Research Article

Erythroxylum Coca In High Altitude Acclimatization And Endurance Performance

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ABSTRACT

The present study was undertaken to elucidate the intervention of Erythroxylum coca (E. coca) in high altitude acclimatization and endurance performance. This study was also programmed to compare and correlate the effect of both E. coca (alkaloid) and a known sulphonamide drug, acetazolamide (as a positive control) in high altitude acclimatization. Experiment was conducted in two phases, Phase-I and Phase-II. In the phase-I study, animals were exposed to a simulated high altitude of 7,620m for 24h duration. Concurrently, E. coca and acetazolamide were prior administered at 25mg/kg body wt, each in separate groups to assess their role in high altitude acclimatization. In the phase-II study, animals were subjected to force swim exercise in order to investigate the role of E. coca in enhancing endurance performance. Both E. coca and acetazolamide could efficiently restore the hypoxia induced biochemical changes at par to that of normoxia. However, E. coca was found to make more of the availability of blood glucose (P<0.01), restore liver glycogen (P<0.01), and lowers the level of blood lactate (P<0.05). Also, the E. coca administration resulted into a more prolonged swimming time, indicating less fatigue in those groups of animals. Histopathological findings demonstrated the absence of muscular damage due to forceful swim exercise in the E. coca treated rats. The present study findings therefore justify the importance of E. coca in high altitude acclimatization and improving endurance performance.
INTRODUCTION:
Travel to high altitudes primarily causes hypoxia, accompanied by acute mountain sickness (AMS) characterized by headache, loss of appetite, nausea, vomiting, disturbed sleep, fatigue and dizziness. A rapid ascent to high altitude puts people at risk of developing high-altitude pulmonary oedema (HAPE), and high-altitude cerebral oedema (HACE) [1]. To circumvent the problems caused by altitude exposure, acclimatization is one of the alternatives practiced by many sojourns. It is a process by which individuals gradually adjust to hypoxia and enhance performance and survival. It is a slow process, taking place over a period of days to weeks. As a thumb rule, in long ascents at altitudes higher than 3000 m, the positive difference of the altitudes between two consecutive nights should not exceed 300m, and there should be two nights at the same altitude at every 3 days [2]. In military point of view, modern military operations frequently require rapid deployment of personnel into extreme environments (altitude, cold, heat) with little or no time for physiological acclimation. Thus, the practice of acclimatization drastically reduces the utilization of military personnel in rapid response military missions that exploit the air mobility and capability of modern military forces to quickly deploy to an area of operations on short notice.

Besides acclimatization, several other therapeutic interventions have been tried to lessen the effect of hypobaric hypoxia. In this direction, acetazolamide has been in use to counteract the effects of acute mountain sickness. Studies have shown that the use of acetazolamide in enhancing the velocity of ascent by reducing the symptoms of AMS and improving physical performance [3]. However, acetazolamide has got a lot of adverse effects that include metabolic acidosis, hypokalemia, and numbness of the extremities, headache, tinnitus, gastrointestinal disturbances, Stevens - Johnson syndrome and reduction in aerobic endurance performance [4, 5]. Other researchers suggested dexamethasone to treat altitude related ailments [6]. However, keeping in view of the adverse effects of steroids such as, dexamethasone, it is very uncommonly used in clinical medicine as a therapeutic modality to treat the altitude related maladies. At present, there are no effective therapeutic modalities available to counteract the altitude associated ailments during rapid ascent to high altitude. Thus, identifying a potential therapeutic modality which can reduce the period of high altitude acclimatization as well as improves work endurance is the need of the hour.

The plant Erythroxylum coca, is widely used by Andean peoples since ages as a performance enhancer at high altitude [7]. Previous reports observed that chewing of E. coca leaves enhances physical performance at high altitude [8]. Scientific interest in E. coca chewing derives from the self reported anecdotal information of coca users, which claim that E. coca enhances tolerance for work at altitude [7]. Despite the apparent use of E. coca leaves to enhance physical performance at high altitude, very little empirical data is available on the E. coca and its role in performance enhancement under hypoxic condition.

To address this issue, present work was undertaken to investigate the potential therapeutic applications of the plant Erythroxylum coca that is hypothesized to enhance acclimatization at high altitude as well as improving endurance performance under strenuous physical exercise.

