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Modulatory effect of seabuckthorn leaf extract on oxidative stress parameters in rats during exposure to cold, hypoxia and restraint (C-H-R) stress and post stress recovery

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Abstract

This study was carried out to examine the antioxidative potential, if any, of seabuckthorn leaf aqueous extract, administered orally in rats at a dose of 100 mg kg^{-1} both in single and five doses, 30 min before cold (5°C)–hypoxia (428 mm Hg)–restraint (C-H-R) exposure. The effect of the extract was studied on lipid peroxidation and antioxidant parameters in liver and gastrocnemius muscle of rats on attaining the rectal temperature (T_{rec}) of 23°C during C-H-R exposure and after recovery (T_{rec}37°C) from C-H-R-induced hypothermia. In untreated rats exposed to C-H-R, there was a significant increase in malondialdehyde (MDA) levels in liver and muscle along with decreased activity of catalase (CAT) and glutathione-S-transferase (GST) in liver and muscle. Single- and five-dose extract treatment restricted the increase in liver and muscle MDA levels and five doses of extract treatment further improved the levels of liver antioxidants, viz. reduced glutathione (GSH), on recovery of T_{rec}37°C, increased superoxide dismutase (SOD) during exposure and recovery, normalized CAT activity in liver during C-H-R exposure and an increase on recovery of T_{rec}37°C. The decreasing pattern of liver and muscle GST levels both in single-dose and five-dose extract treated rats was similar to that in untreated rats. Results suggested that supplementation with seabuckthorn extract helps to reduce oxidative stress in liver and muscle of rats during C-H-R exposure and post-stress recovery.

Introduction

Oxygen is essential and indispensable to most organisms on the earth, with mitochondrial ATP production linked to the reduction of oxygen (O_2) to water being the primary energy-producing pathway of the cell. Increased energy demand during exposure to adverse stressful environmental situations or high-endurance physical activity, especially of the aerobic type, necessitates a multifold increase in oxygen supply to maximize cellular energy yield. Reactive oxygen species (ROS) are formed during increased metabolic states due to partial reduction of O_2. ROS are part and parcel of life. But during chronic or recurrent stress there is increased utilization of energy and more ROS are produced. If the concentration of these ROS exceeds the body's capacity to neutralize them, they begin to harm cells and, in cases of chronic stress, our tissues and organs. ROS also damage mitochondria, the cellular components that produce energy, thereby reducing our capacity to maintain cellular energy levels.

Hence, during stress aerobic organisms have to pay a price for such increase in metabolism, due to generation of ROS, which causes lipid peroxidation, enzyme inactivation and oxidative damage to DNA (Halliwell & Gutteridge 1989; Shewfelt & Purvis 1995). ROS generation occurs in various stressful conditions viz. intermittent exposure to hypoxia (Radak et al 1994), cold (Bhaumik et al 1995) and immobilization (Hisao et al 1993). ROS stimulate the rate of autocatalytic process of lipid peroxidation and damage to biomembranes and muscular structure (Benzi 1993), thereby compromising cell integrity and function. This results in muscular atrophy (Kondo & Itokawa 1994) and muscle fatigue (Barclay & Hansel 1991) leading to decreased performance of an individual.

It is possible to support the body's adaptation during stressful situations by using food supplements, dietary elements, herbs and minerals for increasing physical and mental performance. Such substances have been described as adaptogens (Brekhman & Dardymov

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1969). Supplementation with various macro- and micronutrient and herbal preparations has been shown to possess adaptogenic activity during exposure to stressful environments (Kumor & Srivastava 2000; Kumar et al 1996, 1999, 2002).

Seabuckthorn (*Hippophae rhamnoides* L.), a wild shrub growing at high altitude (2500–4000 m) in adverse climatic conditions, belongs to the family Elaeagnaceae. All parts of the plant (i.e., fruits and leaves) are considered to be good sources of a large number of bioactive substances like vitamins (A, C, E, riboflavin, folic acid and K), carotenoids (α, β, δ-carotene, lycopene), flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glucoside compounds), organic acids (malic acid and oxalic acid), sterols (ergosterol, stigmasterol, lanosterol and amyrins) and some essential amino acids (Pintea et al 2001; Kalio et al 2002). Seabuckthorn leaves and fruits are rich in different flavonoids in addition to other bioactive molecules.

