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Hypobaric hypoxia (HH): Induced alterations of hematological responses in male rats are influenced by naproxen, vitamin C and vitamin E

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Abstract
The physiological systems are affected in humans after exposure to simulated hypobaric hypoxia (HH). The aim of the present study was to investigate the changes of some hematological parameters during simulated hypobaric hypoxic (HHc) condition and to explore the role of prostaglandins (PGs) and/or oxidative stress on HH-induced hematological changes in male rats. The rats were exposed to HH at 18,000ft (380 Torr) in a simulated chamber for 8 h/day for 6 consecutive days. Different doses of naproxen (a non-steroidal anti-inflammatory drug), vitamin C and vitamin E were administered in separate groups of rats in both the HHc and normobaric conditions. The present study indicated that the TC of RBC, hemoglobin concentration and packed cell volume (PCV) were increased in HHc condition in rats. The HH-induced changes of the hematological parameters showed gradual recovery with graded doses of naproxen and vitamin E. But vitamin C at higher dose did not show any recovery of observed parameters indicating absence of anti-oxidant activity at the higher dose. The present study indicates that the observed hematological responses may be explained on the basis of the oxidative stress induced production of PGs and/or reactive oxygen species in HHc condition. The study also indicated the potential role of non-steroidal anti-inflammatory drug (naproxen), and antioxidants (vitamin C and E) on the maintenance of the homeostasis of the physiological impairments in high altitude.

Keywords: hypobaric hypoxia, hematological changes, nsaids, Vitamin C, Vitamin E

1. Introduction
Human beings travel to high altitude (HA) either for recreational activities or for work. The defense personal may have to stay in high altitude for a long time due to their professional requirements. During these periods they may be exposed to chronic and/or intermittent hypoxia and very often suffer from acute mountain sickness (AMS). Sometimes they may suffer from high altitude pulmonary edema (HAPE) and high-altitude cerebral edema (HACE) [1-3]. The oxidative stress in hypobaric hypoxic (HHc) condition generates reactive oxygen species (ROS) that may help in acclimatization. Different physiological systems are altered in HHc condition, which has been reported in humans [2, 4-8] and experimental animals [9-13]. In high altitude condition the increase of red blood cell (RBC), hemoglobin concentration and packed cell volume (PCV) are the main features to elicit the acclimatization and hematological adaptation of the mountaineers.

In high altitude oxidative stress induced changes of hemoglobin concentration is mediated by prostaglandins (PGs) which is increased by higher cyclooxygenase (COX) activity [14-16]. The increased hemoglobin was beneficial for the early phase of high-altitude exposure but after long period of exposure it may turned to be a problem as hemoglobin and RBCs were increased excessively resulting in polycythemia [17, 18]. The high altitude induced problems of polycythemia and other high-altitude sickness in mountaineers and experimental animals were treated with non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen, ibuprofen, aspirin and indomethacin [19, 20]. A NSAID such as aspirin was used by mountaineers at high altitude to facilitate acclimatization [21]. It has been reported that the treatment of indomethacin in rats exposed to simulated high altitude can reduce the high hematocrit value by inhibiting PGs, which stimulate erythropoietin [22]. Thus the peripheral resistance due to polycythemia may be reduced by aspirin in prolonged exposure to high altitude. Naproxen, is another non selective inhibitor of COX enzymes and 20 times potent than aspirin. It is also effective in reducing the HH-induced increment of the total count of RBC and hemoglobin concentration [11].

Foods rich in antioxidant nutrients or consuming moderate amounts of an antioxidant supplement (such as vitamin C and E, selenium and possibly alpha-lipoic acid) during work at altitude in humans may have benefits related to AMS, muscle soreness and oxygenation of peripheral tissues [1]. Vitamin C and E both are the most important antioxidants present in body tissues that protects membranes from free radical (FR) induced damage and are important for normal functioning of the body cells [23, 24]. Vitamin C and E both are known to play an important role in FR quenching process [25]. Clinical trials with vitamin C and E have yielded contrasting results in the prevention of several diseases related to oxidative stress [25]. After supplementation of vitamin C and E, the HH-induced elevation of hemoglobin concentration and increased SOD and CAT activity were partially blocked which indicate the preventive role of vitamin C and E both on the HH-induced

oxidative stress [26].

On the basis of above point of view, the present study explores the effect of naproxen, vitamin C and E on some HH-induced hematological changes in rats. Thereby the study attempts to assess whether naproxen, vitamin C and E could be effective against the oxidative stress induced generation of PGs and/or ROS, which have some role on these changes.

