Different commercial probiotics were administrated orally and groups ware labeled as CP-1 (VSL#3); CP-2 (TruBiotics); CP-3 (Yogut); CP-4 (Propolis Plus). The exposure was carried out in a decompression chamber (Instrumentation India, India) for 7 days.

Result and discussion:

The blood urea and creatinine levels were significantly increased in group HA-I, HA-II and HA-III animals (hypobaric-hypoxic exposure animals) compared to group NC. However, the increase of urea and creatinine was significantly higher in HA-II and HA-III with respect to group HA-I (Table 3.4.1.). Blood urea and creatinine levels are nephrotoxicity biochemical markers (Pathan *et al.*, 2013). In case of renal disease, the level of urea accumulates in the blood as because the rate of urea production exceeds than the rate of clearance. The elevation of urea and creatinine levels in the blood is taken as the index of nephrotoxicity. Creatinine, on the other hand is mostly derived from endogenous sources by the breakdown of creatinine in the tissues. As a result, the serum or blood urea concentration is frequently considered as a more reliable renal function forecaster in respect to plasma creatinine (Vanholder and Smet 1999). So blood urea concentration can be considered as a more dependable renal function indicator than plasma creatinine also. In the study, probiotic treatments on the various group were found to be responsible for the reduction of urea nitrogen (BUN) and creatinine in the plasma as

compared to hypobaric-hypoxic exposed animals (group HA). In this study, hypoxia-induced nephrotoxicity was characterized by marked elevations in the circulating level of BUN and blood creatinine as found in control rat (group HA). In hypoxic induced nephrotoxicity a significant ($P<0.05$) increase of plasma urea and creatinine concentrations in the group of HA (hypoxia induced) rats was found in comparison to normal group (Group NC). Moreover, the oral administration of commercial probiotics responsible for the decreased level of plasma urea and creatinine in group HA-CP-1, HA-CP-2, HA-CP-3 and HA-CP-4 as compared to the HA group. It may be due to the presence of urease positive bacteria in commercial probiotics usually thought to reduce the overall blood uremic toxins through enteric dialysis (Ranganathan *et al.*, 2006).

Table 3.4.1: Changes of uremic profile of rats at different altitude after supplementation of probiotic.

Parameter	Groups	CP-1	CP-2	CP-3	CP-4
Urea (mg/dl)	NC	21.26 ± 0.45^{a}	21.26 ± 0.45^{a}	21.26 ± 0.45^{a}	21.26 ± 0.45^{a}
	NCP	20.06 ± 0.65^{a}	23.89 ± 0.23^{b}	20.56 ± 0.89^{a}	20.78 ± 0.71^{a}
	HA-I	27.56 ± 1.23^{b}	27.56 ± 1.23^{c}	27.56 ± 1.23^{b}	27.56 ± 1.23^{b}
	HA-I-CP	22.16 ± 0.78^{a}	22.07 ± 0.79^{a}	26.78 ± 0.77^{b}	22.14 ± 0.12^{a}
	HA-II	36.87 ± 1.27^{c}	36.87 ± 1.27^{d}	36.87 ± 1.27^{c}	36.87 ± 1.27^{c}
	HA-II-CP	21.89 ± 0.1^{a}	32.89 ± 0.76^{e}	29.12 ± 1.76^{b}	23.34 ± 0.33^{a}
	HA-III	45.76 ± 2.46^{d}	45.76 ± 2.46^{f}	45.76 ± 2.46^{d}	45.76 ± 2.46^{d}
	HA-III-CP	29.78 ± 0.32^{b}	38.16 ± 1.52^{d}	35.98 ± 0.78^{c}	34.56 ± 1.56^{c}
Creatinine (mg/dl)	NC	0.55 ± 0.05^{a}	0.55 ± 0.05^{a}	0.55±0.05a	0.55 ± 0.05^{a}
	NCP	0.56 ± 0.11^{a}	0.48 ± 0.03^{a}	0.53±0.10a	0.57 ± 0.03^{a}
	HA-I	0.59 ± 0.12^{a}	0.59 ± 0.12^{a}	0.59±0.12a	0.59 ± 0.12^{a}
	HA-I-CP	0.53 ± 0.04^{a}	0.51 ± 0.13^{a}	0.54±0.07a	0.57 ± 0.01^{a}
	HA-II	0.92 ± 0.09^{b}	0.92 ± 0.09^{b}	0.92±0.09b	0.92 ± 0.09^{b}
	HA-II-CP	0.55 ± 0.01^{a}	0.58 ± 0.18^{a}	0.59±0.02a	0.67 ± 0.02^{c}
	HA-III	1.12 ± 0.05^{c}	1.12 ± 0.05^{c}	1.12 ± 0.05^{c}	1.12 ± 0.05^{d}
	HA-III-CP	0.76 ± 0.04^{b}	0.96 ± 0.03^{b}	0.93±0.04b	0.59 ± 0.05^{a}

*Values as mean±SD within a colum followed by superscripts (a, b, c, d, e, f) significantly different at $P<0.05$.

In this experiment, the urinary KIM-1 level was significantly ($p<0.05$) increased in animals of HA group as compared to other animals of NC group like HA-I, HA-II and HA-III. Though this alteration was not significant in group HA-I animals (Fig 3.4.1). Here, the of level KIM-1 of group HA-II and HA-III animals were striking ($p<0.05$). The differences of the level KIM-1 were also found in other groups of the animal such as HA-CP-1, HA-CP-2, HA-CP-3 and HA-CP-4. So, the probiotics also prevent the hypoxia induced kidney diseases and maintain the kidney function as well. KIM-1 is a novel biomarker of kidney injury and also predicts the progression of ESRD. KIM-1 helps to indicate injury to the proximal tubule of the kidney. During kidney dysfunction in proximal tubular cells KIM-1 causes the increase of cellular responses. In the lumen, the ectodomain of KIM-1 expresses and serves as a novel urinary marker for renal dysfunctionalities (Han *et al.*, 2002).

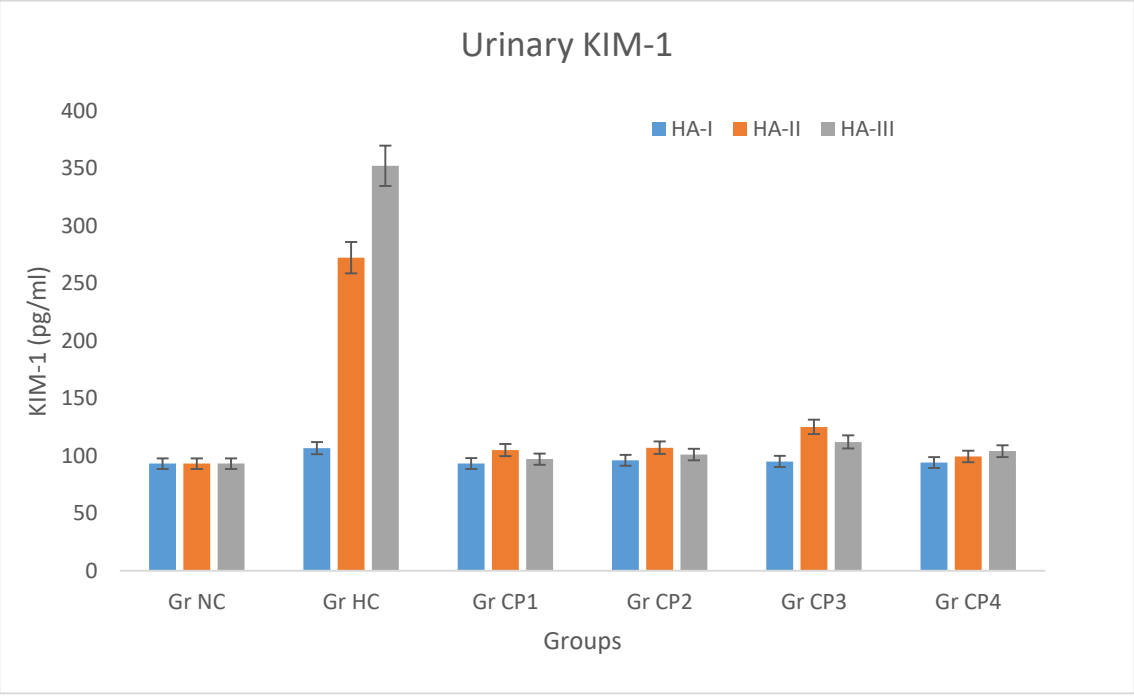


Fig 3.4.1: Effect of therapeutic efficacy of commercialprobioticon urinary KIM- 1 level. Data are expressed as Mean±SE (n=6). ANOVA followed by multiple two tail t-test. Bars for a specific data differ from each other significantly ($p<0.05$).