MATERIALS AND METHODS
Experimental Animals
The experiment was conducted using apparently healthy male Sprague Dawley rats as an animal model. Rats with an average body weight of 200±10g were selected for the experiment. All animal procedures and experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), DIPAS; authorization number; IAEC-08/DIPAS/2013.

Dose optimization of Erythroxylum coca
Erythroxylum coca (E. coca) was purchased from JNSON laboratories, India. A dose dependent study of E. coca in rats was performed by administering orally, starting with a minimum dose of 12.5mg/kg body wt, followed by 25, 50 and 100mg/kg body wt of E. coca freshly dissolved in dimethyl sulfoxide (DMSO-0.5%) and high altitude hypoxic tolerances were
observed in those groups of animals. Normoxic animals were given the solvent (DMSO- 0.5%).

High altitude hypoxic tolerances in the rats (gasp time) was measured by exposing the animals to a simulated altitude of 9,754m (205mmHg) in an animal decompression chamber [9]. The airflow in the chamber was maintained at 2 L/min, while the relative humidity was maintained at 50 – 55 %. The time taken for the appearance of first sign of gasping was recorded using an electronic stopwatch.

During the dose optimization of E. coca, it was observed that, administration of E. coca at all the doses resulted into a significant extended gasping time as compared to the control groups of animals (hypoxia exposure without E. Coca; 25±09 minutes), with the peak level at 25mg/kg body wt dose (155±14 minutes) showing a much longer time of hypoxic tolerance in those groups of animals (Fig. 1).

Therefore, for further studies, rats were administered with E. coca, using optimum dose of 25mg/kg body wt through oral route, 1h prior to high altitude hypoxia exposure in the phase-I study as well as swimming exercise in the phase-II study, in order to investigate the role of E. coca in acclimatization on exposure to high altitude hypoxia as well as endurance performance test through force swim exercise respectively.

**Acetazolamide dose**

The drug acetazolamide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Studies in human subjects have shown that prophylactic administration of acetazolamide at a dose of 250mg twice daily was effective in inducing a significant decline in AMS symptoms over a 24h period of sojourn to high altitude [10]. It increases the amount of bicarbonate excreted in the urine, making the blood more acidic by inhibiting the enzyme carbonic anhydrase [11]. Acidifying the blood drives the ventilation, which is the cornerstone of acclimatization. Acetazolamide dose for the rats were extrapolated from the standard formula set forth by [12], which is based upon the body weight of the animal and body surface area multiplied with that of the human equivalent dose. Therefore the data calculated for the rats was 25mg/Kg body weight.

The drug acetazolamide was administered orally at 25mg/Kg body weight, freshly dissolved in DMSO (0.5%), 1h prior to the high altitude hypoxia exposure as a positive control against E. coca in order to compare and correlate its effect in high altitude acclimatization.

**Details Of High Altitude Hypoxia Exposure**

The rats were exposed to a simulated altitude of 7,620m (280mm Hg) in a hypobaric chamber for 24h duration. Fresh air was flushed continuously into the chamber at 4 l/h and humidity of the chamber was maintained at 50-55 %. The animals were given food and water ad libitum during exposure to hypobaric hypoxia.

**Experimental design**

The experiment was conducted into two phases: Phase I and Phase II.

Phase I experiment was conducted to study the role of E. coca in acclimatization on exposure to hypobaric hypoxia, using acetazolamide as a positive drug control. Animals were divided into 4 groups viz., I. Normoxia, II. Hypobaric hypoxia, III. Hypobaric hypoxia with E. coca and IV. Hypobaric hypoxia with acetazolamide. The solvent DMSO (0.5%) was given to the control rats viz., normoxia and hypobaric hypoxia.

Phase II experiment was conducted in order to investigate the effects of E. coca leaf extract in enhancing endurance performance under exhaustive swim exercise. Here animals were subjected to strenuous physical exercise of forced swimming, considering that the animals were experienced with hypoxic condition while subjecting to swimming exercise. It has been well documented that hypoxia results in slower oxygen uptake kinetics during exercise [13] resulting in severe and repetitive oxygen stress [14].