2. Materials and Methods

2.1 Animals

Adult Charles-Foster male albino rats weighing 200-220 gm were used in this study. Animals were housed individually in polypropylene animal cages with food pellet and water *ad libitum.* Animal room was maintained at the temperature of 25˚ C ± 1˚C with a 12h light dark cycle (light 7 AM to 7 PM). The animal studies were performed in accordance with the guidelines for the care to minimize the pain and discomfort of the animals and use of laboratory animals of the institutional Animal Care and Use Committee (IACUC) and all the animal protocols were approved by the IACUC of the Department of Physiology in University of Calcutta (IAEC/PROP/TKG1/2010 approved on 16/11/2011).

2.2 Exposure to Hypobaric Hypoxia

The rats were exposed to simulated hypobaric hypoxia (HH) at 18,000ft (380 mm of Hg) in a hypobaric hypoxic chamber following the method of Goswami *et al*, [27- 29]. The periods of exposure to the rats in the decompression chamber was limited to 8 hrs per day for 6 consecutive days from 9.00 am to 5.00 pm. By that way the hypoxia was made intermittent. The decompression chamber had metallic body with a glass lid on the top of the chamber and connected to a vacuum pump. The air pressure (380 ± 3 mm of Hg) inside the chamber was maintained by regulating the air flow through the chamber. The pressure and temperature (25˚ C ± 1˚C) inside the chamber monitored continuously. Food and water was available for the animals within the chamber during the exposure schedule. The normobaric rats were also placed in another similar chamber but those rats were not exposed to HH.

2.3 Supplementation of Naproxen, Vitamin C and Vitamin E

Naproxen (RPG Life Science Ltd., India) was dissolved in 10% ethanol water mixture (w/v), vitamin C (SRL, India) was dissolved in distilled water (w/v) and vitamin E (Loba Chem, India) was dissolved in 70% ethanol water mixture (v/v) prior to experiment and it was administered orally through a gastric cannula attached to a 1 ml syringe. Both the groups of rats (HHc and normobaric) were treated with naproxen (6 mg / kg body wt., 12 mg / kg body wt., 18 mg / kg body wt. and 24 mg / kg body wt.), vitamin C (200 mg / kg body wt., 400 mg / kg body and 600 mg / kg body wt.) and vitamin E (20 mg / kg body wt., 40 mg / kg body wt. and 60 mg / kg body wt.) in different doses. The daily dose of naproxen, vitamin C and vitamin E were divided equally into two parts; one part was given orally before the exposure and other part was given after the exposure to hypobaric hypoxia on each day in both the groups of rats. The daily dose of naproxen, vitamin C and vitamin E were similarly given into two parts in different group of rats exposed to normobaric condition.

2.4 Design of Experiments

The different groups of rats in normobaric conditions were, naproxen treated groups: CNP0 (vehicle only, without naproxen or 0 mg/kg body wt. of naproxen), CNP1 (6 mg of naproxen/kg body wt.), CNP2 (12 mg of naproxen/kg body wt.), CNP3(18 mg of naproxen/kg body wt.), CNP4 (24 mg of naproxen/kg body wt.), vitamin C treated groups: CNV0 (vehicle only, without vitamin C or 0 mg/kg body wt. of vitamin C), CNV1 (200 mg of vitamin C/kg body wt.), CNV2 (400 mg of vitamin C/kg body wt.), CNV3 (600 mg of vitamin C/kg body wt.) and vitamin E treated groups: CVE0 (vehicle only, without vitamin E or 0 mg/kg body wt. of Vitamin E), CVE1 (20 mg of vitamin E/kg body wt.), CVE2 (40 mg of vitamin E/kg body wt.) and CVE3 (60 mg of vitamin E/kg body). The different groups of rats in HHc condition were, naproxen treated groups: HHNP0 (vehicle only, without vitamin C or 0 mg/kg body wt. of naproxen), HHNP1 (6 mg of naproxen/kg body wt.), HHNP2 (12 mg of naproxen/kg body wt.), HHNP3(18 mg of naproxen/kg body wt.), HHNP4 (24 mg of naproxen/kg body wt.), vitamin C treated groups: HHCV0 (without vitamin C or 0 mg/kg body wt. of vitamin C, vehicle only), HHCV1 (200 mg of vitamin C/kg body wt.), HHCV2 (400 mg of vitamin C/kg body wt.), HHCV3 (600 mg of vitamin C/kg body wt.) and vitamin E treated groups: HHVE0 (without vitamin E, vehicle only), HHVE1 (20 mg of vitamin E/kg body wt.), HHVE2 (40 mg of vitamin E/kg body wt.), and HHVE3 (60 mg of vitamin E/kg body wt.).