From the recent study, it was revealed that the KIM-1 has proven specific urinary biomarker of renal diseases (Sutton, 2009). This study has revealed that the commercial probiotics decreased the urinary KIM-1 level in the experimental animals and as a consequence, the level of urinary KIM-1 was increased in the hypobaric hypoxia induced groups of the animal as compared to the control. So, the probiotics are an indispensable factor for the maintaining of kidney functions and health at an optimum level.

Conclusion:

Hypobaric hypoxia has an adverse effect and probiotic bacteria has lot of beneficial effects on human health. From the study, it is concluded that

1. Hypoxia induced nephrotoxicity showed a significant ($P<0.05$) increase in the plasma urea and creatinine concentrations in the Group HA (hypoxia induced) rats as compared to the Group NC. Moreover, oral administration of commercial probiotics ($P<0.05$) decreased plasma urea and creatinine in group HA-CP-1, HA-CP-2, HA-CP-3 and HA-CP-4 as compared to the Group HA.
2. In addition to the reduction of uremic profile, probiotic treatment decreased the KIM-1 level as compare to hypoxic treated groups. Hence probiotics inhibit kidney injury and maintain kidney health.

Experiment No 3.5- Histological analysis of kidney and liver tissue by light and scanning microscopy

Materials and methods:

For histological study, kidney and liver of all the animals from all the groups of respective experiment were dissected out and then fixed in Bouin's solution. After fixation tissues were embedded in paraffin wax. Sections were prepared in microtome (weswox) at 5µm thickness. All the sections were finally stained with hematoxylin-eosin as per standard protocol and then examined under compound microscope at 40x magnification for detection any pathological and morphological changes (Mani, 2010; Khorsandi and Orazizadeh, 2008; Adeneye *et al.*, 2008; Lee *et al.*, 2006). For study of scanning electron microscopy fresh intestinal tissue was rinsed with cold saline (0.9% NaCl) and cut into 5 mm×5 mm sections, fixed in 2.5% glutaraldehyde, 10% osmium, and dehydrated in sucrose solution having PBS. Then it was gold coated and observed under scanning electron microscope (ZEISS, IIT-Kharagpur). The arrangement of microvilli, deformed and exfoliated villi and the intercellular space between epithelia were examined (Adak *et al.*, 2014).

Result and discussion:

The histological pattern of control animals showed kidney section with normal tubular and intact glomeruli and Bowman's capsule (Fig. 3.5.1A). Hypobaric hypoxia induced groups treated with probiotics 10^9CFU/mL/100 g of body weight/day for 7 days to group HA-CP-1, HA-CP-2, HA-CP-3 and HA-CP-4 animals also showed control like histo architecture with normal glomerulus and normal tubules as the effect of probiotics prevent the hypobaric hypoxia induced nephrotoxicity (Fig. 3.5.1A). It was showed that kidney sections of hypobaric hypoxic exposed animal of group HA-III showed the massive

glomerular and renal tubular damage by inflamed and necrotic epithelial cells and after treatment with probiotic this was partially repair by probiotics.

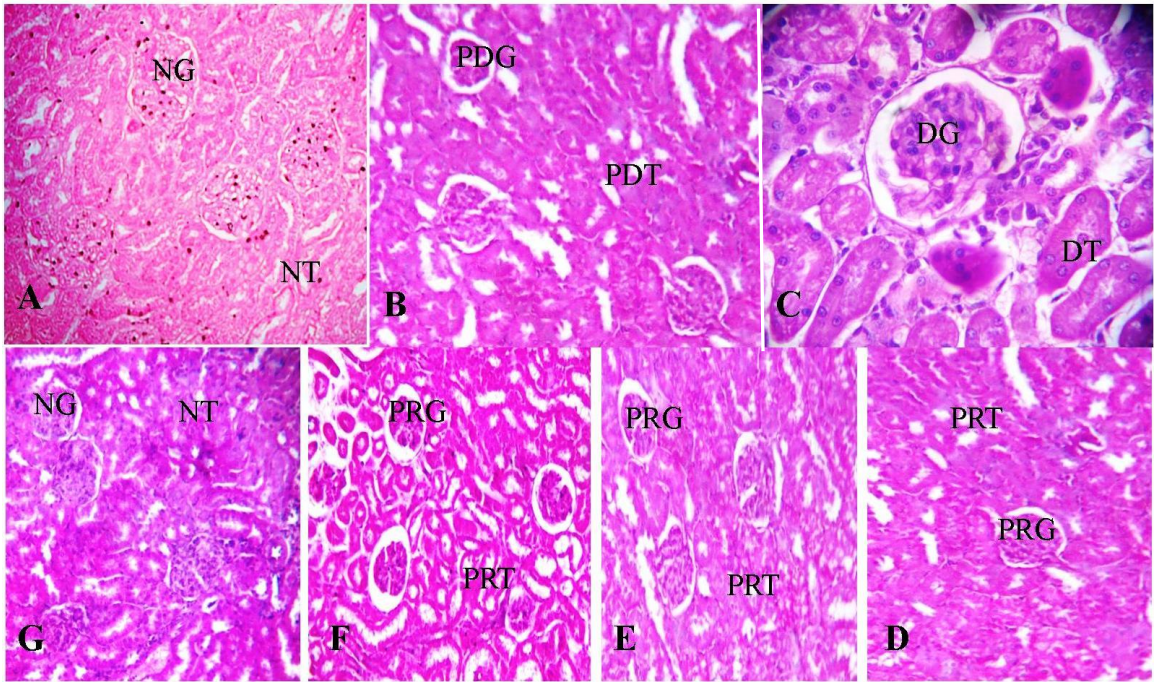


Fig. 3.5.1: Representation of light microscopy of kidney tissue section Hematoxylin-eosin stain X 100. A: Histology of kidney tissue in group C (Control), B: Histology of kidney tissue in group HA-II (12000 feet/3657 m), C: Histology of kidney tissue in group HA-III (18000 feet/5486 m), D: Histology of kidney tissue in group HA-III+CP1, E: Histology of kidney tissue in group HA-III+CP2, F: Histology of kidney tissue in group HA-III+CP3, G: Histology of kidney tissue in group HA-III+CP4

NG: Normal glomerulus, NT: Normal renal tubules with intact well organized cellular boundary that are not affected due to the lower atmospheric pressure, DG: Damaged glomeruli, DT: Damaged tubules are dilated with loss of cellular boundary PD: Partially damaged and PR: Partially repaired.

Histological analysis of different parts of liver tissue of normobaric control (C), hypobaric control (HA-II and HA-III) and probiotic treated groups of animals have been presented in figure 3.5.2 (A-G). Control or group C showed normal architecture with spoke like intact and well organized hepatocytes around central vein (Section A). Liver section of group HA-II and HA-III animals showed degeneration of hepatic cells with disorganization of liver tissue (Section B & C). Co-administration of probiotics in group HA-II and HA-III

animals showed normal arrangement of this tissue and there was better organization of hepatocytes around the central vein then group HA-III+CP4 animals (Section G) and showed well organized liver tissue with normal hepatocytes. So, this commercial probiotic prevents the drug induced hepatic necrosis.

