Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 1, 120–123
www.aaem.pl

SHORT COMMUNICATION

Alcohol- and water-based extracts obtained from *Rhodiola rosea* affect differently the number and metabolic activity of circulating granulocytes in Balb/c mice

Robert Zdanowski[1], Sławomir Lewicki[1], Ewa Skopińska-Różewska[2], Waldemar Buchwald[3], Przemysław Michał Mrozikiewicz[4,5], Wanda Stankiewicz[6]

[1] *Department of Regenerative Medicine, Military Institute of Hygiene and Epidemiology, Warsaw, Poland*
[2] *Department of Pathology Biostructure Center, Warsaw Medical University, Poland*
[3] *Team of Botany and Agriculture of Medicinal Plants, Department of Botany, Breeding and Agriculture, Institute of Natural Fibres and Medicinal Plants, Plewiska, Poland*
[4] *Laboratory of Experimental Pharmacogenetics, Department of Clinical Pharmacy and Biopharmacy Poznań University of Medical Sciences, Poznań, Poland*
[5] *Department of Quality Control of Medicinal Products and Dietary Supplements, Institute of Natural Fibres and Medicinal Plants, Poznań, Poland*
[6] *Department of Microwawe Safety, Military Institute of Hygiene and Epidemiology, Warsaw, Poland*

Zdanowski R, Lewicki S, Skopińska-Różewska E, Buchwald W, Mrozikiewicz PM, Stankiewicz W. Alcohol- and water-based extracts obtained from *Rhodiola rosea* affect differently the number and metabolic activity of circulating granulocytes in Balb/c mice. Ann Agric Environ Med. 2014; 21(1): 120–123.

Abstract

Introduction and objective. *Rhodiola rosea* (RR) rhizomes with root extracts are traditional natural drugs originating from Asia and now commonly used as adaptogens and antidepressants. The aim of the presented study was to examine the *in vivo* effect of aqueous (RRW) and 50% hydro-alcoholic (RRA) extracts on the number and metabolic activity of blood granulocytes in mice.

Material and methods. Mice were fed for 7 days with RR extract at daily doses of 0.05, 0.1, 0.2 or 0.4 mg. The metabolic activity of blood granulocytes was determined by measuring of their luminol-dependent chemiluminescent activity on a scintillation counter, after zymosan stimulation.

Results. The number of blood granulocytes was diminished and their chemiluminescence was enhanced in all groups of mice fed *R.rosea* hydro-alcoholic extract. Aqueous extract (RRW) was ineffective in all doses applied.

Conclusion. The presented study revealed difference in the number and metabolic activity of granulocytes mice fed RRA or RRW extracts. Immune characteristics of some individual compounds from RRA and RRW extracts, selected by HPLC analysis, should be carried out in subsequent experiments.

Key words

Rhodiola rosea, mice, granulocytes, chemiluminescence

INTRODUCTION

Rhodiola rosea (*Crassulaceae*) is an arctic-alpine plant growing in Asia, Eastern Europe and North America. Extracts prepared from the underground parts of this plant are used as traditional drugs, more often as adaptogens and antidepressants. Today, various dietary supplements containing *Rhodiola rosea* are available on the market, on the sale in drugstores and via the Internet. This situation is alarming because RR extracts contain many compounds possessing strong biological activities, acting, among others, as antioxidants and angiogenesis inhibitors [1, 2, 3] and, in the opinion of the authors, should be available only under prescription and used under medical control.

Information about the immunotropic activity of *Rhodiola rosea* is scarce. We previously reported that extracts of *R. rosea* influence *in vivo* tumour angiogenesis and some parameters of *in vivo* specific (lymphocyte- dependent) cellular immunity [4, 5, 6]. The aim of the presented study was to evaluate the effect of aqueous and 50% hydro-alcoholic extracts of RR rhizomes with roots in the experimental model of *in vivo* non-specific granulocyte-mediated immunity in mice.

Granulocytes phagocytize and kill several microbial pathogens, and an important event in the killing process is the generation of reactive oxygen species during the oxidative burst, leading to the emission of light, which may be read on a scintillation counter as chemiluminescence (CL). This is a widely-accepted method for measuring granulocytes metabolic activity and their oxygen-dependent killing potential [7, 8].

