

Effects of various *Eleutherococcus senticosus* cortex on swimming time, natural killer activity and corticosterone level in forced swimming stressed mice

Yoshiyuki Kimura*, Maho Sumiyoshi

Second Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu-cho, Onsen-gun, Ehime 719-0295, Japan

Received 5 March 2004; received in revised form 18 August 2004; accepted 30 August 2004

Abstract

The cortex of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. has been used extensively in Russia, China, Korea and Japan as an adaptogen whose properties are the ability to increase as non-specific body resistance to stress and fatigue. Although it has been reported that *Eleutherococcus senticosus* has anti-fatigue and anti-stress actions, their actions are still unclear on the relationship between immune system, especially natural killer (NK) activity and endocrine system (corticosterone level). We compared the effects of the water extracts (A, B, C, D and E) of five *Eleutherococcus senticosus* cortex on the swimming time, NK activity and blood corticosterone level using forced swimming stressed mice. Among five kinds, C, D and E extracts significantly prolonged the swimming time. C and D extracts inhibited the reduction of NK activity and the corticosterone elevation induced by forced swimming. The contents of eleutheroside E, isoflaxidin and eleutherosides B plus E were in the order C > D > E > B > A and C > E > D > A > B extracts, respectively. Therefore, it is suggested that eleutheroside E may be contributed to the anti-fatigue action, the recovery of the reduction of NK activity and the inhibition of corticosterone elevation induced by swimming stress.

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Keywords: *Eleutherococcus senticosus*; Stress; Forced swimming; Corticosterone; Anti-fatiguing effect; Natural killer activity.

1. Introduction

The root bark or stem bark of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Araliaceae) has been called as “Ezo-ukogi” in Japanese and “Siberian ginseng” in Russian. In China, Japan and Russia (Brekhman and Dardmov, 1969; Frasnsworth et al., 1985), the extract of the root bark of *Eleutherococcus senticosus* has been used for the control of blood pressure, for mental and emotional problems, as analeptic or agents to cope with stress. Red Ginseng (the roots of *Panax ginseng* C.A. Meyer) is one of the most famous herbal medicines, and has been used for tonic and anti-stress medicine. The root bark of *Eleutherococcus senticosus* has also been used as substitute drug of ginseng roots. There

is some literature regarding the stress-induced physiological and physical changes (Takasugi et al., 1985; Nishiyama et al., 1985; Takeda, 1990; Fujikawa et al., 1996). Nishibe et al., (1990) reported that the aqueous extract of the stem bark of *Eleutherococcus senticosus* prolonged swimming time of rats in a forced swimming test. Furthermore, it has been reported that the root bark and stem bark of *Eleutherococcus senticosus* have immuno-stimulating action (Wagner et al., 1985; Han et al., 2003) and the inhibitory actions on mast cell-dependent anaphylaxis (Yi et al., 2002). Recently, Yoon et al., (2004) reported that *Eleutherococcus senticosus* extracts have anti-metastatic activity through the activation of macrophages and natural killer (NK) cells. Moreover, there is some report regarding the immune functions (Bohn et al., 1987; Schmolz et al., 2001; Han et al., 2003) and the effects of *Eleutherococcus senticosus* extracts on the endocrine system (Winterhoff et al., 1993; Gaffney et

* Corresponding author. Tel.: +81 89 960 5254; fax: +81 89 960 5256.
E-mail address: yokim@m.ehime-u.ac.jp (Y. Kimura).