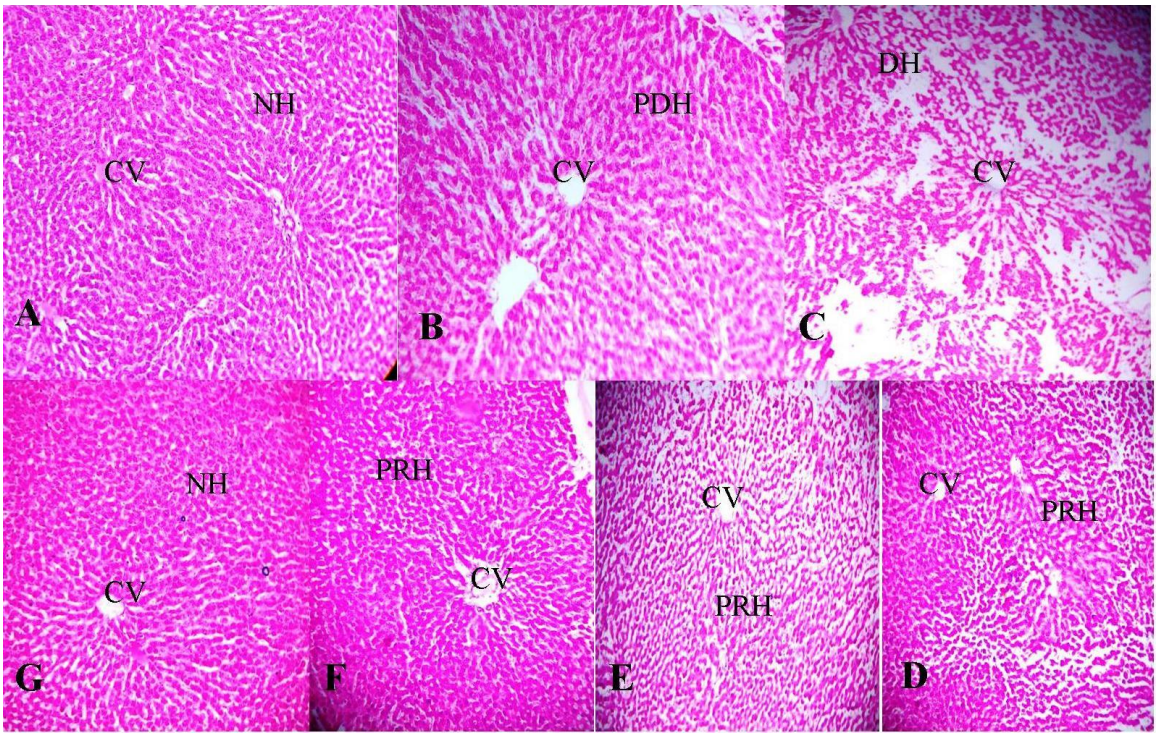


Fig. 3.5.2: Fig. 3.5.1: Representation of light microscopy of liver tissue section Hematoxylin-eosin stain X 100. A: Histology of liver tissue in group C (Control), B: Histology of liver tissue in group HA-II (12000 feet/3657 m), C: Histology of liver tissue in group HA-III (18000 feet/5486 m), D: Histology of liver tissue in group HA-III+CP1, E: Histology of liver tissue in group HA-III+CP2, F: Histology of liver tissue in group HA-III+CP3, G: Histology of liver tissue in group HA-III+CP4

CV: Central vain, NH: Normal hepatocytes, PDH: Partially damaged hepatocytes, DH: Damaged hepatocytes and PRH: Partially repaired hepatocytes.

In rat model, the villus arrangement was regular in C group (Figure 3.5.3 A) but it was disordered in HA-III group after 7 days (Figure 3.5.3 B) of hypoxia. In large intestine, the projection of villus in C group (Figure 3.5.3 A) was higher and ordered whereas in HA-III group it was distorted due to ischemic damage (Figure 3.5.3 B). Whereas the villus arrangement of HA-I and HA-II does not change significantly. Scanning electron

microscopy also revealed severely injured intestinal mucosa as well as evident atrophy and disordered villi in HA-III group in respect to orderly intestinal villi of C group (arrow indicated in Fig. 3.5.3A and Fig. 3.5.3C). This intestinal mucosal atrophy, mucosal barrier dysfunction was also strongly correlated with oxidative damage of epithelial layer. Whereas probiotics supplemented groups showed normal intestinal villi likely to C group (Fig. 3.5.3D).

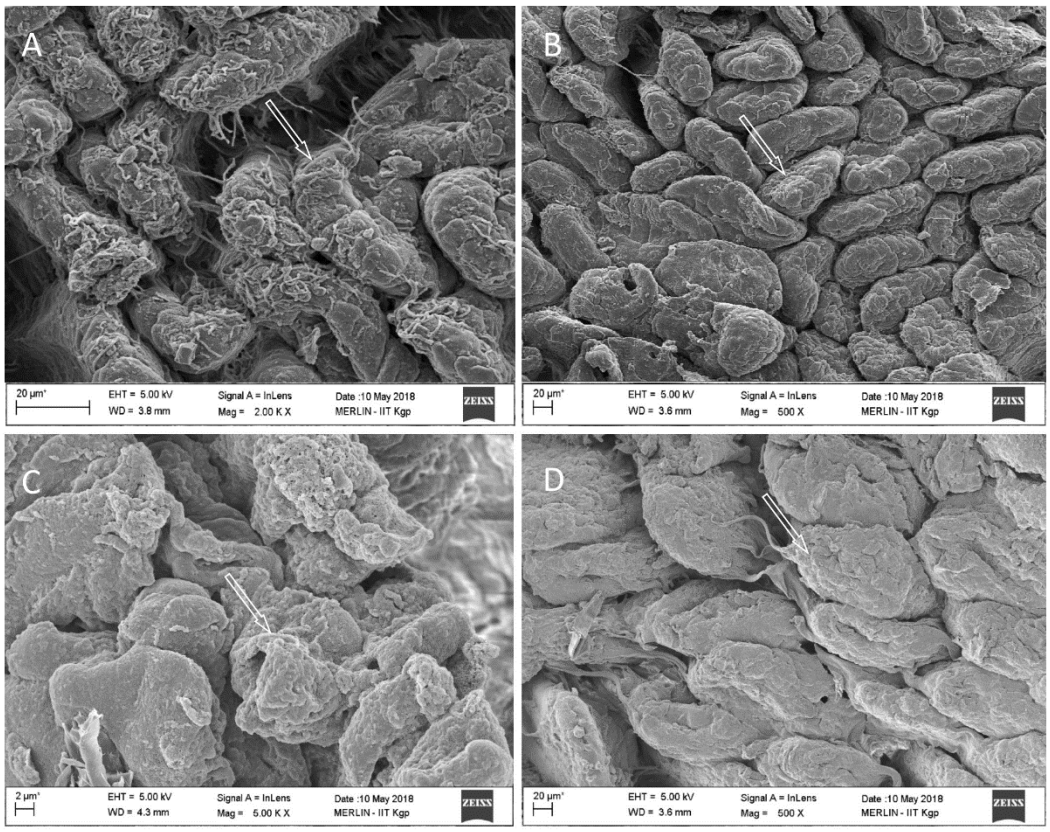


Fig. 3.5.3: Larger villus projection of small intestine with clear structure as well as complete and orderly villi in group C (A) and group HA-II (B), smooth villus projection but with distorted mucosa along disordered villi in HA-III group of small intestine (C). Probiotic (VSL#3) treated group showed smooth villus projection (D) with clear and complete structure as well as orderly villi.