MATERIALS AND METHOD

Cultivation of *Rhodiola rosea L.* Cultivation was established by vegetative propagation, as previously described [6]. Briefly, the seedlings of *Rhodiola rosea* L. originated from many

Address for corespondence: Sławomir Lewicki, Department of Regenerative Medicine, Military Institute of Hygiene and Epidemiology, Bolesławicka 17/9, 03-352 Warsaw, Poland
e-mail: lewickis@gmail.com

Received: 10 January 2013; accepted: 14 March 2013

years' cultivation of the Research Institute of Medicinal Plants (now the Institute of Natural Fibres and Medicinal Plants) in Poznań. The taxonomic status of plants was confirmed on the basis of *Flora of the Soviet Union* (Vol. 9, 1939) and *Flora of China* (Vol. 8, 2001). A voucher specimen is kept in the herbarium of Department of Botany, Breeding and Agriculture in Plewiska near Poznań.

In autumn, the rhizoma with roots of *Rhodiola rosea* were collected for phytochemical and pharmacological analysis. The raw material was washed, cut into thick slices and dried in natural conditions.

Preparation of extracts. Sample extractions were prepared as previously described [6]. Briefly, finely powdered rhizoma with roots were extracted twice with water (RRW), or with 1/1 v/v ethanol/water solution (RRA) at the temperature of 40–45^{0}C. Extracts were lyophilized and stored at -70^{0}C until used.

HPLC analysis was performed as previously described [6], on an Agilent 1100 HPLC system equipped with a photodiode array detector. For all separations, a Lichrospher 100 RP18 column (250.0′4.0 mm, 5 mm, Merck) was used. All separations were performed at a temperature of 25^{0}C. Peaks were assigned by spiking the samples with standard compounds and comparison of the UV-spectra and retention times.

Spectrophotometric analysis of tannins. Performed according to the method of European Pharmacopoeia on UV-Visible Spectrometer Cintra 20 GBC 9 (European Pharmacopoeia, 6th. edn., 2008).

Animals. The study was performed on 8–10-week old female inbred Balb/c mice, 20–22 g body mass, delivered from the Polish Academy of Sciences breeding colony. For all experiments, animals were handled according to the Polish law on the protection of animals and NIH standards. All experiments were accepted by the local Ethical Committee.

Both aqueous and hydro-alcoholic lyophilized *Rhodiola* extracts were dissolved in 10 % ethyl alcohol and administered to groups of 6 mice each *per os,* by Eppendorff pipette, in 40 µl daily doses of 0.05, 0.1, 0.2 or 0.4 mg, for 7 days. These doses corresponded to 25, 50, 100 or 200 mg given to a 70 kg person (applying the coefficient equal 7 for adjusting differences between mouse and human in relation of the surface to body mass). Control mice were fed 40 µl of 10 % ethyl alcohol. On day 8, the mice were bled under anaesthesia from the retro-orbital plexus and sacrificed with Morbital.

Chemiluminescence test (CL). CL was measured according to [9] with some modifications, at room temperature, in a scintillation counter (RackBeta 1218, LKB, Sweden). Briefly: samples of 0.05 ml heparinised blood were diluted 1:4 with PBS (Biomed Lublin, Poland) supplemented with 0.1 %BSA (Sigma-Aldrich, USA) and 0.1% glucose (Polfa, Poland). Next, 0.05 ml of this diluted blood was mixed with 0.2 ml of luminol (Sigma-Aldrich, USA) solution (10^{-5} M) in PBS and placed in a scintillation counter in the 'out of coincidence' mode for background chemiluminescence measurement. The cells were then activated by the addition of 0.02 ml solution of opsonised zymosan (10 mg/ml), and chemiluminescence activity was measured for the next 15 min. Counting of leukocytes and blood smears examination were performed by routine methods and the results were shown as the maximum value of chemiluminescence (cpm) obtained for 10^3 granulocytes.

Statistical analysis. The results were verified statistically by one-way analysis of variance, and the significance of differences between the groups was verified with Tukey's multiple comparisons post-test and unpaired ***t*** test (GraphPad Prism software package).