Seabuckthorn is a thorny nitrogen-fixing deciduous shrub, native to Europe and Asia (Rousi 1971). Many medicinal effects of seabuckthorn against flu, cardiovascular diseases, mucosal injuries and skin disorders (Xiao 1980; Beveridge et al 1999; Eccleston et al 2002) have been suggested, which might be due to its adaptogenic, antioxidative and immunomodulatory activity (Geetha 2004). Recently, a seabuckthorn leaf aqueous extract was shown to possess potent adaptogenic activity in rats during exposure to cold (5°C)–hypoxia (428 mmHg)–restraint (C-H-R) with no acute or subacute toxicity in rats. In an acute toxicity study the LD50 of the extract was observed to be more than 10 g kg^{-1} (Saggu et al 2007). The extract was also found to be safe with respect to heavy metal toxicity (Saggu et al 2006).

This study was undertaken to examine the antioxidant effect of seabuckthorn dry leaf aqueous lyophilized extract administration, if any, at an oral dose of 100 mg kg^{-1} (Saggu et al 2007) on attaining the rectal temperature (T_{rec}) of 23°C during exposure to C-H-R (Ramachandran et al 1990) stress and after recovery (T_{rec}37°C) from C-H-R-induced hypothermia.

Materials and Methods

Experimental animals

Male inbred Sprague–Dawley rats, 12–14 weeks old, weighing 150 ± 10 g, from the animal colony of the Defence Institute of Physiology and Allied Sciences (Delhi, India), were used for the study. The rats were maintained under a controlled environment in the institute's animal house at 22 ± 1°C, 55% ± 1% humidity and 12-h light–dark cycle. The rats had free access to standard animal food pellets and water. The experiments were performed in accordance with the regulations specified by the institutional animal ethical committee and conformed to national guidelines on the care and use of laboratory animals, India.

Plant material

Seabuckthorn (*Hippophae rhamnoides* L.) leaves were collected in the month of September from hilly regions of the western Himalayas, India, where the plant grows widely under natural conditions. The Field Research Laboratory, Leh, India, where the voucher specimen of the plant material is preserved in the herbarium, carried out the ethanobotanical identification of the plant. Fresh leaves of seabuckthorn were cleaned and washed thoroughly with water and re-washed with distilled water. Washed fresh leaves were dried under shade in a clean, dust free environment and crushed using a laboratory blender.

Extract preparation

Aqueous lyophilized extract of seabuckthorn dried leaves was prepared by a cold percolation method as described earlier (Saggu et al 2007). One gram of dried seabuckthorn leaves produced 0.137 g of lyophilized seabuckthorn aqueous extract powder. The HPLC profile of the extract was studied and there was no batch-to-batch variation in the prepared extract. The HPLC profile of the extract has been published earlier (Saggu et al 2007).

Experimental design

All the experiments were done on healthy male rats fasted overnight. In total, 54 rats were used in the study and divided into three groups. Eighteen rats were included for a control group; 18 rats were used for a single oral extract dose (100 mg kg^{-1})-treated group and 18 rats were used for a five oral extract doses (100 mg kg^{-1}, single dose daily)-treated group of rats. The 18 rats included in each of the three above-mentioned groups were further divided into three sub-groups, 6 rats in each sub-group as follows: rats not exposed to C-H-R (unexposed group); rats exposed to C-H-R up to fall of T_{rec} to 23°C (C-H-R stress group); and rats exposed to C-H-R (T_{rec}23°C) and recovered to T_{rec}37°C (C-H-R recovery group).

The rats of control group exposed to C-H-R were orally administered through a gastric cannula 0.5 mL distilled water as vehicle, 30 min before exposure, while for unexposed rats 30 min before sacrifice. The second group of rats was orally administered a single oral extract dose (100.0 mg kg^{-1} body weight) in 0.5 mL volume by gastric cannula, 30 minute before C-H-R exposure. The multiple five oral dose group of rats were administered by gastric cannula multiple oral doses (100.0 mg kg^{-1}) of seabuckthorn dry-leaf aqueous lyophilized extract in 0.5 mL volume for five days (a single dose daily). The rats treated with multiple doses of the extract were exposed to C-H-R, 30 min after the fifth dose administration on day five. The unexposed rats, both single- and five-dose groups were administered 0.5 mL extract, 30 min before sacrifice.