**Biochemical analysis**

**Liver and muscle glycogen**

Following high altitude hypoxia exposure and force swimming exercise, the animals were anaesthetized with thiopentone sodium at 50mg kg/ body wt, intra-peritonealy to perform further experiments.
Liver and muscle glycogen was determined by the phenol-sulphuric acid method for total reducing carbohydrate [15].

**Blood glucose, blood lactate, triglycerides and lactate dehydrogenase**

Blood samples were collected in heparinised tubes from both the phases of animals and were measured for the above parameters using commercial kit (RANDOX, U.K.) as per manufacturer’s instructions.

**2, 3-Diphosphoglycerate (2, 3 -DPG)**

2, 3-Diphosphoglycerate in the plasma samples were measured using commercial kit (Sincere Biotech Co., Ltd, China) as per manufacturer’s instructions.

**Force swim exercise**

Swimming exercise was performed in a cylindrical shaped glass water tank. Temperature of the water for swimming exercise was maintained at 37°C in order to maintain the optimum core body temperature of the rats, as rats maintain a relatively constant temperature under these conditions [16]. The animals were allowed to swim with attached weight equivalent to 2% of the total body weight [17]. The uncoordinated movements and staying under the water for 10 seconds without swimming at the surface were accepted as the exhaustion criteria of the rats [18].

**Tissue processing and histological analysis**

Each gastrocnemius muscle was cut in the middle, transversally and fixed in 10% formalin solution. Tissues were embedded in paraffin wax. Later, serial slices of 5-7µm were allocated in paraffin wax and allowed to dry at room temperature. Histochemical staining procedure of cross-sections was carried out with haematoxylin-eosin (HE) techniques [19]. The slide images were obtained in a light microscope (Olympus BX50) connected to a computer.

**Protein expression studies (Phase-I and Phase-II)**

Briefly, the lung tissue samples of high altitude hypoxia exposed animals and skeletal muscle tissue samples of force swimmmed animals were homogenized in (10 homogenate buffer; 0.154M KCl – 1ml, PMSF - 10µl, DTT - 10µl, Cocktail mix. – 5 µl), followed by centrifugation and the supernatant was collected and stored at - 80°C. Protein estimation was carried out by method of Lowry et al. (1951). After estimating the amount of protein present in the lung tissue and skeletal muscle extracted fractions, the samples were analysed for Hypoxia inducible factor (HIF-1α), Erythropoietin (EPO), Glucose transporter (GLUT-4), Monocarboxylate lactate transporter (MCT-1), Vascular endothelial growth factor (VEGF), Glycogen synthase (GS-1) through western blotting.

**Statistical analysis**

Results were expressed as mean±SD. Differences between the four groups of animals; normoxia, hypoxia, and the drug administered hypoxia exposed animals (E. coca and acetazolamide) were assessed by using one-way ANOVA followed by Student’s–Newman–Keuls test for multiple comparisons among groups. Comparisons between control swimmmed and E. coca administered swimmmed animals were assessed by using a t-test applying the Bonferroni correction. Differences were considered statistically significant at P<0.05. All statistical tests were performed with the SPSS statistical software, version 12.0 for Windows (SPSS Inc, Chicago, Ill).

**RESULTS**

**Phase-I study: High Altitude Acclimatization Study**

**Changes in biochemical parameters through E. coca administration**

Biochemical analysis from the plasma samples of different groups of animals has been summarized in Table 1. Our results indicate that hypoxia exposure at an altitude of 7,620m for 24h duration resulted into a significant decrease in blood glucose level at P<0.05, increase in lactate level at P<0.001 and a decrease in triglyceride level at P<0.0001, as compared to the normoxic animals. The liver and muscle glycogen content was found to be significantly depleted through high altitude hypoxia exposure at P<0.001, as compared to normoxic animals. The liver and muscle glycogen content was found to be significantly depleted through high altitude hypoxia exposure at P<0.001, as compared to normoxic animals. On the contrary, E. coca and acetazolamide administration resulted into a significant restoration in the liver glycogen content of the 24 h hypoxia exposed animals at P<0.01 and P<0.05, respectively, as compared to the control (hypoxia exposure without drug intervention). Further, the 2, 3-diphosphoglycerate (DPG) level increased from normoxic level of 8.51±0.61nmol/L to 19.67±2.44nmol/L at 24h hypoxia exposure, with a
significant difference of P<0.05. Further, administration of E. coca and acetazolamide prior to high altitude hypoxia exposure resulted into an improvement in the changes in all the biochemical parameters in both the groups of animals.