RESULTS

The results of chemical analysis (HPLC) of the extracts are presented in the Table 1. Using HPLC, the presence was determined of gallic acid, rosavin, rosarin, rosin, salidroside, tyrosol, chlorogenic acids and tannins in both types of extracts. Interestingly, the concentration of gallic acid was higher in the aqueous extract than in the hydro-alcohol extract of *Rhodiola rosea*, whereas the concentration of rosavin, salidroside, tyrosol, chlorogenic acid and tannins was substantially lower in the aqueous extract than in the hydro-alcohol extract.

Table 1. The results of HPLC (gallic acid, rosarin, rosavin, rosin, salidroside, tyrosol, chlorogenic acid) and spectrophotometric analysis (tannins) of *Rhodiola rosea* aqueous (RRW) and hydro-alcoholic (RRA) extracts (% amount)

Extracts	*Gallic acid*	*Rosarin*	*Rosavin*	*Rosin*	*Salidroside*	*Tyrosol*	*Chlorogenic acid*	*Tannins*
R. rosea aqueous (RRW)	1.03	0.23	0.003	0.16	0.46	0.07	0.07	3.27
R. rosea hydro-alcoholic (RRA)	0.30	0.17	0.277	0.14	0.73	0.12	0.11	8.37

The results of the experiments performed with hydroalcoholic extract (RRA) are presented in Figure 1 (chemiluminescence) and Figure 2 (granulocytes number). Granulocytes collected from mice fed with this extract for 7 days presented significantly higher metabolic activity than granulocytes obtained from the blood of mice belonging to the control group. The best stimulation was observed after feeding mice with daily dose of 0.4 mg hydro-alcoholic extract ($p<0.001$). The lowest stimulation occurred in mice fed daily with 0.1mg of the extract ($0.05<p<0.1$; the difference was on the border of statistical significance). At the same time, a lower number of granulocytes was observed in the blood of animals treated with RRA (hydro-alcoholic) extract than in the blood of control mice ($p<0.05$).

The results of the chemiluminescence tests performed with RRW (aqueus) extract are presented in Figure 3. There is a tendency to lower granulocytes activity by RRW feeding, but the difference from the control group was on the border of statistical significance in the group fed the 0.1 mg daily dose only. No effect on granulocytes number was observed (control 294+/-59; 0.05 mg 322+/-44; 0.1mg 294+/-28; 0.2mg 343+/-55; 0.4mg 282+/-57).

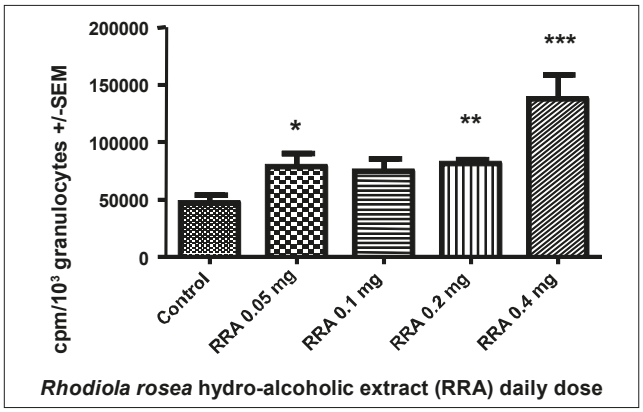

Figure 1. Granulocytes chemiluminescence in mice fed with *Rhodiola rosea* hydro-alcoholic extract for 7 days, measured with luminol in scintillation counter (RackBeta 1218, LKB, Sweden)

*p<0.05; **p<0.01; ***p<0.001

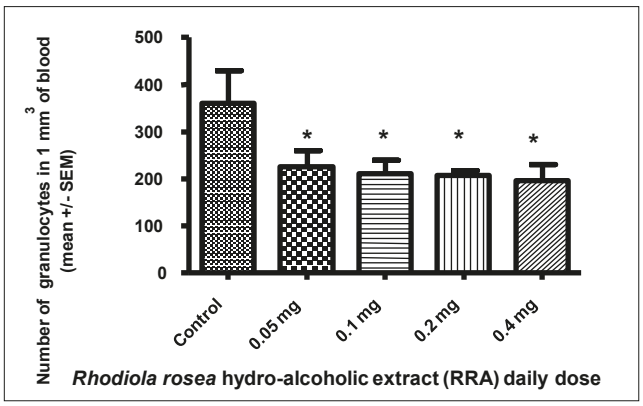

Figure 2. Number of blood granulocytes in mice fed with *Rhodiola rosea* hydro-alcoholic extract for 7 days.