al., 2001a, 2001b). Some reviews have been reported regarding the active substances and pharmacological effects of *Eleutherococcus senticosus* (Frasnsworth et al., 1985; Davydov and Krikorian, 2000; Deyama et al., 2001). Fujikawa et al. (2002) reported that the oral administration of the extracts of *Eleutherococcus senticosus* for 2 weeks preferentially acted on the frontal cortex and anterior hypothalamus of rats to enhance the noradrenaline level and its turnover, and further the long-term administration of their extracts stimulated dopamine and its turnover in the striatum and anterior hypothalamus. They suggested that the effects of *Eleutherococcus senticosus* extract on the noradrenaline and dopamine may be useful in the prevention of some stress-related diseases. There are some reports that stress-induced changes in biogenic monoamine levels in the brain are involved in gastric ulcer formation (Glavin et al., 1991), and that exposure to stress increases turnover of noradrenaline, dopamine and 5-hydroxytryptamine in specific brain region (Stanford, 1995). It is well known that glucocorticoids are major mediators of the stress response and modulated many signaling events in the immune response. Glucocorticoids (e.g. corticosterone) modulate antigen presentation, cytokine production, T-cell expansion, and natural killer cell activity (Belsito et al., 1982; Snyder and Unanue, 1982; Chrousos and Gold, 1992; Bonneau et al., 1997; Maes et al., 1998; Steer et al., 1998; Wiegers and Reul, 1998). Thus, stress causes various disorders in relation to bio-regulatory, autonomic nervous, endocrine and immune system. Although the extracts of *Eleutherococcus senticosus* have anti-fatiguing and anti-stress actions, their actions are still unclear on the relationship between immune system especially natural killer (NK) activity and endocrine system (e.g. the secretion of corticosterone). In the present study, we compared the anti-fatiguing effects of the various extracts of the different habitat of *Eleutherococcus senticosus* in stress induced by forced swimming mice. In addition, the NK activity and plasma corticosterone after orally administered *Eleutherococcus senticosus* extract were also determined in stress-induced mice.

2. Material and methods

2.1. Plant material

Five kinds of the root bark of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. were provided from Nippon Funmatsu Co. Ltd. (Osaka, Japan). The five kinds of *Eleutherococcus senticosus* root cortex were designed as A (Lot No. 02121823, Hokkaido, Japan), B (Lot No. 4091209, China), C (Lot No. 020611, Russia), D (Lot No. 021212 AG, Hokkaido, Japan) and E (Lot No. SQ 020221, China). Red ginseng powder was purchased from Korean Ginseng Monopoly Bureau (Seoul, Korea). Each voucher specimen was deposited in the Second Department of Medical Biochemistry, School of Medicine, Ehime University. The five *Eleutherococcus senticosus* root cortex (100 g) and red ginseng powder (100 g)

were extracted with distilled water (1 L) for 3 h under boiling, and concentrated by freeze-drying. The extract yields of A, B, C, D, E and red ginseng were about 11, 10, 12, 11, 12 and 20%, respectively. The determination of eleutherosides B and E and isoflaxidin (Fig. 1) of five *Eleutherococcus senticosus* extracts were performed using reverse-phase high performance liquid chromatography (HPLC) [YMC-Pack, ODS, acetonitrile–H₂O (1:5); flow rate: 1 mL/min, detection: UV 210 nm]. The contents of the above substances were calculated from a standard curve of each authentic sample. The results of quantitative determination of eleutherosides B and E and isoflaxidin of five *Eleutherococcus senticosus* root cortex were shown in Table 1.

2.2. Material

RPMI 1640 medium was obtained from Nissui Pharmaceutical Co. (Tokyo, Japan). Fetal bovine serum (FBS) and antibiotic and antimycotic solution ($\times 100$) were purchased from Gibco BRL (Auckland, New Zealand) and Sigma Chemical Co. (St. Louis, MO), respectively. 3'-*o*-Acetyl-2',7'-bis(carboxyethyl)-4- or 5-carboxylfluorescein acetoxymethylester (BCECF-AM) was purchased from Dojin Co. (Kumamoto, Japan). The 6- and 96-well plates were purchased from Corning Glass Works (NY) and Nalge Nunc International (Denmark), respectively. Other chemicals were of reagent grade.

2.3. Cells

YAC-1 cells (natural killer cell-sensitive target cells) were obtained from Riken Gene Bank (Tsukuba, Japan) and maintained in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 units/mL), streptomycin (100 μ g/mL) and amphotericin B (0.25 μ g/mL).