2.5 Blood Collection

Blood was collected (1.5 ml, between 5.00 to 5.30 p.m.) from the heart of deeply anesthetized rat (Na- thiopentone, 50 mg/kg body wt, i.p.) by a syringe and mixed with an anticoagulant ethylene diamine tetra-acetic acid (EDTA) (Merck, India) for measurement of TC of RBC, hemoglobin concentration and packed cell volume (PCV).

2.6 TC of RBC

Twenty microliter of blood was taken and mixed with 2 ml of RBC diluting fluid, the dilution of RBC is 200 times. The blood and RBC diluting fluid were mixed well. Then the drop of diluted blood mixture was charge to the cover glass placed over the Neubauer's Haemocytometer. Allow some time for cells to settle down in the counting chamber so that all the cells present will be in the same plane. The charged Neubauer's chamber is now kept under the microscope. Count the RBCs in four medium sized corners and one medium sized central square. Let, 'N' be the total number of RBCs counted in medium sized squares i.e. in 1/50 cumm of the diluted blood. Therefore, the number of RBCs in 1 cumm of undiluted blood = N x 50 x 200 (Dilution factor) [30].

2.7 Hemoglobin Concentration (gm/dl)

Hemoglobin concentration was measured by following the cyanmethemoglobin method [30]. An aliquot of well-mixed whole blood is taken and reacted with a solution of potassium cyanide and potassium ferricyanide (called Drabkin's solution). The chemical reactors yields a product of stable colour-the cyanmethemoglobin. The intensity of the colour is proportional to the hemoglobin concentration and obeys Beer's Law. The tubes were marked with "B" for blank solution and "T such as T1, T2 etc" for test samples.

In a tube (B marked tube) only 5 ml of Drabkin's solution was taken and in another test tube (T marked tube) 0.02 ml of blood was diluted with the 5 ml of Drabkin's solution. 15 minutes time was required for proper mixing of the blood sample with the solution. The spectrophotometer (shimadzu-1601, Japan) was set to zero with the solution present in the "B" marked test tube and finally the optical density of different test samples (solution present in T marked test tubes) were measured in using a length of 540 mm. After that the concentration of hemoglobin present in the blood samples (gm/dl) were calculated by the following formula.
Hemoglobin concentration in gm/dl = Absorbance of the test solution / Absorbance of the Standard ×15.

2.8 Packed Cell Volume (PCV)

Anticoagulant blood (whole blood) when centrifuged at a standard speed, erythrocytes which are heavier than white cells, platelets and plasma, sediment at the bottom. This column of red cells is called haematocrit or packed red cell volume (PCV) which is expressed as fraction of the whole blood (level of plasma). The Wintrobe tube was filled with the anti-coagulant mixed blood up to 100 marks by the Pasteur pipette. Then it was placed into a centrifugation tube and tightened with cotton and centrifuged in 3000 rpm for 30 minutes. After centrifugation the reading of the sediment was noted. The reading (%) of sediment is the packed cell volume (PCV) which includes the total number of RBC and WBC [30].

3. Results

3.1 TC of RBC

3.1.1 Naproxen treated groups

The TC of RBC was significantly increased in HH exposed rats (HHNP0) compared to that of normobaric rats without naproxen (CNP0) [F (9, 50) = 24.737, p<0.001]. The effect of naproxen on the TC of RBC had no significant role at any dose in normobaric rats (graded doses of naproxen CNP1, CNP2, CNP3, and CNP4 compared to CNP0). But the HH-induced enhanced TC of RBC was gradually inhibited with higher doses of naproxen. The HH-induced enhanced TC of RBC returned to normal level at the dose of 18 mg of naproxen/kg body wt. and remained at normal level at the dose of 24 mg of naproxen/kg body wt. The TC of RBC was significantly low in HHNP1 compared to CNP1 [F (9, 50) = 24.737, p<0.001] and without naproxen (CNP0) but not with the HHNP0. Similarly the TC of RBC was decreased in HHNP2 compared to CNP2 [F (9, 50) = 24.737, p<0.001] and to CNP0 but not with the HHNP0. The TC of RBC of HHNP3 and HHNP4 were not significantly changed compared to normobaric rats with corresponding doses of naproxen (CNP3 and CNP4) and without naproxen (CNP0). (Fig 1A)