The C-H-R exposure was performed in an animal decompression chamber maintained at 5°C and 428 mmHg pressure, equivalent to an altitude of 4572 m. The wind flow was 2 L min^{-1}. The rectal probe was inserted 2 cm past the rectum of the rat and retained there with the help of adhesive plaster. The rat was kept in a restrainer. The rectal temperature of the rat was monitored continuously, every minute by Isothermax Temperature Recorder (Columbus Instruments, USA). When the rat attained a rectal temperature of 23°C, it was taken out of the chamber and allowed to recover to normal rectal temperature of 37°C at normal atmospheric pressure and ambient

temperature 32 ± 1°C. However, the rat continued to be in the restrained state. Constant room temperature was maintained because the recovery time to T_{rec}37°C of rats depends on the room temperature. The cooling of the rats to 23°C was taken as the termination point of the C-H-R exposure, as a further fall in the rectal temperature was found to result in high mortality. The time and pattern of T_{rec} to fall to 23°C and its recovery to 37°C were used as a measure of endurance (Ramachandran et al 1990).

The rats of the first sub-group of all the three groups of rats were not exposed to C-H-R, while the rats of second sub-group were exposed to C-H-R up to a fall to T_{rec}23°C. The third sub-group of rats was exposed to C-H-R up to a fall to T_{rec}23°C and recovered to attain T_{rec}37°C at normal atmospheric pressure and ambient temperature 32 ± 1°C. The rats continued to be restrained during the recovery period (Ramachandran et al 1990). After the experiments, rats of all the three groups (control, single-dose treated and five-dose treated) were anaesthetized with anaesthetic ether. Using capillary tubes, blood samples were obtained from the orbital sinus (Riley 1960) in tubes with and without ethylenediamine-tetra-acetic acid (EDTA), as per requirement. Rats were dissected and liver and gastrocnemius muscle tissues were quickly excised, washed in ice-cold saline and extraneous tissue material was removed. A 10% homogenate of the tissue was prepared using a Metrex tissue homogenizer (Metrex Scientific Instruments, Pvt. Ltd, Delhi) and sonicated (given 10 bursts, for 5 s each interval) using a sonicator (IMECO Ultrasonics; Sonitron, Bombay, India). The assays of enzyme activity were performed at 25 ± 1°C. The estimation of biochemical metabolite parameters and enzyme assays were carried out as described below.

Biochemical assays

Assay for lactate dehydrogenase activity

Lactate dehydrogenase (LDH, E.C. 1.1.1.27), as a marker of cell membrane permeability, was estimated in liver and muscle tissue homogenates by the method of Kornberg (1955). The assay mixture consisted of 2.8 mL of pyruvate buffer (sodium pyruvate 10 mM in sodium phosphate buffer, 0.1 M, pH 7.0), 0.1 mL of sample and 0.1 mL of NADH (4.2 mM) in a total volume of 3.0 mL. Enzyme activity was recorded at 340 nm for 3.0 min. The LDH activity was calculated based on oxidation of NADH, using a molar extinction coefficient of 6.22×10^{-3} M^{-1} cm^{-1}. The LDH activity has been expressed as nmol NADH oxidized per minute per mg protein (nmol min^{-1} (mg protein)$^{-1}$).

Estimation of lipid peroxidation

Malondialdehyde (MDA), a marker for lipid peroxidation, was measured in liver and muscle tissue homogenates by the method of Dousset et al (1983). The reaction mixture in a total volume of 1.0 mL contained 0.25 mL of sample and 0.375 mL of working thiobarbituric acid (TBA) solution. The reaction mixture was boiled for 15 min in a water bath, cooled in ice and then 0.75 mL of n-butanol was added. The tubes were centrifuged at 4000 rev min^{-1} for 15 min. The supernatant was taken and the optical density (OD) was read at 531 nm to measure the amount of MDA formed in each of the samples. A standard curve using 1,1,3,3-tetraethoxy propane was prepared and MDA concentrations in the experimental samples were extrapolated from the standard curve. The MDA results were expressed as μmol (mg protein)$^{-1}$.