In rat model, the villus arrangement was regular in group C and HA-II animals (Fig. 3.5.3 A & Fig. 3.5.3 B) but it was disordered in HA-III group after 7 days (Fig. 3.5.3 C) of

hypoxia due to ischemic damage. SEM photography confirmed that small intestinal inflammation with epithelial barrier dysfunction. This may lead to the activation of the mucosal and lymphoid associated immune system due to infiltration of a broad range of luminal insults into systemic. Treatment with commercial probiotic VSL#3 inhibits the mucosal damage and infiltration of endotoxin into the systemic circulation. According to Iji & Tivey (1998), Probiotic bacteria may bind in the intestinal mucosa, and the colonization in the intestine reduced pathogenic bacteria. Therefore, besides a lower infection incidence, there is an increase in the absorption of available nutrients, a mechanism that directly affects the recovery of the intestinal mucosa (Velasco, 2006, Zhou *et al.,* 2011). This also reflected the impairment of intestinal innate immune barrier in hypoxic state to protect against the intestinal pathogens (Deplancke & Gaskins, 2001).

Conclusion:

Lower level of antioxidants imparts oxidative damage that is evident from histological study.

1. The damage of renal tubules and hepatocytes in HA-III groups were more severe than HA-I and HA-II group.
2. Probiotic treatment improves the damage of kidney and liver in HA-I and HA-II groups in contrast to HA-III group.
3. The scanning electron microscopic analysis revealed that the hypoxic state diminished the proliferation of small intestinal epithelia. Whereas, probiotic (VSL#3) supplemented group showed normal intestinal villi as found in the control group. Damage of intestinal epithelia restored by probiotic treatment may protect the infiltration of toxin into the blood.

Comparative study of intestinal enzymes before and after probiotic supplementation of experimental animals at hypobaric condition.

Aims of study: The present study was carried out to measure the alteration of intestinal enzymes including gas formation abilities of intestinal microbial consortium in relation to probiotic supplementation of experimental animals at hypobaric condition.

Experiment No. 3.6 - Metabolic activities of gut after probiotic supplementation of experimental animals at hypobaric condition

Materials and methods:

The cultivable microflora was enumerated on agar plates on the basis of colony-forming units (CFU/g). CFU represent the actual number of bacteria present in the faecal samples. These CFU values were converted to their logarithmic value and compared with the corresponding experimental set of specified conditions. Details experimental procedure is discussed in chapter 2.

Result and discussion:

Analysis of faecal sample-

The population of total aerobes was 5.37 $\log_{10}$CFU/g with positive GDI 1.06, in the faecal matter of NC group (without exposure animals) after seven days of experiment. But it was reduced drastically in HA-I, HA-II and HA-III groups ($p<0.05$) by 4.12, 3.90 and 3.67 with final GDI of –1.19, –1.34 and −1.45 ($\log_{10}$C/ $\log_{10}$HA-15) respectively (Table 3.6.1). The quantity of total anaerobes was increased in HA-I, HA-II, and HA-II groups with the positive GDI of +1.16, +1.31 and +1.60 respectively (Table 3.6.2). The ratio (ratio of $\log_{10}$CFU/g) of total aerobe and anaerobic population was altered from 1:1.5 (control) to 1:1.66 (HA-I), 1:2.07 (HA-II) and 1:2.76 (HA-III) accordingly after 7 days of exposure at hypobaric-hypoxic environment. Though the ratio (ratio of $\log_{10}$CFU/g) of total aerobe and anaerobic population was not altered significantly in HA-I group. It leads to the higher

anaerobic state of intestinal epithelia and alterations of GI mucosal microenvironment which are the major limiting factors for such group (Maity *et al.*, 2012).

Table 3.6.1: Alteration of total aerobes populations of intestine luminal content and its changes with GDI after 7 days of hypobaric hypoxia at different altitude

Microbial parameters	Different Groups	Exposure duration		GDI ($log_{10}NC0/log_{10}HA7$)
		Day 0	Day 7	
Total aerobes	NC	5.04±0.12	5.37±0.07	1.06
	NCP1	5.05 ±0.02	5.21 ±0.14	1.03
	NCP2	4.95 ±0.06	2.51 ±0.16	-2.07
	NCP3	4.93 ±0.05	3.56 ±0.12	-1.41
	NCP4	4.83 ±0.15	3.98 ±0.10	-1.21
	HC-I	4.93±0.36	4.12±0.08	-1.19
	HA-I-CP1	4.92±0.11	3.75±0.03	-1.31
	HA-I-CP2	4.91±0.01	4.25±0.13	-1.16
	HC-I-CP3	4.81±0.04	4.21±0.14	-1.17
	HA-I-CP4	4.92±0.11	3.79±0.08	-1.3
	HC-II	5.24±0.12	3.90±0.15	-1.34
	HA-II-CP1	5.12±0.14	2.60±0.14	-2.01
	HA-II-CP2	5.16±0.04	3.60±0.04	-1.45
	HC-II-CP3	5.15±0.08	4.60±0.14	-1.13
	HA-II-CP4	5.13±0.02	4.53±0.11	-1.15
	HC-III	5.35±0.05	3.67±0.12	-1.45
	HA-III-CP1	5.21±0.07	2.14±0.05	-2.5
	HA-III-CP2	5.27±0.04	3.04±0.08	-1.74
	HC-III-CP3	5.22±0.03	3.14±0.05	-1.7
	HA-III-CP4	5.19±0.17	2.94±0.02	-1.76

**Microbial population density was expressed (mean of $log_{10}CFU/g$±SD).*

In case of probiotics (CP1, CP2, CP3 and CP4) supplemented groups this kind of alteration was not significant. The *E. coli* content was increased with the positive GDI in hypoxic condition. It was well known that the *E. coli* population was generally found to be 10^2 times higher than the total aerobes in the faces. Total aerobes, facultative anaerobes (*E. coli*) and total anaerobes present in the ratio of $4.36:1:4.03\times10^5$. But this may differ within species and even between individuals in the same species (Maity *et al.*, 2009). Researcher has revealed that immobilization stress for 6-h initiates the increase of the population of *E. coli* in the proximal sections (the duodenum and the jejunum) of the

digestive tract. This rapid expansion of *E. coli* population might encourage the growth of other strict anaerobes (*Bacteroidetes* sp. *and Lactobacillus* sp.) and other pathogens (Gombošov *et al.*, 2011). But it is not clear why the growth of lactic acid bacteria was lower than the other anaerobes. The quantity of strict anaerobes like *Bacteroides* sp. and *Bifidobacterium* sp. were increased with the GDI of +1.03; +1.1; +1.19 and +1.01; +1.08; 1.11 after seven days of exposure at HA-I, HA-II and HA-III respectively (Table 3.6.4 & Table 3.6.6). Normally the aforementioned strict anaerobes were present (at base level) in the fecal sample at a ratio of 1:1.03 (ratio of log_{10}CFU/g) which were changed to 1:1.09 (HA-I), 1:1.19 (HA-II) and 1:1.41 (HA-III) respectively after giving hypoxic stress (Table 3.6.6).