3.1.2 Vitamin C treated groups

The TC of RBC was significantly increased in HH exposed rats without vitamin C (HHCV0) compared to that of normobaric rats without vitamin C (CNV0). The effect of vitamin C on the TC of RBC was not significant at any dose in normobaric rats with graded doses of vitamin C (CNV1, CNV2 and CNV3) compared to CNV0 (normobaric rats without vitamin C). But the HH-induced enhanced TC of RBC returned to normal level at the dose of 400 mg of vitamin C /kg body wt. The TC of RBC was significantly high in HHCV1 compared to CNV1 [F (7, 40) = 24.401, p<0.001] and without vitamin C (CNV0) [F (7, 40) = 24.401, p<0.001] but not with the HHCV0. The TC of RBC of HHCV2 were not significantly changed compared to normobaric rats with corresponding doses of vitamin C (CNV2) and also with HHCV0. But the TC of RBC of HHCV3 again increased significantly [F (7, 40) = 24.401, p<0.05] compared to normobaric rats with corresponding dose of vitamin C (CNV3) and CNV0. (Fig 1B)

3.1.3 Vitamin E treated groups

The TC of RBC was significantly increased in HH exposed rats without vitamin E (HHVE0) compared to that of normobaric rats without vitamin E (CVE0) [F (7, 40) = 24.450, p<0.01]. The effect of vitamin E on the TC of RBC was not significant at any dose in normobaric rats with graded doses of vitamin E (CVE1, CVE2 and CVE3) compared to CVE0. But the HH-induced enhanced TC of RBC returned back to normal level at the dose of 400 mg of vitamin E /kg body wt. The TC of RBC was significantly higher in HHVE1 compared to CVE1 [F (7, 40) = 24.450, p<0.01] and without vitamin E (CVE0) [F (7, 40) = 24.450, p<0.01] but not with the HHVE0. The TC of RBC of HHVE2 was not significantly changed compared to normobaric rats with corresponding doses of vitamin E (CVE2) and CVE0. (Fig 1C)

3.2 Hemoglobin Concentration

3.2.1 Naproxen treated groups

The hemoglobin (Hb.) concentration (gm/dl) was significantly increased in HH exposed rats without naproxen (HHNP0) compared to that of normobaric rats without naproxen (CNP0) [F (9, 50) = 20.128, p<0.001]. The effect of naproxen on the Hb. concentration was not significant at any dose in normobaric rats with graded doses of naproxen (CNP1, CNP2, CNP3, and CNP4) compared to CNP0. But the HH-induced enhanced Hb. concentration was gradually inhibited with higher doses of naproxen and returned to normal level at the dose of 18 mg of naproxen/kg body wt. and remained at normal level at the dose of 24 mg of naproxen/kg body wt. The Hb. concentration was significantly high in HHNP1 compared to CNP1 [F (9, 50) = 20.128, p<0.001] and without naproxen (CNP0) but not with the HHNP0. Similarly the Hb. concentration was increased in HHNP2 compared to CNP2 [F (9, 50) = 20.128, p<0.001] and to the normobaric rats without naproxen (CNP0) but not with the HHNP0. The Hb. concentartion of HHNP3 and HHNP4 were not significantly changed compared to normobaric rats with corresponding doses of naproxen (CNP3 and CNP4) and without naproxen (CNP0). (Fig 2A)

3.2.2 Vitamin C treated groups

The hemoglobin (Hb.) concentration (gm/dl) was significantly increased in HH exposed rats without vitamin C (HHCV0) compared to that of normobaric rats without vitamin C (CNV0) [F (7, 40) = 17.234, p<0.001]. The effect of vitamin C on the Hb. concentration was not significant at any dose in normobaric rats with graded doses of vitamin C (CNV1, CNV2 and CNV3) compared to CNV0. But the HH-induced enhanced Hb. concentration returned to normal level at the dose of 400 mg of vitamin C/kg body wt. The Hb. concentration was enhanced in HHCV1 compared to CNV1 [F (7, 40) = 17.234, p<0.001] and without vitamin C (CNV0) [F (7, 40) = 17.234, p<0.001] but not with the

HHCV0. The Hb. concentration of HHCV2 was not significantly changed compared to normobaric rats with corresponding dose of vitamin C (CNV2). But the Hb. concentration of HHCV3 again increased significantly [F (7, 40) = 17.234, p<0.001] compared to normobaric rats with corresponding dose of vitamin C (CNV3) and CNV0. (Fig 2B)