2.4. Animal

Male C57 BL/6J strain mice (5 weeks old) were obtained from Clea Japan (Osaka, Japan). These mice housed for 1 week in a room maintained at $(25 \pm 1)^\circ\text{C}$ with 60% relative humidity and provided with free access to laboratory standard diet and water. The room lights were on for 12 h/day starting at 7:00 h. Mice were treated according to the ethical guidelines of the Animal Center, School of Medicine, Ehime University. The experimental protocol was approved by the Animal Studies Committee of Ehime University.

2.5. Measurement of swimming time in mice

The water extract (1 g/kg body weight) of five *Eleutherococcus senticosus* root cortex was administered orally daily (7:00 h) for consecutive 9 days. The swimming stress was given to mice for four trials as follows; The four swimming test was carried out day 3, 5, 7 and 9 after the oral administration of the water extracts (1 g/kg) of five *Eleutherococcus*

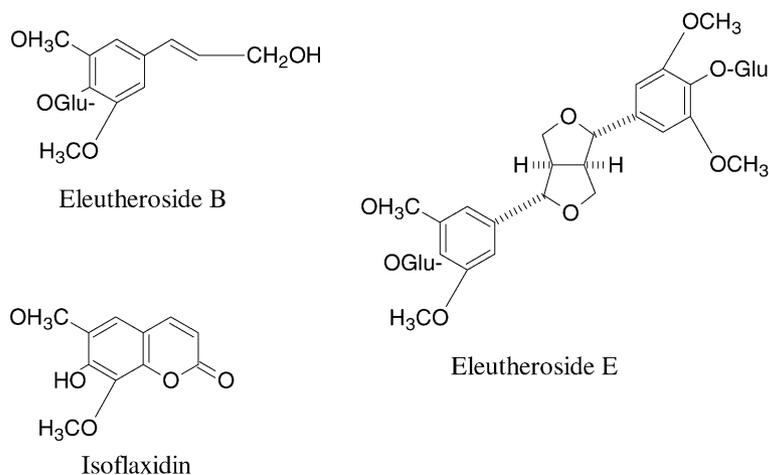


Fig. 1. The structure of eleutherosides B and E and isoflaxidin.

senticosus and red ginseng, respectively. The swimming time of untreated and the drug-treated mice were measured.

2.6. Measurement of corticosterone in chronic swimming stressed mice

On day 10 after the first administration of *Eleutherococcus senticosus* cortex, blood was obtained by venipuncture from swimming stressed mice under pentobarbital anesthesia, and then the spleen was removed. Blood samples were chilled in test tubes containing heparin and centrifuged to give plasma. The plasma corticosterone was determined by radioimmunoassay (RIA) using rat corticosterone [^{125}I] Biotrack Assay System (Amersham Biosciences, Buckinghamshire, UK).

2.7. Isolation of splenic lymphocytes in chronic swimming stressed mice

Splenic lymphocytes were isolated using methods described previously (Kimura and Okuda, 2001). Briefly, the spleen was gently teased to release through dissection in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 units/mL), streptomycin (100 $\mu\text{g}/\text{mL}$) and amphotericin B (0.25 $\mu\text{g}/\text{mL}$). The cell suspension (5 mL)

was layered onto 5 mL of Lympholytes-Mouse (Dainippon Pharm. Co., Osaka, Japan) and centrifuged at $1500 \times g$ for 30 min. The lymphocyte band at the interface was recovered, and the cells were rinsed three times with the above medium.

2.8. Preparation of BCECF-labeled YAC-1 (natural killer cell-sensitive target cells)

Loading of BCECF into the YAC-1 cells was carried out using a modification of the method described previously (Kimura and Okuda, 2000, 2001; Kimura, 2002). Briefly, 3 μM BCECF-AM was added to the YAC-1 cell suspension (1×10^6 cells/mL) in RPMI 1640 medium supplemented with 10% FBS; the cells were incubated for 30 min at 37°C with gentle agitation in a water bath. After the incubation period; the cells were then washed twice with RPMI 1640 medium supplemented with 10% FBS, penicillin, streptomycin and amphotericin B.