Table 3.6.2: Alteration of total anaerobes populations of intestine luminal content and its changes with GDI after 7 days of hypobaric hypoxia at different altitude

Microbial parameters	Different Groups	Exposure duration		GDI (log_{10}NC0/log_{10}HA7)
		Day 0	Day 7	
Total anaerobes	NC	6.1±0.15	6.18±0.15	1.01
	NCP1	5.75±0.06	7.26±0.07	1.26
	NCP2	5.45±0.16	6.46±0.17	1.18
	NCP3	5.65±0.13	6.66±0.11	1.17
	NCP4	5.37±0.10	6.76±0.09	1.25
	HC-I	5.87±0.04	6.86±0.12	1.16
	HA-I-CP1	5.64±0.14	7.43±0.06	1.31
	HA-I-CP2	6.04±0.07	7.13±0.05	1.18
	HC-I-CP3	5.94±0.04	6.99±0.15	1.17
	HA-I-CP4	6.14±0.12	7.03±0.08	1.14
	HC-II	6.16±0.13	8.1±0.11	1.31
	HA-II-CP1	6.26±0.11	7.78±0.09	1.24
	HA-II-CP2	6.11±0.13	7.55±0.09	1.23
	HC-II-CP3	5.51±0.17	7.13±0.11	1.29
	HA-II-CP4	5.91±0.10	7.10±0.18	1.2
	HC-III	6.34±0.18	10.15±0.03	1.6
	HA-III-CP1	5.74±0.19	8.07±0.31	1.4
	HA-III-CP2	5.76±0.16	8.17±0.21	1.41
	HC-III-CP3	6.16±0.15	8.15±0.32	1.32
	HA-III-CP4	6.34±0.12	8.25±0.03	1.3

**Microbial population density was expressed (mean of log_{10}CFU/g±SD).*

Upon application of probiotics (CP1, CP2, CP3 and CP4), the GDI of *E. coli* was found *to* move towards the negative direction, whereas, GDI of anaerobes like *Bacteroides* sp., *Bifidobacterium* sp., and lactic acid bacteria were moved in the positive direction which was near to the ratio of control microbial population. It is well known that probiotics promote gut health by influencing enterocyte turnover, opposing the growth of pathogenic bacteria, and producing bacteriostatic components which control the growth of pathogenic bacteria (Farthing, 2004; Manning and Gibson, 2004). Probiotics are thought to work largely through direct or indirect effects on the gut microbiota and associated environments in the host. It was also revealed that probiotics also accelerate the production of anti-microbial substances against deadly invading pathogens (Todoriki *et al.,* 2000).

Table 3.6.3: Alteration of *Escherichia coli* populations of intestine luminal content and its changes with GDI after 7 days of hypobaric hypoxia at different altitude

Microbial parameters	**Different Groups**	**Exposure duration**		**GDI (log_{10}NC0/log_{10}HA7)**
		Day 0	**Day 7**	
Escherichia coli	NC	2.52±0.05	2.63±0.06	1.04
	NCP1	2.78±0.04	2.43±0.06	-1.14
	NCP2	2.71±0.12	2.33±0.21	-1.16
	NCP3	2.76±0.11	2.11±0.06	-1.3
	NCP4	2.46±0.16	2.21±0.05	-1.11
	HC-I	2.56±0.08	3.43±0.05	1.33
	HA-I-CP1	2.65±0.09	2.11±0.03	-1.25
	HA-I-CP2	2.75±0.13	2.07±0.06	-1.32
	HC-I-CP3	2.67±0.13	2.45±0.14	-1.08
	HA-I-CP4	2.73±0.10	2.35±0.09	-1.16
	HC-II	2.62±0.12	4.57±0.02	1.74
	HA-II-CP1	2.63±0.18	3.24±0.12	1.23
	HA-II-CP2	2.67±0.16	4.24±0.10	1.58
	HC-II-CP3	2.95±0.07	3.67±0.12	1.24
	HA-II-CP4	2.68±0.16	3.68±0.09	1.37
	HC-III	2.57±0.11	6.16±0.21	2.39
	HA-III-CP1	2.47±0.17	4.06±0.01	1.64
	HA-III-CP2	2.68±0.09	3.76±0.09	1.4
	HC-III-CP3	2.78±0.19	4.26±0.16	1.53
	HA-III-CP4	2.61±0.21	3.26±0.29	1.24

**Microbial population density was expressed (mean of log_{10}CFU/g±SD).*

Kajander *et al* (Kajander *et al.*, 2005) conducted a 6-month feeding study on 42 irritable bowel syndrome (IBS) patients. In his study, the probiotic used was comprised of a blend of four strains, *Lactobacillus rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS, and *B. Breve* Bb99. A few specific changes in microbes were detected with the help of quantitative polymerase chain reaction (*Clostridium* and *Ruminococcus* groups), but more interestingly probiotic supplementation was promoted stabilization of the microbiota (as revealed with an increased overall similarity index). A similar finding was also reported in the group of patients suffering from Japanese cedar **pollinosis (Kubota *et al.*, 2009).**

Table 3.6.4: Alteration of *Bacteroidetes* sp. populations of intestine luminal content and its changes with GDI after 7 days of hypobaric hypoxia at different altitude

Microbial parameters	**Different Groups**	**Exposure duration**		**GDI (log_{10}NC0/log_{10}HA7)**
		Day 0	**Day 7**	
Bacteroidetes sp.	NC	3.06±0.06	3.14±0.02	1.02
	NCP1	3.02±0.14	2.34±0.03	-1.29
	NCP2	3.01±0.09	2.74±0.06	-1.09
	NCP3	3.05±0.02	2.54±0.09	-1.2
	NCP4	3.04±0.06	2.34±0.19	-1.29
	HC-I	3.11±0.04	3.23±0.6	1.03
	HA-I-CP1	3.09±0.03	2.44±0.21	-1.26
	HA-I-CP2	3.01±0.02	2.54±0.07	-1.18
	HC-I-CP3	3.05±0.09	2.51±0.03	-1.21
	HA-I-CP4	3.07±0.05	2.38±0.10	-1.28
	HC-II	3.12±0.07	3.45±0.11	1.1
	HA-II-CP1	3.10±0.04	2.24±0.22	-1.38
	HA-II-CP2	3.03±0.02	2.26±0.07	-1.34
	HC-II-CP3	3.06±0.10	2.47±0.06	-1.23
	HA-II-CP4	3.10±0.08	2.10±0.09	-1.47
	HC-III	3.18±0.03	3.81±0.13	1.19
	HA-III-CP1	3.14±0.05	1.94±0.20	-1.61
	HA-III-CP2	3.09±0.01	2.09±0.17	-1.06
	HC-III-CP3	3.15±0.06	2.12±0.08	-1.48
	HA-III-CP4	3.06±0.08	2.20±0.08	-1.39

**Microbial population density was expressed (mean of log_{10}CFU/g±SD).*

Table 3.6.5: Alteration of total lactic acid bacterial populations of intestine luminal content and its changes with GDI after 7 days of hypobaric hypoxia at different altitude

Microbial parameters	Different Groups	Exposure duration		GDI (log_{10}NC0/log_{10}HA7)
		Day 0	Day 7	
Total Lactic Acid Bacteria	NC	5.05±0.08	5.16±0.08	1.02
	NCP1	5.55±0.11	7.05±0.14	1.27
	NCP2	5.48±0.04	6.38±0.11	1.16
	NCP3	5.35±0.09	6.38±0.10	1.19
	NCP4	5.54±0.11	7.15±0.14	1.29
	HC-I	5.05±0.08	4.69±0.12	-1.07
	HA-I-CP1	5.56±0.03	7.03±0.0.5	1.26
	HA-I-CP2	5.49±0.02	6.68±0.15	1.21
	HC-I-CP3	5.39±0.17	6.71±0.0.8	1.24
	HA-I-CP4	5.61±0.10	7.15±0.13	1.27
	HC-II	5.05±0.08	4.56±0.06	-1.1
	HA-II-CP1	5.6±0.13	7.56±0.27	1.35
	HA-II-CP2	5.52±0.11	6.88±0.10	1.24
	HC-II-CP3	5.42±0.27	7.41±0.9	1.36
	HA-II-CP4	5.51±0.09	7.34±0.26	1.33
	HC-III	5.05±0.08	3.04±0.29	-1.66
	HA-III-CP1	5.45±0.12	7.98±0.11	1.46
	HA-III-CP2	5.32±0.04	6.98±0.19	1.31
	HC-III-CP3	5.23±0.07	7.01±0.31	1.34
	HA-III-CP4	5.42±0.11	7.25±0.13	1.33

**Microbial population density was expressed (mean of log_{10}CFU/g±SD).*

From the study, it is clear that the results of GDI at hypobaric hypoxic condition for total aerobes like *Bacteroides* sp was in the negative direction and the GDI of anaerobes and lactic acid bacteria resided in the positive direction in comparison to control group. At the time of probiotic supplementations, the GDI value of *total* aerobes and *E. coli* was in the positive direction and this value for the anaerobes and lactic acid bacteria has remained in the positive direction in respect to HC group. In the earlier time, microbiologists and cell biologists have gathered advanced knowledge on microorganisms, especially on pathogens including the addressing of the importance of epithelial cells, particularly enterocytes to get better insight in human beings (Wick *et al.,* 1991).