Estimation of reduced glutathione

Reduced glutathione (GSH) was measured in liver and muscle tissue homogenates by the method of Beutler et al (1963). To 0.1 mL of sample was added 0.9 mL distilled water and 1.5 mL of precipitating reagent (3.34 g metaphosphoric acid, 0.4 g EDTA and 60.0 g NaCl). Tubes were shaken and allowed to stand for 5 min at room temperature (25 ± 1°C). The mixture was centrifuged for 15 min at 4000 rev min^{-1} at 4°C. Supernatant (1.0 mL) was taken and to it 4.0 mL of phosphate solution (0.3 M disodium hydrogen phosphate) and 0.5 mL 5-5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (80 mg in 1% sodium citrate) were added. The developed yellow complex was read immediately at 412 nm on a spectrophotometer (Bio-rad spectrophotometer, Smart Spec-3000). A standard curve using GSH was prepared and GSH concentrations in the experimental samples were extrapolated from the standard curve. GSH concentrations were calculated and expressed as μmol of GSH per mg protein (μmol (mg protein)$^{-1}$).

Assay for superoxide dismutase (SOD) activity

The SOD (E.C. 1.15.1.1) activity was estimated in liver and muscle tissue homogenates by the method of Marklund & Marklund (1974). The total SOD assay volume (3.0 mL) consisted of 1.5 mL of 50 mM Tris-cacodylate buffer pH 8.2 (pH adjusted with 0.1 M HCl), 0.3 mL of nitro blue tetrazolium salt (NBT) (1 mM in water), 0.3 mL of Triton-X-100 (0.01%), 0.8 mL of water, 0.1 mL of sample and 0.01 mL of pyrogallol (60 mM in water). A blank was run simultaneously consisting of 0.1 mL water instead of 0.1 mL sample. Enzyme kinetic activity was recorded at 540 nm for 3 min and change in OD per minute (Δ OD) was used to calculate % auto-oxidation inhibition to derive SOD units (U). One U of SOD was defined as 50% inhibition of the auto-oxidation caused by a certain value of enzyme. The results of SOD activity have been expressed as U (mg protein)$^{-1}$.

Assay for catalase (CAT) activity

The CAT (E.C. 1.11.1.6) activity was measured in liver and muscle tissue homogenates by the method of Aebi (1984). The reaction mixture consisted of 2.9 mL buffer substrate (containing 0.1% H_2O_2 in 50 mM sodium potassium phosphate buffer, pH 7.0) and 0.1 mL sample in the final 3.0-mL assay volume. Change in absorbance was recorded for 150 s (every 15 s) at 240 nm. Catalase activity was calculated using an extinction coefficient of 0.041 cm^2 (μmol)$^{-1}$ and expressed as μmol H_2O_2 consumed per minute per mg of protein (μmol min^{-1} (mg protein)$^{-1}$).

Assay for glutathione S-transferase (GST) activity

The GST (EC 2.5.1.18) activity in liver and muscle tissue homogenates was determined by the method of Habig et al (1974). The reaction mixture consisted of 1.0 mL potassium phosphate buffer (0.3 M, pH 6.5), 0.05 mL 1-chloro-2,4-dinitrobenzene (CDNB) (7.5 mM in ethanol), 0.4 mL reduced glutathione (7.5 mM), 0.2 mL of sample and 1.35 mL of water in

a total assay volume of 3.0 mL. The changes in absorbance were recorded at 340 nm. The enzymatic activity was calculated using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1} and expressed as nmol CDNB conjugate formed per minute per mg of protein (nmol min^{-1} (mg protein)$^{-1}$).

Total protein estimation

Total protein in tissue homogenates was estimated by the method of Lowry et al (1951) for calculating the specific activity of the enzymes. To 0.25 mL of water and 2.5 mL of copper reagent (50 mL of 0.1 M NaOH, 1.0 mL of 1% $CuSO_4$. $5H_2O$ and 1.0 mL of 1% sodium potassium tartrate, ratio 50:1:1) was added to 0.25 mL of sample. Tubes were incubated for 15 min at 37°C. Folin-Ciocalteu reagent (FCR, 1 N, 0.25 mL) was added and tubes were again incubated at 37°C for 30 min. A standard graph was prepared using bovine serum albumin stock solution (1 mg mL^{-1}) and concentrations of 20.0–150.0 μg were used to plot the graph. Absorbance was read at 750 nm. The protein concentration in the test samples were extrapolated from the standard graph and expressed as mg mL^{-1}.

Statistical analysis

The results were analysed by one-way analysis of variance with correction for multiple comparisons using Bonferroni multiple range test, using Graph Pad Prism 2.01. The results are presented as mean ± s.e. $P < 0.05$ was considered as statistically significant.

Results

The data on liver MDA, GSH, SOD, CAT and GST in control and single and multiple extract dose administered rats unexposed and exposed to C-H-R (T_{rec}23°C) and after recovery (T_{rec}37°C) are given in Table 1.