2.9. Cytotoxic activity of splenic lymphocyte against YAC-1 cell

Isolated splenic lymphocytes (effector cells) were placed in RPMI 1640 medium containing 10% FBS, penicillin, streptomycin and amphotericin B, at 4×10^5 cells in 96-well culture plates, and then BCECF-labeled YAC-1 cells (target cells; 4×10^3 cells) were added to the effector cells and incubated with them for 2 h; then these cell mixtures were centrifuged at $410 \times g$ for 10 min. The fluorescence intensity of the supernatant was measured by fluorimetry (FP-777, JASCO, Tokyo, Japan) with excitation at 500 nm and emission at 540 nm. The total fluorescence intensity of the target cells (BCECF-labeled YAC-1 cells) was determined after solubilizing the cells by adding 0.25% Triton X-100. The specific

Table 1
Eleutherosides B and E and isoflaxidin of various *Eleutherococcus senticosus* root cortex

Various <i>Eleutherococcus senticosus</i> cortex	Eleutheroside B (%)	Eleutheroside E (%)	Isoflaxidin (%)
A (Japan)	1.081	0.048	0.001
B (China)	0.03	0.26	0.007
C (Russia)	0.61	3.32	0.090
D (Japan)	0.86	0.51	0.080
E (China)	1.54	0.32	0.044

cytotoxic activity was calculated as follows: percent specific cytotoxicity = (fluorescence intensity of target cell treated with splenic lymphocytes isolated from experimental group – fluorescence intensity of spontaneous release of target cells) / (total fluorescence intensity of target cells – fluorescence intensity of spontaneous release of target cells) × 100.

2.10. Statistical analysis

All values are expressed as means ± S.E. Data were analyzed by one-way ANOVA, and then differences among means were analyzed using Fisher's protected least-significant differences (LSD) multi-comparison test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Anti-fatigue actions of various *Eleutherococcus senticosus* root cortex extracts

The extracts (1 g/kg) of A, C, D, E and red ginseng significantly prolonged the swimming time compared to untreated mice (control groups) in the first (day 3) and second (day 5) swimming tests, but extract B had no effect (Fig. 2a and b). The swimming time of C extract was significantly longer than that of red ginseng extracts in the second swimming test (Fig. 2b). In the third (day 7) and fourth (day 9) swimming test, C, D and E extracts significantly prolonged the swimming time, but A and B extract had no effect (Fig. 2c). The swimming time of C- and D-treated mice was longer than that of red ginseng-treated mice in the fourth trials (Fig. 2d).