3.2.3 Vitamin E treated groups

The hemoglobin (Hb) concentration (gm/dl) was significantly increased in HH exposed rats without vitamin E (HHVE0) compared to that of normobaric rats without vitamin E (CVE0) [F (7, 40) = 29.940, p<0.01]. The effect of vitamin E on the Hb. concentration was not significant at any dose in normobaric rats with graded doses of vitamin E (CVE1, CVE2 and CVE3) compared to CVE0. But the HH-induced enhancement of Hb concentration returned to normal level at the dose of 400 mg of vitamin E /kg body wt. The Hb concentration was significantly high in HHVE1 compared to CVE1 [F (7, 40) = 29.940, p<0.01] and without vitamin E (CVE0) [F (7, 40) = 29.940, p<0.01] but not with the HHVE0. The Hb. concentration of HHVE2 and HHVE3 were not significantly changed compared to normobaric rats with corresponding dose of vitamin E (CVE2 and CVE3) and CVE0. (Fig 2C)

3.3 Packed Cell Volume (PCV)

3.3.1 Naproxen treated groups

The packed cell volume (PCV) (%) was significantly increased in HH exposed rats without naproxen (HHNP0) compared to that of normobaric rats without naproxen (CNP0) [F (9, 50) = 15.383, p<0.001]. The effect of naproxen on the PCV was not significant at any dose in normobaric rats with graded doses of naproxen (CNP1, CNP2, CNP3, and CNP4) compared to CNP0. But the HH-induced enhanced PCV was gradually inhibited with higher doses of naproxen and returned to normal level at the dose of 18 mg of naproxen/kg body wt. and remained at normal level at the dose of 24 mg of naproxen/kg body wt. The PCV was significantly enhanced in HHNP1 compared to CNP1 [F (9, 50) = 15.383, p<0.001] and without naproxen (CNP0) but not with the HHNP0. Similarly the TC of RBC was increased in HHNP2 compared to CNP2 [F (9, 50) = 15.383, p<0.01] and to CNP0 but not with the HHNP0. The PCV of HHNP3 and HHNP4 were not significantly changed compared to normobaric rats with corresponding doses of naproxen (CNP3 and CNP4) and without naproxen (CNP0). (Fig 3A)

3.3.2 Vitamin C treated groups

The packed cell volume (PCV) (%) was significantly increased in HH exposed rats without vitamin C (HHCV0) compared to that of normobaric rats without vitamin C (CNV0) [F (7, 40) = 9.962, p<0.001]. The effect of vitamin C on the PCV was not significant at any dose in normobaric rats with graded doses of vitamin C (CNV1, CNV2 and CNV3) compared to CNV0. The HH-induced enhanced PCV returned to normal level at the dose of 400 mg of vitamin C/kg body wt. The PCV was significantly high in HHCV1 compared to CNV1 [F (7, 40) = 9.962, p<0.01] and without vitamin C (CNV0) [F (7, 40) = 9.962, p<0.05] but not with the HHCV0. The PCV of HHCV2 was not significantly changed compared to normobaric rats with corresponding dose of vitamin C (CNV2). But the PCV of HHCV3 again increased significantly [F (7, 40) = 17.234, p<0.01] compared to normobaric rats with corresponding dose of vitamin C (CNV3) and CNV0. (Fig 3B)

3.3.3 Vitamin E treated groups

The percentage of packed cell volume (PCV) was significantly increased in HH exposed rats without vitamin E (HHVE0) [F (7, 40) = 15.810, p<0.01] compared to that of normobaric rats without vitamin E (CVE0). The effect of vitamin E on the PCV was not significant at any dose in normobaric rats with graded doses of vitamin E (CVE1, CVE2 and CVE3) compared to CVE0. The HH-induced elevation of percentage of PCV returned to normal level at the dose of 400 mg of vitamin E/kg body wt. The PCV was significantly low in HHVE1 compared to CVE1 [F (7, 40) = 15.810, p<0.01] and without vitamin E (CVE0) [F (7, 40) = 15.810, p<0.05] but not with the HHVE0. The PCV of HHVE2 and HHVE3 were not significantly changed compared to normobaric rats with corresponding dose of vitamin E (CVE2, CVE3 and CVE0). (Fig 3C)