Table 3.6.6: Alteration of *Bifidobacterium* sp. populations of intestine luminal content and its changes with GDI after 7 days of hypobaric hypoxia at different altitude				
Microbial parameters	**Different Groups**	**Exposure duration**		**GDI (log_{10}NC0/log_{10}HA7)**
		Day 0	**Day 7**	
Bifidobacterium sp.	NC	5.03±0.49	5.13±0.13	1.01
	NCP1	5.05±0.03	6.13±0.18	1.21
	NCP2	5.06±0.05	6.73±0.10	1.33
	NCP3	5.03±0.03	6.23±0.14	1.23
	NCP4	5.13±0.16	6.98±0.08	1.36
	HC-I	5.33±0.17	5.41±0.11	1.01
	HA-I-CP1	5.15±0.05	6.11±0.17	1.18
	HA-I-CP2	5.08±0.02	6.81±0.04	1.34
	HC-I-CP3	5.19±0.07	6.34±0.15	1.22
	HA-I-CP4	5.16±0.13	6.94±0.24	1.34
	HC-II	5.14±0.16	5.57±0.26	1.08
	HA-II-CP1	5.12±0.03	5.45±0.07	1.06
	HA-II-CP2	5.17±0.11	6.77±0.13	1.3
	HC-II-CP3	5.13±0.15	6.35±0.05	1.23
	HA-II-CP4	5.21±0.19	6.54±0.14	1.25
	HC-III	5.28±0.04	5.90±0.02	1.11
	HA-III-CP1	5.3±0.07	7.54±0.07	1.42
	HA-III-CP2	5.41±0.02	7.86±0.08	1.45
	HC-III-CP3	5.42±0.12	7.98±0.14	1.47
	HA-III-CP4	5.38±0.19	7.91±0.11	1.47
**Microbial population density was expressed (mean of log_{10}CFU/g±SD).*				

This interaction between micro-organisms and epithelial cells has been termed as 'bacterial-epithelial cross-talk'. Probiotics exhibit antimicrobial effect against pathogens by secreting an array of antibacterial substances which includes organic acids, hydrogen peroxide and bacteriocins. These compounds mainly help to reduce the viable count of pathogens as well as alters their metabolisms which include toxin production through the reduction of luminal pH and by the production of SCFAs. By the advent of the physiologic receptor, the microorganism can use epithelial cellular pathways to assist its re-translocation into the host's intravascular spaces which ultimately helps in the pathogenesis of gastroenteritis and sepsis causing bacteria.

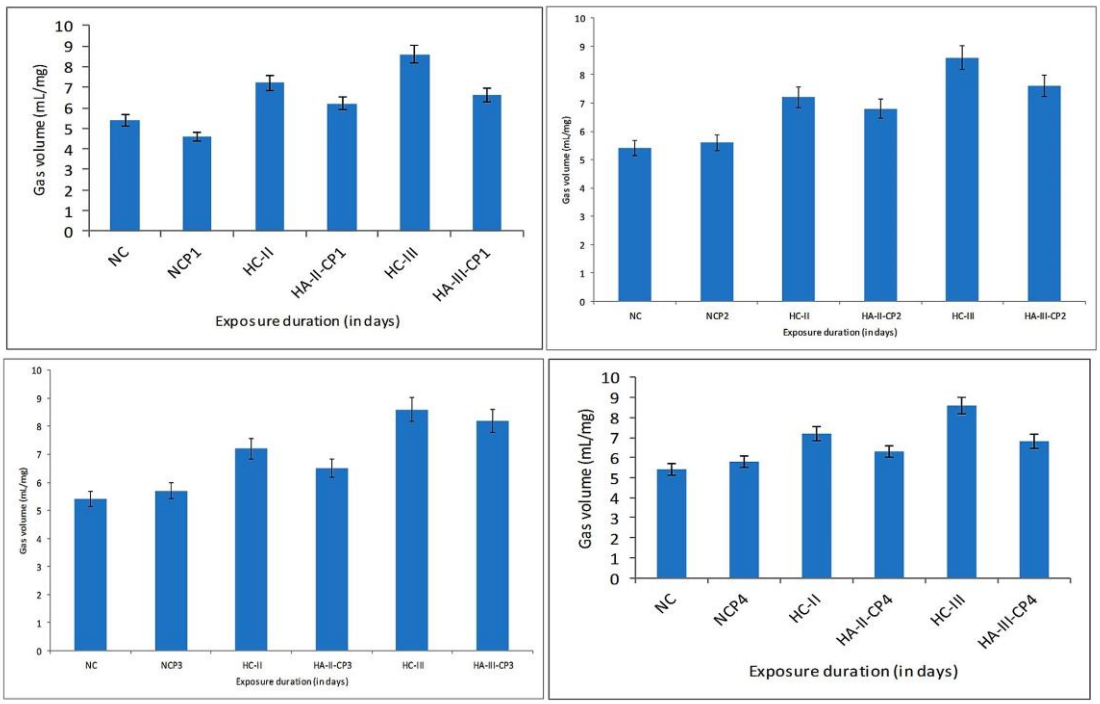


Fig. 3.6.1: Gas formation ability by the composite bacterial populations in rat small intestine of control and after different probiotic supplementation during seven days of experiment.

Metabolic activity of microbial consortium-

Hypoxia is responsible for the lower GI motility and at the same time it induces the overgrowth of gram negative bacterial population which seemed to be associated with the gas formation and the accumulation of acids. In this study it was observed that in small intestine the capability of gas-formation by composite microflora in NC group was 5.4 mL/g, that increased to 7.2 mL/g (HA-II) and 8.6 mL/g (HA-III). At the time of administration of probiotics (CP1, CP2, CP3 and CP4) in different groups the gas formation was decreased significantly in respect to HA group and tends to normobaric group (NCP1: 4.6 mL/g; HA-II-CP1: 6.2 mL/g; HA-III-CP1: 6.6 mL/g; NCP2: 5.6 mL/g; HA-II-CP2: 6.8 mL/g; HA-III-CP2: 7.6 mL/g; NCP3: 5.7 mL/g; HA-II-CP3: 6.5 mL/g; HA-III-CP3: 8.2 mL/g; NCP4: 5.8 mL/g; HA-II-CP4: 6.3 mL/g; HA-III-CP4: 6.8 mL/g) (Fig. 3.6.1).