The liver MDA values increased significantly in all the three groups of rats (i.e., untreated (267.4%), single dose (74.5%) and five doses (50.0%) extract treated), on attaining T_{rec}23°C during C-H-R exposure (Table 1). The liver GSH values showed no change either in untreated or single-dose extract treated rats on attaining T_{rec}23°C during C-H-R exposure and recovery (T_{rec}37°C), in comparison with unexposed rats of the respective groups. But five-dose oral extract treatment to rats resulted in significant increases in GSH values (Table 1) both on attaining T_{rec}23°C (41.1%) and on recovery (40.1%) of T_{rec}37°C.

The SOD activity on attaining T_{rec}23°C, in single-dose extract treated rats, significantly increased (24.3%) but five-dose extract treatment significantly increased SOD values both on attaining T_{rec}23°C (16.4%) and on recovery (31.7%) of T_{rec}37°C, in comparison with respective unexposed controls (Table 1). The CAT activity in untreated control rats significantly decreased both on attaining T_{rec}23°C (22.1%) during C-H-R exposure and on recovery (9.1%) of rats from C-H-R induced hypothermia. In both single- and five-dose extract treated rats the CAT activity showed no decreases on attaining T_{rec}23°C during C-H-R exposure, but it increased significantly on recovery of T_{rec}37°C in comparison with unexposed rats of the respective group (Table 1). The GST activity decreased significantly in untreated rats on attaining T_{rec}23°C (28.6%) during C-H-R exposure as well as on recovery (15.4%) from hypothermia. A similar pattern of GST activity was observed both in single-dose and five-dose extract treated rats on attaining T_{rec}23°C (21.5%, 18.7%) and recovery (16.2%, 16.4%) from hypothermia, in comparison with the respective group of unexposed rats (Table 1).

The results on muscle MDA, GSH, SOD, CAT and GST in control and single and multiple extract dose administered rats unexposed and exposed to C-H-R (T_{rec}23°C) and after recovery (T_{rec}37°C) are given in Table 2.

The muscle MDA values increased significantly in the control (188.8%) and single-dose (109.4%) treated rats, on

Table 1 Effect of seabuckthorn dry-leaf aqueous lyophilized extract administration on MDA, GSH, SOD, CAT and GST activity in rat liver

Group	MDA (μmol (mg protein)$^{-1}$)	GSH (μmol (mg protein)$^{-1}$)	SOD (U (mg protein)$^{-1}$)	CAT (μmol min^{-1} (mg protein)$^{-1}$)	GST (nmol min^{-1} (mg protein)$^{-1}$)
Untreated					
Unexposed	0.43 ± 0.04	11.17 ± 1.30	54.17 ± 1.25	966.15 ± 7.40	604.92 ± 19.60
C-H-R stress (T_{rec}23°C)	1.58 ± 0.23*	13.48 ± 0.71	57.76 ± 1.68	752.53 ± 5.95*	431.89 ± 6.47*
C-H-R recovery (T_{rec}37°C)	0.33 ± 0.03	9.10 ± 0.63	60.52 ± 2.16	878.16 ± 6.71*	511.43 ± 16.6*
Single-dose treated					
Unexposed	0.47 ± 0.02	12.46 ± 0.89	51.15 ± 2.66	954.76 ± 13.99	617.90 ± 5.73
C-H-R stress (T_{rec}23°C)	0.82 ± 0.08*[a]	16.56 ± 1.66	63.76 ± 2.91*	940.29 ± 14.65[a]	485.35 ± 17.7*[a]
C-H-R recovery (T_{rec}37°C)	0.49 ± 0.02	13.76 ± 1.04[a]	62.05 ± 3.90	1063.16 ± 6.21*[a]	517.64 ± 14.9*
Five-dose treated					
Unexposed	0.46 ± 0.03	11.62 ± 1.15	51.23 ± 2.28	965.06 ± 12.81	619.76 ± 6.99
C-H-R stress (T_{rec}23°C)	0.69 ± 0.12*[a]	16.40 ± 1.60*	59.65 ± 1.82*	955.17 ± 9.09[a]	503.80 ± 14.00*[a]
C-H-R recovery (T_{rec}37°C)	0.41 ± 0.01	16.28 ± 0.78*[a]	67.49 ± 2.01*	1013.31 ± 8.90*[ab]	518.17 ± 4.58*

Values are mean ± s.e. of six rats in each group. *$P < 0.05$, compared with respective group of unexposed rats. [a]$P < 0.05$, compared with respective control exposed (T_{rec}23°C) and recovered (T_{rec}37°C) rats. [b]$P < 0.05$, compared with single-dose treated exposed (T_{rec}23°C) and recovered (T_{rec}37°C) rats.