4. Discussion

Many physiological changes have been reported in humans [1, 5, 31] and in animals [10, 11, 13] in HH. These changes are induced by the oxidative stress in HH. The simulated HH of laboratory condition may provide standard environment of HH and would provide specific effects of HH on some hematological parameters. However scanty reports are available in this regard. In the present study, the hematological responses were measured in rats exposed to simulated altitude (18,000ft; daily 8 hours for 6 days). In hypobaric hypoxic (HHc) condition the TC of RBC, hemoglobin (Hb) concentration and percentage of packed cell volume (PCV) were increased in rats after exposure to hypobaric hypoxia. It has been reported that the hypobaric hypoxia induced stimulation of erythropoietin production and subsequent recruitment of red cells is mainly mediated by the prostaglandins (PGs) [22]. The PGs production is also increased in HHc condition probably from the polyunsaturated fatty acids elaborated from the mitochondria of the hypoxic tissue [32]. Richalet *et al*, [33] reported that after exposure to acute high altitude hypoxia at 4,350 m for 1-8 days, the plasma concentration of prostaglandin E2 (PGE2) and prostaglandin F2α were increased in humans. The urinary prostaglandin Fα (PGFα) was also found to be high in high altitude residents (4,300 m altitude) and in residents of sea level exposed to the high altitude for 48 hrs [34]. It has been already established that PGs stimulate the production of red blood cell or erythropoiesis directly at the marrow cell level. The enhancement of the number of red blood cell, hemoglobin concentration and the percentage of packed cell volume might be due to hemoconcentration. The present study was in agreement with the study of Biswas *et al*, [11]. Similar increase in these parameters was observed in rats [35, 36] and in humans [5] after exposure to continuous and intermittent exposure to HH.

The contention that the PGs are the regulator of the hematological responses in HHc condition can be supported from the observed changes of the hematological parameters after administration of naproxen at graded doses in HHc condition. The synthesis of PGs are inhibited by non-steroidal anti-inflammatory drug naproxen by inhibiting the cyclooxygenase enzymes [37-39]. The increase of TC of RBC,

haemoglobin (Hb) concentration and percentage of packed cell volume (PCV) in rats after exposure to simulated high altitude were reduced by naproxen. A dose dependent graded effect was noted in naproxen treated rats. While 12 mg/kg body wt. p.o., naproxen showed a very little effect to block the increase of hematological parameters in hypobaric hypoxic (HHc) condition however 18 mg/kg body wt. and 24 mg/kg body wt. showed a greater effect. The high altitude induced changes were almost blocked by naproxen at the dose of 18 mg/kg body wt. and it maintain the control level at dose of 24 mg/kg body wt. This was indicated that when rats were treated with naproxen the exposure to hypobaric hypoxia fails to stimulate sufficiently to increase the hematological parameters. The dose dependent inhibition of the HH-induced increased TC of RBC, hemoglobin (Hb) concentration and percentage of packed cell volume (PCV) by the administration of graded doses of naproxen probably indicates the gradual inhibition of PGs production. In normobaric condition the naproxen was not able to influence the above parameters at any dose because in that condition the production PGs was not stimulated like that of HHc condition. Thus it appears that the naproxen might be block the HH induced increased parameters by inhibiting the production of excess PGs.

The oxidative stress is the initiators of the physiological changes in HH, the mechanism of these changes are very complex which is still not clearly known. It is well established that the reactive oxygen species (ROS) are generated in HH due to the oxidative stress (OS) along with increased prostaglandins (PGs). According to Edmonds and Blake, [40] exposure to HH imposes the oxidative stress (OS). Exposure to HH is responsible for production of various oxyradicals [reactive oxygen species (ROS) etc], if these are not neutralized, they may cause damage of the cell-membrane [41]. The inhibition of ROS level by the administration of antioxidants in HH might block these changes and might indicate the role of ROS on the HH-induced hematological changes. Vitamin C and vitamin E are well known antioxidants. Both vitamin C and E have the ability to sequester of the singlet oxygen radical [42]. There are also reports that both the vitamin C and E might inhibit the generation of ROS [43-45]. In the present study the HH-induced hematological changes were blocked by vitamin E and vitamin C. While vitamin C was able to block the HH-induced hematological responses at the dose 400 mg/Kg body wt, vitamin E showed inhibition of HH-induced hematological responses at the dose of 40 mg/kg body wt and it maintained the control level at dose of 60 mg/kg body wt. At the higher dose of vitamin C (600 mg/kg body wt) this blocking effect on HH induce hematological responses was not observed but Vitamin E was able to show its blocking effect even at 60 mg/kg body wt. It appears that vitamin E is a suitable ROS inhibitor for a wide range of administered dose but vitamin C at higher dose may not work as an antioxidant. It has been reported that the supplementation of vitamin C at high doses (above 500 mg/kg body wt.) can serve as a pro-oxidant through the formation of ascorbyl radical [46]. In the present study, vitamin C at higher dose (600 mg/kg body wt) did not show any pro-oxidant effect on the HH-induced hematological responses but its antioxidant effect was not evident at this dose and similar observation was noted in our previous experiments [28]. Thus it appears from the present study that the higher ROS in oxidative stress of high altitude play an important role on the observed HH-induced hematological responses.