Table 1. Changes in biochemical parameters through E. Coca administration.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Phase I study</th>
<th>Phase II study</th>
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<tbody>
<tr>
<td></td>
<td>Normoxia</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>125±10.29</td>
<td>103.94±8.29 *</td>
</tr>
<tr>
<td>Blood lactate (mg/dl)</td>
<td>11.81±2.37</td>
<td>33.74±5.04 †</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>127.23±5.34</td>
<td>90.75±3.73 ‡</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>118.32±7.76</td>
<td>282.70±8.75 †</td>
</tr>
<tr>
<td>Liver glycogen (mg/g tissue)</td>
<td>0.496±0.13</td>
<td>0.096±0.07 ‡</td>
</tr>
<tr>
<td>Muscle glycogen (mg/g tissue)</td>
<td>0.298±0.08</td>
<td>0.054±0.04 ‡</td>
</tr>
<tr>
<td>2,3-DPG (nmol/L)</td>
<td>8.51±0.61</td>
<td>19.67±2.44 †</td>
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Protein expression studies

**Hypoxia inducible factor (HIF-1α) expression**

In the present study, hypoxia exposure for 24h duration resulted into a striking and marked up regulation in the HIF-1α protein expression pattern (Fig. 2 a-i). However, when the E. coca and acetazolamide treated hypoxia exposed group of animals were compared, there was a marked and appreciable difference in the HIF-1α expression pattern with a higher level of expression (P<0.001) in the E. coca treated hypoxia exposed group of animals.

**Erythropoietin (EPO) expression**

High altitude hypoxia exposure resulted into a distinct up regulation in the EPO protein expression pattern (Fig. 2 a-ii). Further, densitometry analysis showed a statistically higher EPO level in the lung tissues of rats administered prior with E. coca as well as acetazolamide as compared to hypoxia exposure without any drug intervention. A statistically non significant difference existed in between the E. coca and acetazolamide treated hypoxia exposed groups of animals.

**Vascular endothelial growth factor (VEGF) expression**

There was a noticeable up regulated expression of VEGF in the lung tissues of rats through high altitude hypoxia exposures (Fig. 2 a-iii). Densitometry analysis revealed a statistically significant difference of P<0.001 in the hypoxia exposed groups of animals as compared to the control (normoxia). A statistically non significant difference existed in between the E. coca and acetazolamide treated hypoxia exposed groups of animals.

**Phase-II study: Endurance performance Study**

**Force Swim Test**

The phase-II experimental study results showed a significantly longer swimming time (P<0.001) in the E. coca treated animals as compared to the animals that were subjected to swim exercise without administering with E. coca (control).

**Histological changes of skeletal muscle tissue samples through E. coca administration**

Histological comparison between the gastrocnemius muscles of the control and E. coca treated groups of animals has been depicted in Fig. 4. The gastrocnemius muscle biopsy cut of the rats subjected to force swim test (Fig. 4 a, b) showed rounding, loss of cytoplasm, atrophy of fibres with irregularity in shape and increased inter fascicular...
space. However muscle biopsy cut of the E. coca treated groups of animals (Fig. 4 c, d) showed uniformly sized angular muscle fibres with a mild atrophy. The inter fascicular space was found to be minimal.

**Biochemical Parameters**

Administration of E. coca in the rats prior to force swim exercise resulted into a significantly higher level of blood glucose (P<0.001), lower blood lactate level (P<0.01), a higher plasma triglyceride level (P<0.001) and a statistically non-significant difference in the plasma LDH, liver glycogen, as well as 2, 3-DPG level, as compared to the animals that were subjected to force swim exercise without prior administering with E. coca (control) (Table 1).