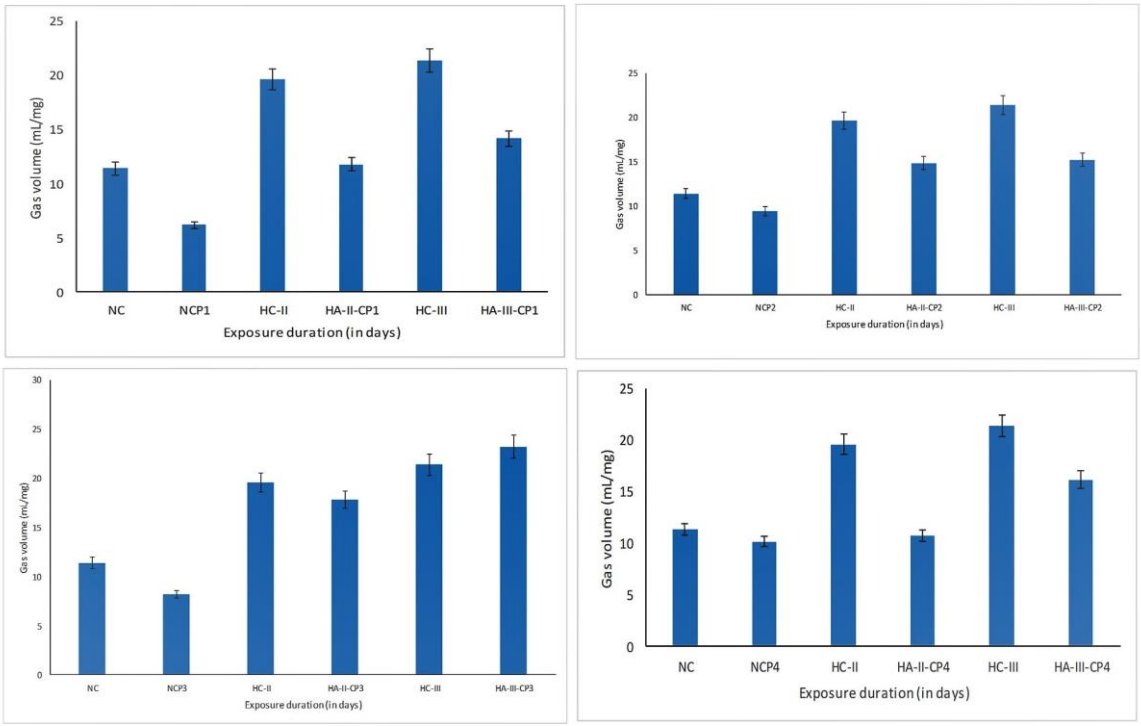


Fig. 3.6.2: Gas formation ability by the composite bacterial populations in rat large intestine of control and after different probiotic supplementation during seven days of experiment.

In case of large intestine microflora could produce NC group was 11.4 mL/g that increased to 19.6 mL/g (HA-II) and 21.4 mL/g (HA-III). When probiotics (CP1, CP2, CP3 and CP4) was administrated to different groups the gas formation was decreased significantly with respect to HA group (NCP1: 6.2 mL/g; HA-II-CP1: 11.8 mL/g; HA-III-CP1: 14.2 mL/g; NCP2: 9.4 mL/g; HA-II-CP2: 14.8 mL/g; HA-III-CP2: 15.2 mL/g; NCP3: 8.2 mL/g; HA-II-CP3: 17.8 mL/g; HA-III-CP3: 23.2 mL/g; NCP4: 10.2 mL/g; HA-II-CP4: 10.8 mL/g; HA-III-CP4: 16.2 mL/g) (Fig. 3.6.2).

The huge proliferation and growth of anaerobes were found to transform the gut microenvironment into a more anoxic condition which has promoted acid accumulation in higher level and gas formation in GI lumen as well. The visual observation also revealed mild colonic bleeding with irregular dystrophy related inflammation sign in the small and

large intestine on 7th day in case of HA-II and HA-III groups. The inflammation of both kinds of intestine is indicated for higher gas formation in the lumen which may create a gaseous pressure to the gut wall. The significant functions of colonic bacteria are nutrient metabolism of substrates. This function leads to the accumulation of gas and SCFA, particularly SCFA are an important energy source and trophic regulator of colonic enterocytes (Guarner and Malagelada; 2003), and associated for the induction of propulsive and accelerated transit or enhancement of liquid along with the absorption of sodium molecules. At HA, lower the motility rate of intestine is responsible for the reduction of self-cleaning capabilities and extension of the retention time of microbes. Alteration of the colonic flora with the administration of probiotics modify fermentation processes and as a results control the gas production, colonic transit and fluid effluxes (Bausserman and Michail 2005).

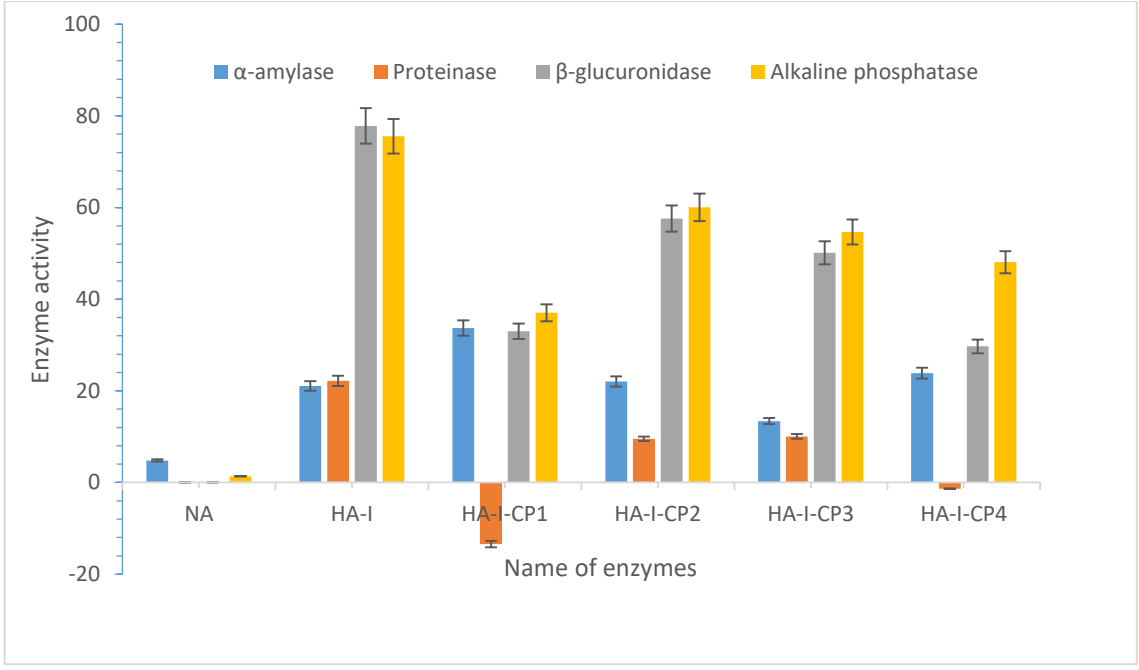
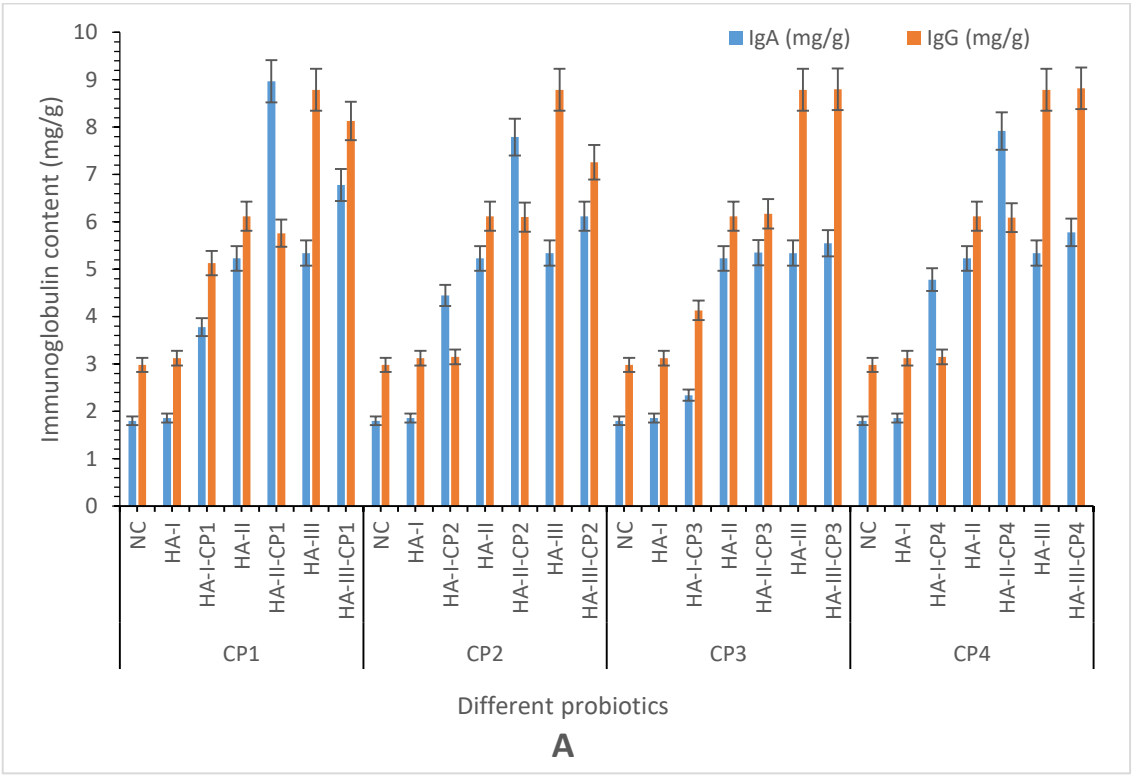