The HH-induced changes of hematological parameters (TC of RBC, Hb concentration and PCV) probably indicated that the exposure to HH was sufficient to generate the oxidative stress. Exposure to HH for long duration would have added some information on the adaptive nature of the observed changes, if any. It appears from the study that the oxidative stress in HH might be responsible for the generation of ROS which is responsible for the production of PGs by upregulating COX. The observed changes of hematological responses due to exposure to HH may be explained on the basis of the production of PGs and/or generation of ROS. The production of PGs and/or ROS level in HHc condition has stimulatory effect on the TC of RBC, Hb concentration and PCV. The specific influence of ROS and PGs on the HH-induced hematological responses cannot be ascertained from the study. However, the observed HH-induced changes may play an important role for the pathogenesis of high altitude induced disorders. The present study also indicates the potential role of NSAID (naproxen), and antioxidants (vitamin C and E) on the maintenance of the homeostasis of the physiological impairments in high altitude.

5. Figures with Legends

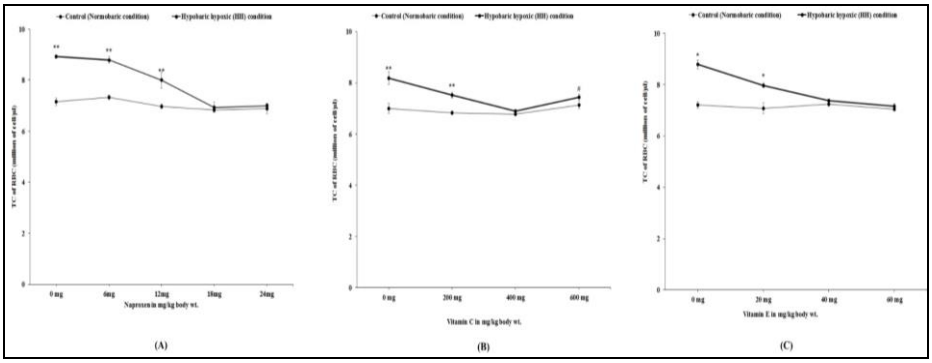

Fig 1: The TC of RBC in rats exposed to normobaric condition and hypobaric hypoxic (HHc) condition with the different doses of (A) Naproxen (6, 12, 18 and 24 mg/kg body wt.), (B) vitamin C (200, 400 and 600 mg/kg body wt.) and of vitamin E (20, 40 and 60 mg/kg body wt.) and without naproxen (vehicle only i.e., 0 mg/kg body wt. of naproxen), vitamin C (vehicle only i.e., 0 mg/kg body wt. of vitamin C) and vitamin E (vehicle only i.e., 0 mg/kg body wt. of vitamin E). The TC of RBC was increased significantly in hypobaric hypoxic exposed rats compared to that of corresponding doses in normobaric rats and normobaric rats without naproxen, vitamin C and vitamin E (vehicle only i.e., 0 mg/kg body wt. of naproxen, vitamin C and vitamin E) at ** $p \leq 0.001$, *$p \leq 0.01$ and # $p \leq 0.05$. Values are expressed in mean ±

SEM (n=6 in each group).