**Protein expression studies**

When the rats were subjected to force swimming exercise, we observed a marked up regulated expression of the transcription factor HIF-1α with a significantly higher level in the E. coca treated groups of animals (P<0.05). The downstream genes regulated by HIF-1α with respect to glucose metabolism viz., GS-1, GLUT-4 and MCT-1 were also found to be substantially higher in the E. coca treated groups of animals. These changes in protein expression pattern have been depicted in the figure 5, representing western blot (a) as well as densitometry analysis (b).

*Figure 1. Dose optimization of E. coca in hypoxia exposed rats (high altitude hypoxic tolerance-gasping time parameter).*

Values are mean ±SD (n=7). ** P<0.001; as compared to control (hypoxia without E. coca ; 0 mg/kg body wt). # P<0.05; as compared to hypoxia with E. coca treated group (12.5 mg/ kg body wt). NS; as compared to hypoxia with E. coca treated group (50, 100 mg/kg body wt).
Figure 2. Protein expression studies in lungs of rats exposed to hypobaric hypoxia (7620 m) for 24 h duration.

(a) represents western blot analysis and (b) represents densitometry analysis. Values are mean ±SD (n=7). *P<0.05, **P<0.01, ***P<0.001, NS-Non significant. (Normoxia Vs Hypoxia; Hypoxia Vs Hypoxia + E. coca; Hypoxia Vs Hypoxia + Acetazolamide; Swim Vs Swim + E. coca). *P<0.05, **P<0.01, @P<0.001, NS Non significant (Hypoxia + E. coca Vs Hypoxia + Acetazolamide).

Figure 3. Changes in force swim exercise test through Erythroxylum coca administration.

Values are mean ±SD (n=7). ***P<0.001; Swim Vs Swim + E. coca
Figure 4. Histological changes of skeletal muscle tissue samples through E. coca administration as observed by hematoxylin and eosin staining (40x).

(a): Force swim exercised rat (Control)-Transverse section: High power photomicrograph of muscle biopsy cut in transverse section showing mild atrophy of muscle fibres with widening of inter fascicular space. A few fibres showed rounding and loss of cytoplasm.

(b): Force swim exercised rat (Control)-Longitudinal section: High power photomicrograph of muscle biopsy cut in longitudinal section showing atrophy of fibres with shape irregularity and increased inter fascicular space.

(c): Force swim exercised rat with E. coca (Treated)-Transverse section: High power photomicrograph of muscle biopsy cut in transverse section showing uniformly sized angular muscle fibres with peripheral nuclei. The inter fascicular space is minimal.

(d): Force swim exercised rat with E. coca (Treated)-Longitudinal section: High power photomicrograph of muscle biopsy cut in longitudinal section showing uniformly sized muscle fibres with peripheral nuclei. A single hypertrophic fibre (H) is seen in the centre of the photomicrograph.
Figure 5. Protein expression studies in the skeletal muscles of rats subjected to force swim test.

(a) Represents western blot analysis; (b) Represents densitometry analysis. Values are mean ± SD (n= 7). *P<0.05, **P<0.01; Swim Vs Swim + E. coca
**DISCUSSION**

Under an exhaustive condition of high altitude and exercise induced hypoxia, cellular utilization of oxygen increases 10 to 15-folds [20], which could further induce a variety of physiological and biochemical changes. Following are the changes observed in the biochemical parameters through high altitude as well as exercise induced hypoxic rats.

In the present study, high altitude hypoxia exposure at 7,620m resulted into a decrease in blood glucose level as compared to the normoxia animals. However, administration of E. coca and acetazolamide prior to the high altitude hypoxia exposure resulted into a higher blood glucose level in both the groups of animals as compared to the control (high altitude hypoxia exposure without any drug).