Fig. 3.6.3: Changes in the enzyme profiles (luminal enzymes activities) after 7 days of experiment before and after probiotics supplementation.

It was noted that the specific activities of microbial associated enzymes like alkaline phosphatase, proteinase, β-glucuronidase and α-amylase were increased at 7th day of acclimatization at HA (Fig. 3.6.3). The alkaline phosphatase activity was reported to remove the phosphate from glutamine from the lipid for the reduction of LPS toxicity and the creation of a less toxic environment (Bates *et al.,* 2006). The α-amylase activity usually digests the complex polysaccharides to salvage the energy and facilitates the accumulation of acid in the colon (Gloster *et al.,* 2008). On the other hand, probiotics are able to regulate the enzyme production by acting on hepatic lipogenic enzymes through influencing the production of short-chain fatty acids (SCFAs).

Earlier studies suggested that the probiotics can influence digestive processes by enhancing the population of beneficial microorganisms, improving the intestinal microbial balance and microbial enzyme activity, improving the digestibility and absorption of food and its utilization (Suzer *et al.,* 2008). The current study also revealed similar phenomenon as described above like increased in digestive capabilities by increasing the total protease activity of the gut as found in probiotics treated groups (Munilla *et al.,* 1990; Ziaei-Nejad *et al.,* 2006) besides activating the fabrication of endogenous enzymes (Ochoa-Salano & Olmos-Soto, 2006; Wang, 2007). Additionally, the exogenous enzymes have a broader pH range than endogenous enzymes which lengthens the digestion time and permits the hydrolysis of substrates in optimum level.



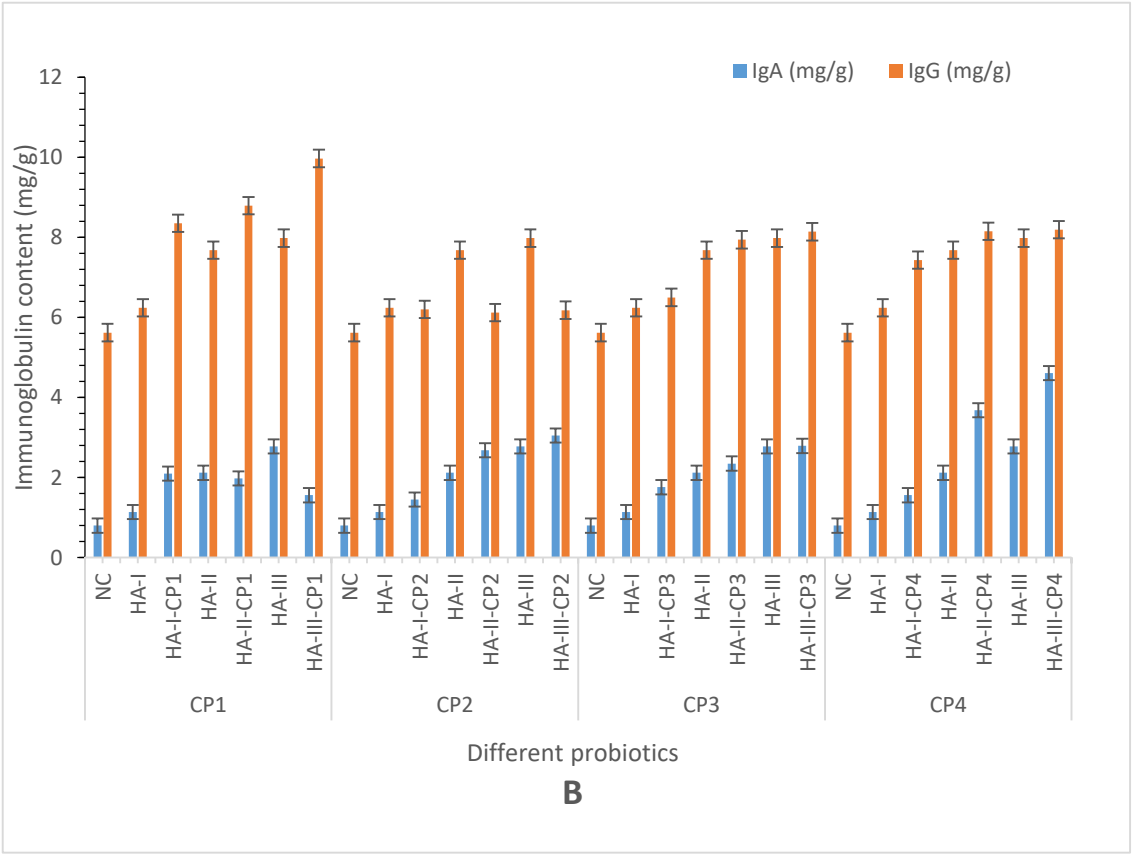


Fig. 3.6.4: In albino rat model, the level of IgG and IgA in luminal content of Control (C; 14.7 psi) and HA (11.8 psi; 9.3 psi; 7.3 psi) groups after 7th day of hypobaric hypoxic stress. **A.** Small intestine; **B.** Large intestine.

Evaluation of different immunoglobulins-

The immunoglobulin, IgA and IgG level of large and small intestine was found to be progressively increased at different HA (Fig. 3.6.4). This indicated that hypoxia induced inflammatory responses lead to the increased production of IgA and IgG in the mucosal and lymphoid associated immune organs as an immune-protective measurement (Fig. 3.6.4A; Fig. 3.6.4B). Probiotic mediated immunological exaggerations are also manifested by the increased rate of production of immunoglobulins through enhancing the activity of macrophages and lymphocytes, which in turn stimulates the production of interferon and is known to be beneficial for maintaining the gut health.

Conclusion:

From the study, it can be concluded that environmental hypoxia and probiotic treatment have the following effects-

1. During hypoxic stress, hypobaric hypoxia increased the population density of total strict and facultative anaerobes whereas decreased the total aerobes.
2. Ingestion of probiotics during hypoxic stress prevent the alteration of above microbial population and try to maintain the microbial population like normobaric condition.
3. Altered microbial population in probiotic treated groups decreased acid and gas formation and regulates the microbial associated enzymes.
4. Among the four commercial probiotics, VSL#3 showed the better activity hypobaric hypoxic stress.