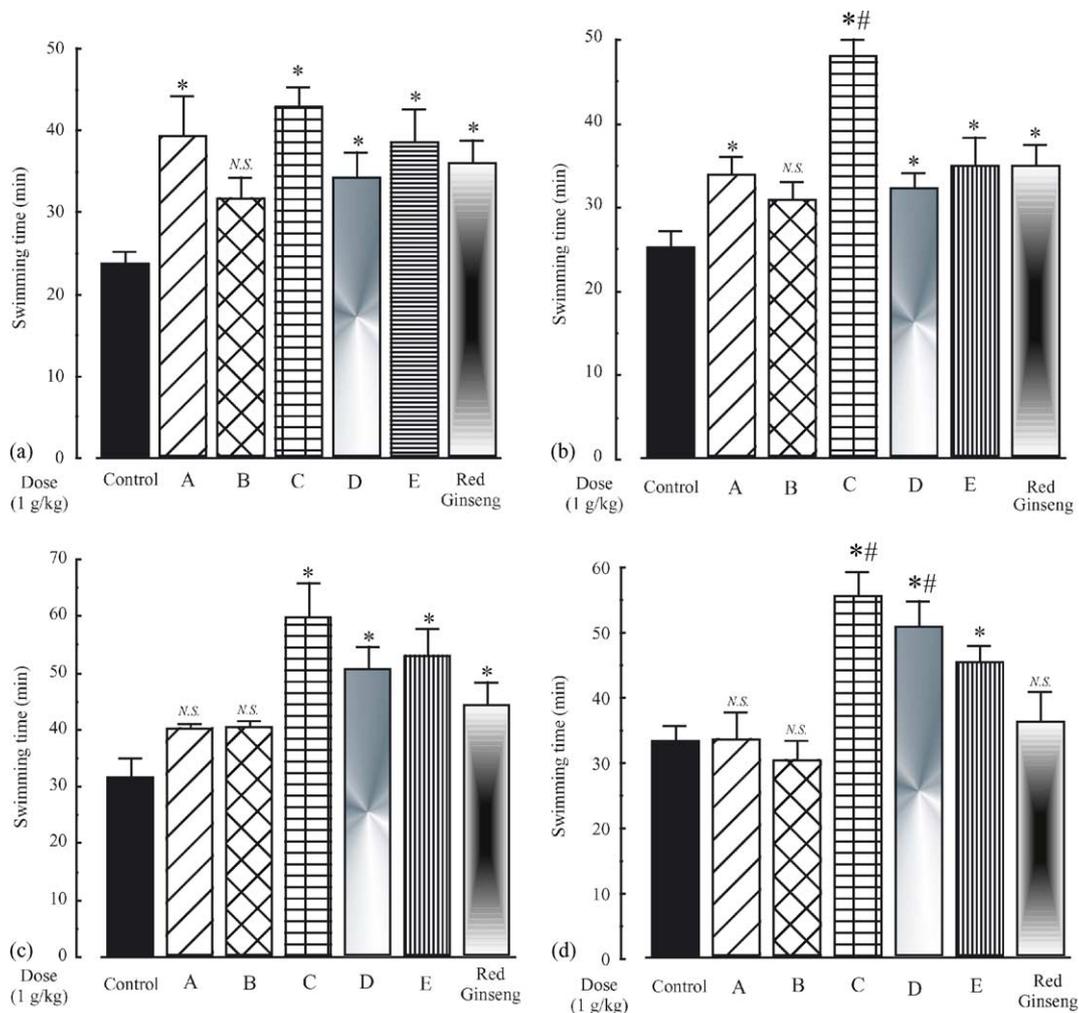


Fig. 2. Effects of five *Eleutherococcus senticosus* cortex and red ginseng on the swimming time in forced swimming stressed mice. (a) The first trial swimming test at day 3 after the oral administration of five *Eleutherococcus senticosus* and red ginseng. (b) The second trial swimming test at day 5 after the oral administration of five *Acanthopanax senticosus* and red ginseng. (c) The third trial swimming test at day 7 after the oral administration of five *Eleutherococcus senticosus* and red ginseng. (d) The fourth trial swimming test at day 9 after the oral administration of five *Eleutherococcus senticosus* and red ginseng. Values are means ± S.E. of 7–14 mice. Normal groups consisted of 10 mice; control, D extract (1 g/kg) of *Eleutherococcus senticosus* and red ginseng-treated (1 g/kg) groups consisted of 14 mice each; A, B, C and E extracts (1 g/kg) of *Eleutherococcus senticosus* cortex-treated groups consisted of seven mice each. (*) Significantly different from control groups; (#) significantly different from red ginseng-treated mice; (N.S.) not significant.