Similar to our result, previous reports also confirmed that chewing of coca leaves at high altitude resulted into a rise in blood glucose concentration in humans [8]. An increased dependence on blood glucose after acclimatization to 4,300m was observed in humans [21]. Therefore, the present study decrease in blood glucose level due to high altitude hypoxia exposures might be the consequence of the high altitude acclimatization process. Concomitantly, the present study with increase response in blood glucose level due to E. coca administration while subjecting the rats to force swim exercise, might have resulted into a ready availability of energy source which might have delayed the onset of fatigue in those groups of rats, as seen with an extended swimming time.
Parallel to the blood glucose level, blood lactate concentrations reflect the balance between lactate production and clearance. A high blood lactate level is an early sign of tissue hypoxia [22]. In our study the rise in blood lactate level due to high altitude hypoxia exposure indicated a compensatory adjustment to the stress of hypoxic exposure, signalling the lack in the tissue oxygen level. The lower blood lactate level in the E. coca treated hypoxia exposed as well as, in the animals subjected to force swim test, indicated that there was an early clearance of lactate from the circulation providing the substrate as gluconeogenic precursor (Cori cycle pathway). The same was not observed in the acetazolamide treated high altitude hypoxia exposed groups of animals, showing a higher blood lactate level. Evidences relate the lactate exchange between tissue sites of production and removal, representing an important means for delivery of oxidizable substrate as well as gluconeogenic precursor during exercise [21]. Reports have stated that accumulation of lactic acid in the muscle is usually related to fatigue and muscle soreness [23]. Therefore, better performance is often related to lactate clearance from the blood.

Further, the present study showed a decreased level of triglycerides in the control groups of animals (high altitude hypoxia exposure and exercised rats), which therefore, indicated an increase in fat oxidation during exposure to high altitude, and utilization of fat as an energy source for the forceful swimming exercise. Decrease in triglyceride level indicated an increase in fatty acid oxidation using fat as an energy source [24]. However, higher level of triacylglycerols in the E. coca and acetazolamide treated group of hypoxia exposed animals indicates a substantial role of both the drugs in high altitude acclimatization, thereby conserving the substrate in the yield of ATP per mole of oxygen.

The current investigation of elevated level of LDH in the plasma of high altitude exposed rats and force swummed control rats indicates that the rats were experiencing with oxidative stress due to hypoxic stress of high altitude as well as stress of forceful swimming exercise. It has been known that extracellular appearance of LDH in the serum or plasma is used to detect oxidative stress, cell damage or cell death [25]. This was probably minimized through E. coca administration, indicating that E. coca curtails oxidative stress or cell damage.

The present study exposure of the rats to high altitude hypoxia resulted into a marked increase in the glycolytic metabolite, 2, 3- DPG. It has been stated that hypoxia would appear to be the primary stimulus for 2, 3-DPG release [26]. The probable reason behind the marked rise in the 2, 3-DPG in our study, in all the three hypoxia exposed groups of animals might be as an early adaptive mechanism to hypoxia. Previous studies showed that there exists a parallel relationship in between the increase in erythrocyte 2, 3-DPG level and the decrease affinity of Hb to bind to oxygen, thereby releasing more numbers of oxygen available to the tissues for its oxygenation [27].

The lower level of 2, 3-DPG in the drug administered rats might be attributed with a decreased sensitivity of the 2, 3-DPG response to hypoxia after high altitude acclimatization, indicating the role of E. coca and acetazolamide in high altitude acclimatization process.

The master regulator of body's adaptive responses to hypoxia is the heterodimeric transcription factor, hypoxia inducible factor -1 (HIF-1α) [28]. The HIF-1α dependent pathway regulates the expression of proteins important for anaerobic glycolysis, angiogenesis, and cell survival [29].

The present study results of hypoxia inducible factor (HIF-1α) dependent pathway in high altitude acclimatization showed that exposure of the rats to high altitude hypoxia at 7,620m above sea level resulted into a marked up regulation of the HIF-1α expression. Furthermore, it was also observed in the present study that there was a marked up regulation in the HIF-1α regulated genes, viz., Erythropoietin (EPO) and Vascular Endothelial Growth Factor (VEGF) through high altitude hypoxia exposure. However, when the control groups (24h hypoxia exposed animals) were compared with that of the treated groups (24h hypoxia exposed animals treated with E. coca and acetazolamide), there existed a marked and considerable difference in their protein expression pattern with a higher level of expression in the HIF-1α and EPO. On the other hand, the VEGF expression was found to be lowered through the E. coca administration.