Table 2 Effect of seabuckthorn dry-leaf aqueous lyophilized extract administration on MDA, GSH, SOD, CAT and GST activity in rat gastrocnemius muscle

Group	MDA (μmol (mg protein)$^{-1}$)	GSH (μmol (mg protein)$^{-1}$)	SOD (U (mg protein)$^{-1}$)	CAT (μmol min^{-1} (mg protein)$^{-1}$)	GST (nmol min^{-1} (mg protein)$^{-1}$)
Untreated					
Unexposed	0.54 ± 0.12	6.68 ± 0.36	23.29 ± 1.17	17.99 ± 2.98	16.06 ± 1.14
C-H-R stress (T_{rec}23°C)	1.56 ± 0.07*[a]	4.78 ± 0.48	26.79 ± 1.91	18.39 ± 2.17	11.51 ± 0.45*
C-H-R recovery (T_{rec}37°C)	0.32 ± 0.05	6.71 ± 1.01	26.49 ± 1.28	17.20 ± 1.98	13.92 ± 2.40
Single-dose treated					
Unexposed	0.53 ± 0.11	6.88 ± 0.63	25.38 ± 1.15	16.81 ± 0.44	16.69 ± 1.08
C-H-R stress (T_{rec}23°C)	1.11 ± 0.07*[a]	7.52 ± 0.60	28.74 ± 2.79	20.77 ± 2.78	12.07 ± 0.90*
C-H-R recovery (T_{rec}37°C)	0.51 ± 0.08	8.36 ± 2.15	42.91 ± 2.65*[a]	23.96 ± 3.59	14.44 ± 0.49
Five-dose treated					
Unexposed	0.54 ± 0.09	6.58 ± 0.55	25.87 ± 0.618	15.08 ± 0.93	15.95 ± 1.44
C-H-R stress (T_{rec}23°C)	0.39 ± 0.06[b]	7.38 ± 0.69	43.15 ± 7.58*	30.25 ± 0.84*[a]	12.84 ± 0.97
C-H-R recovery (T_{rec}37°C)	0.51 ± 0.02	8.56 ± 1.01	46.48 ± 3.83*[a]	28.75 ± 1.29*[a]	18.41 ± 1.89

Values are mean ± s.e. of six rats in each group. *$P < 0.05$, compared with respective group of unexposed rats. [a]$P < 0.05$, compared with respective control exposed (T_{rec}23°C) and recovered (T_{rec}37°C) rats. [b]$P < 0.05$, compared with single-dose treated exposed (T_{rec}23°C) and recovered (T_{rec}37°C) rats.

attaining T_{rec}23°C, in comparison with the respective group of unexposed rats (Table 2). The GSH values showed no changes in any of the three groups of rats (i.e. untreated and single-dose and five-dose treated), both on attaining T_{rec}23°C during C-H-R exposure and recovery of T_{rec}37°C from hypothermia (Table 2).

There was no change in SOD values of untreated rats both on attaining T_{rec}23°C and on recovery of T_{rec}37°C, in comparison with unexposed rats. The single-dose extract treatment rats had significantly increased (69.1%) SOD values on recovery of T_{rec}37°C but oral treatment of rats with five doses of extract resulted in significantly increased SOD values on attaining T_{rec}23°C (66.7%) during C-H-R exposure, as well as on recovery (79.7%) of T_{rec}37°C, in comparison with unexposed rats (Table 2).

The CAT activity in five-dose extract treated rats significantly increased both on attaining T_{rec}23°C (100.6%) during C-H-R exposure and on recovery (90.6%) of T_{rec}37°C from hypothermia (Table 2), in comparison with the respective group of unexposed rats. The GST activity decreased significantly in untreated (13.3%) and single-dose extract treated (27.7%) rats on attaining T_{rec}23°C during C-H-R exposure. The decreasing pattern of liver and muscle GST levels both in single-dose and five-dose extract treated rats was similar to that observed in untreated rats (Table 2).