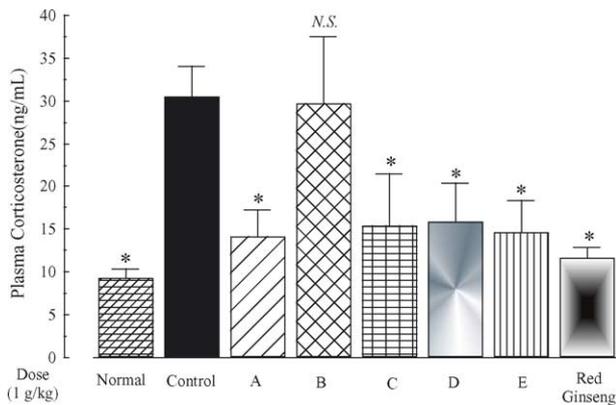


Fig. 3. Effects of five *Eleutherococcus senticosus* cortex and red ginseng on the plasma corticosterone level in forced swimming stressed mice. Values are means \pm S.E. of 7–14 mice. Normal groups consisted of 10 mice; control, D extract (1 g/kg) of *Eleutherococcus senticosus* and red ginseng-treated (1 g/kg) groups consisted of 14 mice each; A, B, C and E extracts (1 g/kg) of *Eleutherococcus senticosus* cortex-treated groups consisted of seven mice each. (*) Significantly different from control groups; (N.S.) not significant.

There is no significant difference between E-treated and red ginseng-treated mice. Among five extracts, C extract prolonged the swimming time, most strongly.

3.2. Effects of various *Eleutherococcus senticosus* root cortex extract on blood corticosterone in swimming stressed mice

Blood corticosterone levels were increased by the swimming stress compared to normal mice. The increase of blood corticosterone levels were inhibited by the orally adminis-

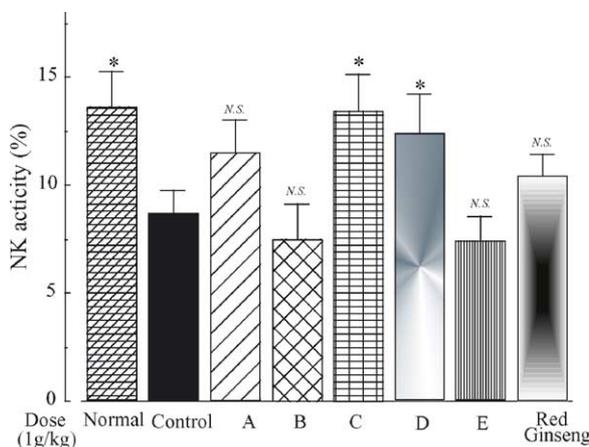


Fig. 4. Effects of five *Eleutherococcus senticosus* cortex and red ginseng on the natural killer (NK) activity in forced swimming stressed mice. Values are means \pm S.E. of 7–14 mice. Normal groups consisted of 10 mice; control, D extract (1 g/kg) of *Eleutherococcus senticosus* and red ginseng-treated (1 g/kg) groups consisted of 14 mice each; A, B, C and E extracts (1 g/kg) of *Eleutherococcus senticosus* cortex-treated groups consisted of seven mice each. (*) Significantly different from control groups; (N.S.) not significant.

tered A, C, D, E and red ginseng for 9 days at a dose of 1 g/kg (Fig. 3).

3.3. Effects of various *Eleutherococcus senticosus* cortex extract on natural killer (NK) activity in swimming stressed mice

NK activity was reduced by the tolerance of swimming stress compared to normal mice. The reduction of NK activity in forced swimming stressed mice was inhibited by the orally administered C and D extracts, but A, B, E and red ginseng extracts did not affect (Fig. 4).