It has been reported by previous studies that the primary transcriptional response factor for acclimatization to hypoxic stress is the hypoxia-inducible factor-1α (HIF-1α), which facilitates glucose uptake and metabolism, angiogenesis and erythropoiesis [30]. It has been known that hypoxia induces tissue-specific gene products such as Erythropoietin and
Vascular Endothelial Growth Factor, which modulates the erythropoiesis and vascularisation in the tissues in order to adapt cells to reduced oxygen availability [31]. Moreover, it has been well established that high altitude exposure induced changes in erythropoiesis is mainly characterized by an increase in red blood cells and haemoglobin concentration and these changes are common adaptable criteria for high altitude acclimatization [32]. It has been observed in our study that the administration of E. coca prior to the high altitude hypoxia exposure resulted into a noticeable higher expression of HIF-1α and erythropoietin. The higher accumulation of HIF-1α and erythropoietin in the lung tissues of hypoxia exposed E. coca treated groups of animals indicated that, E. coca might promote adaptive mechanisms to high altitude hypoxia exposed maladies under extreme altitude exposure of 7,620m above sea level by improving the peripheral oxygen supply through improved erythropoiesis and vascularisation.

Studies revealed that the transcription factor, HIF-1α has been recognized to cause an increased expression of VEGF under hypoxic condition [33]. Although the VEGF is known to be an important signal protein produced by the cells that stimulates vasculogenesis and angiogenesis and restores the oxygen supply to the tissues when blood circulation is inadequate, however its overexpression is known to induce high altitude pulmonary edema [34] and cerebral edema [35] under exposure to high altitude hypoxic condition. The present study lower expression of VEGF in the E. coca as well as acetazolamide treated groups of rats as compared to the control groups indicated that there was a less destruction of the vascular basement membrane in those groups of animals which otherwise would have resulted in vascular permeability. The VEGF over expression and vascular permeability has been discussed in the previous reports as well [36]. Our study therefore indicated the possible role of E. coca in preventing HAPE and HACE.

Parallel to the high altitude acclimatization studies, our study for physical performance showed that oral administration of E. coca seems to alleviate fatigue as observed with a much extended swimming time. Fatiguing respiratory muscle elicits sympathetically mediated vasoconstriction in limb muscle vasculature which compromises leg blood flow [37]. Reductions in muscle oxygen transport attenuate the maximal oxygen consumption (VO2 max), exaggerate the rate of fatigue development and deteriorate endurance exercise performance [38]. It has been reported that high intensity endurance exercise in some fit athletes caused a time dependent decrease in saturation of inspired oxygen (SaO2) of greater than 5% from resting level of 98% to an extreme drop of 80% [39], which resulted in reduced endurance capacity in athletes [40].

Our result therefore indicated that under exhaustive swimming exercise, the rats were experiencing tissue hypoxia, followed by cellular stress and muscle fatigue as observed with a higher blood lactate level in the exercised groups of animals. However, prior administration of E. coca might have reduced the hypoxic fatigue condition in the force swim exercised rats, as observed with a lesser lactate level in the E. coca treated groups of rats.

Previous reports also stated that chewing of E. coca leaves enhance physical performance at high altitude [8, 41] and delays the onset of fatigue during prolonged exercise by potentially enhancing tolerance for work [42]. Furthermore, chewing of the E. coca leaves was found to be more common amongst mineworkers [43]. It has been suggested that anti-fatigue agents are essential to enhance the physical performance in persons engaged in heavy manual work, sports personnel, or soldiers who will be performing physical work continuously [44].

Therefore, the present study showing more extended swimming time in the E. coca administered groups of animals indicated that the animals were experiencing less exhaustion, which evidences the use of E. coca as an anti-fatigue agent in enhancing endurance performance. Parallel study on histological changes in the skeletal muscle tissue samples through force swim exercise showed muscular damage, as seen in the histological tissue sections of the gastrocnemius muscle (Fig. 4 a,b). The reason behind the skeletal muscle tissue damage during heavy exercise was better explained by the earlier findings of [45], who reported that glycogen synthase kinase 3β is responsible for the induction of skeletal muscle atrophy. Also, the reactive oxygen species generated during tissue hypoxia of heavy exercise have been implicated in the damage of cell proteins, DNA, and lipids through oxidation and thus have been related with muscle damage and muscle wasting and various pathological conditions [46].
Therefore, it becomes confirmed through our report as well as the earlier findings that under strenuous physical activity, skeletal muscle tissue damage takes place [47]. However the use of E. coca will minimise the intensity of muscular damage occurred through exhaustive swimming, as observed in the histological tissue sections.