Discussion

Exposure of organisms to hypoxia and cold stress, a situation which exists at high altitude, requires a higher rate of metabolism to cope with increased energy demands. The stressors, such as hypoxia (Chattopadhyay 1974), cold (Balkrishna & Bhatia 1968) and immobilization (Hisao et al 1993), produce neuro-humoral and metabolic changes, including oxidative damage (Simmons et al 1990; Yoshikawa et al 1982), which result in mal-adaptation and decreased performance in such stressful situations.

Effect on lipid peroxidation and antioxidants

Increased energy demand during stressful adverse environmental situations or physical exercise, especially of the aerobic type, necessitates a multifold increase in oxygen supply to active tissues to maximize energy yield. The increased metabolic rate results in generation of reactive oxygen species (ROS) free radicals in excessive amounts, causing oxidative stress due to imbalance between ROS production and antioxidant defence. We have studied MDA, a lipid peroxidation product, as a marker of oxidative damage and some of the important antioxidants, viz. GSH, SOD, CAT and GST, to evaluate the antioxidative effect, if any, of seabuckthorn dry-leaf aqueous lyophilized extract in rats exposed to C-H-R up to fall to T_{rec}23°C and post-stress recovery to T_{rec}37°C.

In this study in untreated and exposed control rats to C-H-R, there was a significant increase of MDA levels in liver and muscle along with decreased levels of catalase and GST in liver. The findings are in agreement with the previous observations of increased levels of lipid peroxides in liver and muscle and decreased liver GST levels of hypoxia-exposed rats (Singh et al 2001).

It was observed that the increase in liver MDA levels in untreated (267.4%) rats on attaining T_{rec}23°C was restricted by single-(74.5%) and five (50.0%)-dose treatment. Single-dose extract treatment normalized liver GSH and CAT activity and increased SOD (24.3%) activity on attaining T_{rec}23°C. Five doses of extract treatment further improved the status of liver antioxidants, viz. increased GSH values both on attaining T_{rec}23°C and recovery of T_{rec}37°C, increased SOD during exposure and recovery, normalized catalase activity during C-H-R exposure and an increase on recovery of T_{rec}37°C (Table 1). In the skeletal muscle also

(Table 2), single-dose treatment of extract restricted the rise of MDA levels (109.4%) as compared with untreated rats (188.8%) and increased (69.1%) SOD values on recovery of $T_{rec}37°C$ (Table 2). Five-dose treatment (Table 2) resulted in significantly increased muscle SOD values on attaining $T_{rec}23°C$ (66.7%) during C-H-R exposure, as well as on recovery (79.7%) of $T_{rec}37°C$; muscle CAT activity also significantly increased both on attaining $T_{rec}23°C$ (100.6%) and on recovery (90.6%). Increase in antioxidants, viz. SOD, is indicative of an acclimatization process in the adverse situation of oxidative stress. The activity of SOD, which decreased in liver during emotional stress, increased 65% during adaptation to moderate intermittent hypoxia (Tverdokhlib et al 1988). The observed findings are also in agreement with the reported literature. It is reported that even during short-term acclimatization, to increase flux capacity, the activity of oxidative enzymes must be increased (Hochachka et al 1982). The enzyme CAT, which catalyses the neutralization of hydrogen peroxide, was observed to increase as a compensatory intensification in human subjects during high altitude exposure (Wozniak et al 2001).

The plants, which grow in adverse climatic conditions, acquire molecules that help them to sustain life in that situation (Divekar et al 1996). A root extract of high-altitude-growing plant *Rhodiola imbricate* has been shown to possess potent antioxidative properties (Gupta et al 2007). Seabuckthorn is also a high-altitude-growing plant and perhaps it also acquired various biomolecules (Pintea et al 2001; Kalio et al 2002) possessing bioactivity, viz. antioxidant action. Seabuckthorn leaf extracts and flavonoids have been shown previously to possess potent wound-healing properties due to antioxidative activity (Gupta et al 2005, 2006). In this study also Seabuckthorn leaf extract was found to possess antioxidative properties during C-H-R stress and post-stress recovery from C-H-R-induced hypothermia and the findings are in agreement with the earlier studies. The seabuckthorn leaf extract that was used in this study was earlier found to possess potent adaptogenic activity (Saggu et al 2007). Treatment with adaptogenic substances has been shown to decrease lipid peroxidation and increase SOD and CAT activity (Bhattacharya et al 1997; Panda & Kar 1997). The findings of this study indicate that the administration of seabuckthorn leaf extract, an adaptogen, has potent antioxidative properties, which further improve on multiple doses.

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