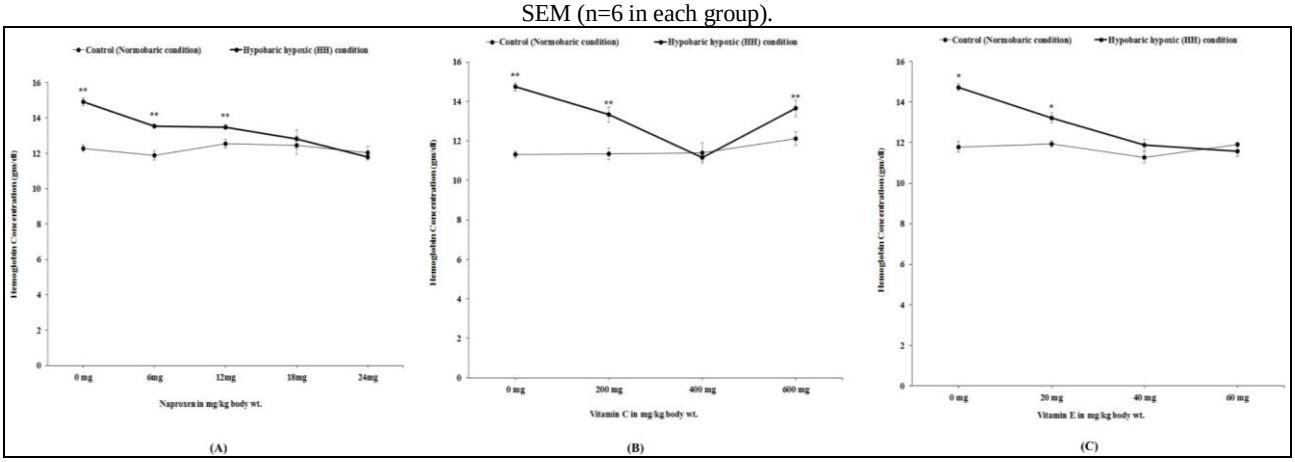

Fig 2: The hemoglobin concentration in rats exposed to normobaric condition and hypobaric hypoxic (HHc) condition with the different doses of (A) Naproxen (6, 12, 18 and 24 mg/kg body wt.), (B) vitamin C (200, 400 and 600 mg/kg body wt.) and of vitamin E (20, 40 and 60 mg/kg body wt.) and without naproxen (vehicle only i.e., 0 mg/kg body wt. of naproxen), vitamin C (vehicle only i.e., 0 mg/kg body wt. of vitamin C) and vitamin E (vehicle only i.e., 0 mg/kg body wt. of vitamin E). The hemoglobin concentration was increased significantly in hypobaric hypoxic exposed rats compared to that of corresponding doses in normobaric rats and normobaric rats without naproxen, vitamin C and vitamin E (vehicle only i.e., 0 mg/kg body wt. of naproxen, vitamin C and vitamin E) at ** $p \leq 0.001$, and *$p \leq 0.01$. Values are expressed in mean ± SEM (n=6 in each group).

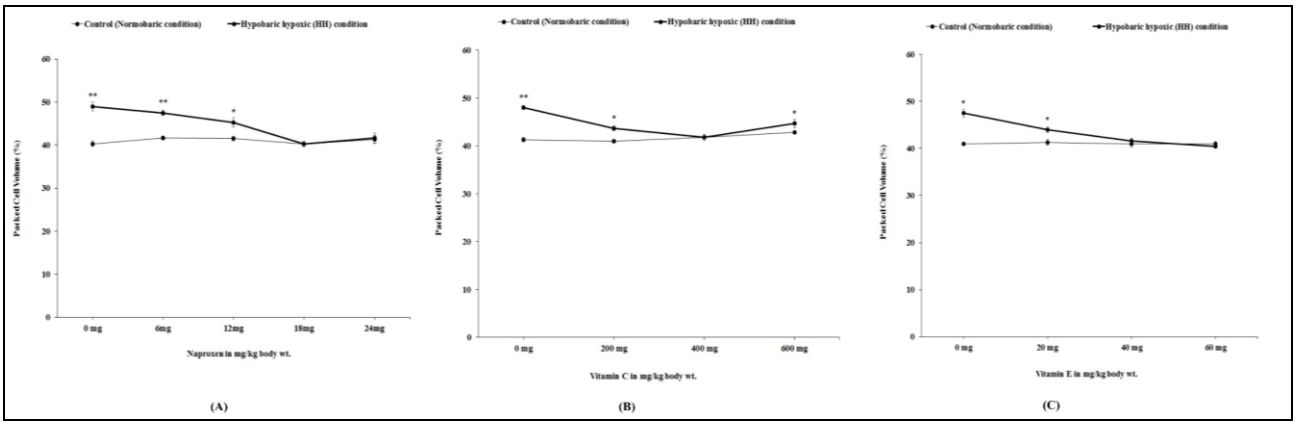

Fig 3: The Packed cell volume (PCV) in rats exposed to normobaric condition and hypobaric hypoxic (HHc) condition with the different doses of (A) Naproxen (6, 12, 18 and 24 mg/kg body wt.), (B) vitamin C (200, 400 and 600 mg/kg body wt.) and of vitamin E (20, 40 and 60 mg/kg body wt.) and without naproxen (vehicle only i.e., 0 mg/kg body wt. of naproxen), vitamin C (vehicle only i.e., 0 mg/kg body wt. of vitamin C) and vitamin E (vehicle only i.e., 0 mg/kg body wt. of vitamin E). The PCV was increased significantly in hypobaric hypoxic exposed rats compared to that of corresponding doses in normobaric rats and normobaric rats without naproxen, vitamin C and vitamin E (vehicle only i.e., 0 mg/kg body wt. of naproxen, vitamin C and vitamin E) at ** $p \leq 0.001$, and *$p \leq 0.01$. Values are expressed in mean ± SEM (n=6 in each group).

6. Conclusion

The HH-induced changes of haematological parameters observed in the present study is recovered by the administration of a NSAID (naproxen), which is a blocker of the PGs production and antioxidants (vitamin C and E), which have ROS quenching effects. The present study also indicates that the ROS are generated in HH due to the oxidative stress (OS) along with increased PGs may be involved in the HH-induced hematological changes in male rats.

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