Also, it is known that in exercising skeletal muscle, the physiological flux of oxygen is extreme as evidences revealed that during exercise under normoxia, the partial pressure of oxygen in the muscle tissue has been shown to decrease [48]. Hypoxia is thus a critical parameter in muscle function under exercise, influencing production of ATP, utilization of energy producing substrates, and manufacture of exhaustion-inducing metabolites. It has been known that a switch from aerobic to anaerobic metabolism occurs during strenuous exertion, as the muscles need more oxygen than breathing can provide, and glycolysis is the central source of anaerobic energy in animals under hypoxic condition of exercise [14]. This shift in the metabolic pathway from aerobic to anaerobic glycolysis is regulated under low-oxygen conditions by the transcription factor hypoxia-inducible factor 1α (HIF-1α).

The present trial result with higher HIF-1α in the E. coca treated group implicated the role of E. coca in the HIF-1α regulation of metabolic function along with normal physiological oxygenation even during strenuous physical exertion of force swim exercise. Also, the much enhanced upregulated expression of the HIF-1α regulatory genes, GS-1, GLUT-4 and MCT-1, indicated that subjecting the rats with E. coca, prior to swimming exercise will assist in better utilization of the circulating blood glucose as observed with a higher blood glucose level. This also signifies with a higher muscle oxidative capacity in the same groups of animals. Since, previous studies in human subjects reported that higher GLUT-4 levels [49] and MCT-1 levels [50] are associated with higher muscle oxidative capacity. Therefore, factors that are contributing to the rapid phase of glycogen synthesis is an up regulation of the transcription of HIF-1α with an increase in the percentage of GS-1 and GLUT-4, which will be followed by an increase in the muscle cell membrane permeability to glucose, which is true in case of E. coca treated swummed rats.

This has also evidenced that the E. coca treated groups of animals have a higher oxidative and endurance capacity as observed with a much lower lactate level and a higher MCT-1 expression. Taken together a hypothesis has been drawn from the present study findings on the role of E. coca in enhancing endurance performance (Fig. 6.). The present study subjecting the rats to forceful swim exercise resulted into an episode of reduced oxygen availability while in exercise. Reduction in oxygen availability will further result in metabolic shift from aerobic to anaerobic respiration by the key transcription factor, HIF-1α, transcribing the genes responsible for glucose metabolism viz., glucose transporter (GLUT-4; consequential in an increased glycolytic flux) and glycogen synthase (GS-1; ensuing in accumulation of glycogen). The up regulated HIF-1α also regulates the cellular transport of the glycolytic substrates pyruvate and lactate via monocarboxylate transporter (MCT-1). HIF-1α, therefore makes the availability of monocarboxylates (pyruvate and lactate) for further glycolysis cycle, which helps in faster generation of ATP, as required during the exhaustive swimming exercise. In the present study, the higher level of HIF-1α, GS-1, GLUT-4 and MCT-1 in the E. coca administered force swim animals as compared to the control groups indicated that E. coca might have stirred the glycolysis cycle while in swimming exercise, followed by faster clearance of blood lactate thereby making the availability of the substrate lactate and its utilisation in the re-synthesis of glucose (gluconeogenesis) as an energy source in the liver. The same was not seen in the control group of rats subjected to swimming exercise without E. coca (as seen with a much higher blood lactate level and lower MCT-1 expression). Eventually administration of E. coca prior to strenuous physical activity might enhance the endurance performance, which needs further extensive study.

CONCLUSIONS

In conclusion, our study therefore suggested that E. coca supplementation improves acclimatization to high altitude hypoxia in rats, probably by facilitating biochemical and molecular adaptation to hypoxia as observed with an up regulated expression of HIF-1α transcription factor. Simultaneously, it promotes the better utilization of the circulating level of increased blood glucose by glycogen synthesis through GLUT-4 and Glycogen synthase-1 and better lactate utilization through MCT-1 expression. The present study outcome also reveals that administration of E. coca exhibits anti-
fatigue properties, possibly by modulating at the biochemical and molecular level, and thereby might possibly improve endurance performance capacity in an individual.

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