4. Discussion and conclusions

Eleutherococcus senticosus cortex has been used extensively in Russia as an adaptogen whose properties are the ability to increase as non-specific body resistance to stress and fatigue (Brekhman and Dardmov, 1969). Recently, it is suggested that the enhancing effects of *Eleutherococcus senticosus* on the noradrenaline and dopamine levels may be useful in the prevention of gastric ulcer induced by stress (Fukikawa et al., 2002). Glucocorticoids are major mediators of the stress response and directly suppress the natural killer (NK) activity (Shakhar and Blumenfeld, 2003). Thus, the response for body the stress is complex, involving metabolic, inflammatory, neuroendocrine, and immunological aspects. In this study, we examined the effects of five kinds of *Eleutherococcus senticosus* root cortex on the swimming time, NK activity and plasma corticosterone levels in forced swimming stressed mice. In the first trial (day 3) of swimming test, the strength of the prolonging time was in the order C>A>E>D extracts. In the second trial (day 5), the strength of the prolonging time in forced swimming mice was in the order of C>A=E>D extracts. In the third trial (day 7), the strength of the prolonging time was in the order C>E>D extracts, but A and B extracts did not affect compared to the control groups. In the final trial (day 9), the strength of the prolonging time was in the order C>D>E extracts, but A and B extracts did not affect compared to the control groups. Thus, among five kinds of *Eleutherococcus senticosus* root cortex, C extract prolonged the swimming time, most strongly, in the forced swimming mice. On the other hand, NK activity was reduced by the forced swimming stress for 9 days. The reduction of NK activity was recovered to the NK activity of normal mice by the oral administration of C and D extracts, but A, B and E extracts did not affect the NK activity compared to the control groups. Conversely, blood corticosterone level was increased by the forced swimming stress. The increase of blood corticosterone induced by swimming stress was inhibited by the oral administration of A, C, D and E extracts. Therefore, it seems likely that the inhibitory effects of C and D extracts on the elevations of blood corticosterone levels induced by forced swimming stress may be

associated with the recovery of the reduction of NK activity. The contents of eleutheroside E or isoflaxidin were in the order C>D>E>B>A. On the other hand, the contents of eleutheroside B were in the order E>A>D>C>B. The contents of eleutheroside B of A, D and E extracts were larger than those of eleutheroside E. The contents of eleutheroside B plus eleutheroside E were in the order C>E>D>A>B extracts. Nishibe et al. (1990) reported that the water extract of the stem bark of *Eleutherococcus senticosus* and (+)-syringaresinol-di-*o*- β -D-glucoside (eleutheroside E) showed the prolonging effect on the exercise time to exhaustion in chronic swimming stressed rats. Furthermore, Fujikawa et al. (1996) reported that the extracts of *Eleutherococcus senticosus*, and their components such as chlorogenic acid and eleutheroside E prevented the occurrence of gastric ulcer in rats exposed to restraint stress in water. Therefore, these findings suggest that the content of eleutheroside E of *Eleutherococcus senticosus* root cortex may be partly contributed to the anti-fatigue action, the recovery of the reduction of NK activity and the inhibition of corticosterone elevation induced by swimming stress. Extracts A and E, which are a higher content of eleutheroside B, had no effect on the reduction of NK activity induced by swimming stress. On the other hand, extracts A and E inhibited the blood corticosterone elevation induced by the swimming stress. Therefore, the between recovery of the reduction of NK activity and the inhibition of corticosterone elevations induced by swimming stress could not sufficiently be explained by the content of eleutheroside B. On the other hand, extract B did not affect the swimming time, NK activity and blood corticosterone level compared to untreated mice. Among five extracts, the contents of eleutherosides B and E, and isoflaxidin of extract B was lower than those of other extracts. This finding also suggests that eleutheroside E of *Eleutherococcus senticosus* may be contributed to the anti-fatigue action, the recovery of the reduction of NK activity and the inhibition of corticosterone elevation induced by swimming stress. The effects of eleutheroside B or isoflaxidin on the reduction of NK activity and corticosterone elevation induced by swimming stress are unknown from the present results. Therefore, studies are further needed to clarify the relationship between NK activity and corticosterone level by eleutherosides B, E and isoflaxidin, and to isolate the active components as well as isoflaxidin, eleutherosides E and B. Since the five extracts of *Eleutherococcus senticosus* were prepared from the root bark and used in this study, stem bark of *Eleutherococcus senticosus* was not used. Therefore, further studies are needed to clarify the compared effects of stem bark and root bark on the swimming time, NK activity and blood corticosterone level in forced swimming stressed mice.

In conclusion, the extracts of *Eleutherococcus senticosus* cortex have the stress-reducing actions and consequently, they may have anti-fatigue, and the releasing actions on the reduction of NK activity and blood corticosterone elevations induced by stress.

Acknowledgements

This work was supported by Research Grant from Nippon Funmatsu Co. Ltd. (Osaka, Japan).

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