* p<0.05

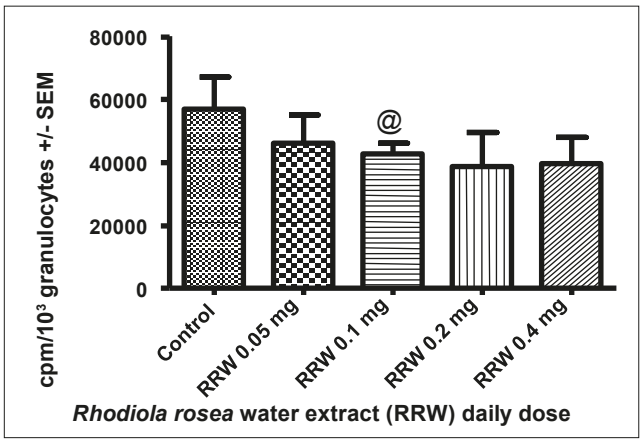

Figure 3. Granulocytes chemiluminescence in mice fed with *Rhodiola rosea* water extract for 7 days, measured with luminol in scintillation counter (RackBeta1218, LKB, Sweden)

@ 0.1>p>0.05

DISCUSSION

Elimination of pathogens (bacteria, viruses or others) is one of the most important functions of the immunological system. The first defence against the spread of pathogens in the body are the white blood cells, especially the granulocytes fraction. There are many factors which are able to modify, by both increasing as well as decreasing, the phagocytic properties of granulocytes. Some of the most popular are natural herbs, and/or their extracts. Their popularity is connected with their beneficial, broad scale of action and, usually, no side- effects. Therefore, researchers are still searching for new herbs or their extracts which may be useful for this purpose. Previously, we have reported some immunomodulatory properties of plants of the *Rhodiola* family [10, 11]. Interestingly, depending on the *Rhodiola* species and immunity model, aqueous and hydro-alcoholic extracts presented various modulatory properties. Significant differences were observed in the content some compounds between RR extracts. HPLC analysis revealed in aqueous extract an increased concentration of gallic acid, and a decreased concentration of rosavin, salidroside, and tannins in comparison to hydro-alcoholic concentration. The chemical differences observed between extracts might be responsible for the differences observed in their effect on the number and activity of blood granulocytes.

In the presented study, the results obtained in mice supplemented for 7 days with *R.rosea* aqueous extract did not differ from the results of the control group. However, hydro-alcoholic extract 7 days supplementation caused significant differences in both number (decrease) and metabolic activity (increase) of granulocytes in comparison to the control values. The questions arise: 1) Which biologically active compounds of *R.rosea* extracts might be responsible for the observed effects; 2) Does RRA-induced lowering of the number of granulocytes reflect diminished granulopoiesis, increased apoptosis, or increased migration from blood vessels to the tissues.

It has been noted that the major biologically-active components of *Rhodiola rosea* are phenylpropenoids (rosavin, rosarin and rosin), salidroside, and salidroside precursor tyrosol [12, 13].

The first compound, the concentration of which differs in RRW and RRA extracts, is phenylpropenoid rosavin. In the hydro-alcoholic extract, which influences granulocytes activity and number, it was found in a concentration about 100 times higher than in the aqueous extract. It is known that rosavin exerts adaptogenic and anti-stress effects [14], but nothing is known about immune properties of rosavin.

The second active compound of *Rhodiola rosea* which distinguishes both extracts, is salidroside. Salidroside is able to attenuate inflammatory responses, both in the number of immunological cells as well as the secretion of inflammatory cytokines in induced disorders in mouse [15]. Studies performed by Zhang et al. revealed that salidroside may positively affect bone marrow (BM) function. This applies to both recovery of haematopoietic BM function as well as modulating the number of peripheral white blood cells in bone marrow depressed mice [16]. Salidroside could promote the recovery of the haematopoietic function of BM depressed anemic mice by increasing the expression and activity of metalloproteinases, and releasing the cytokines from extracellular matrix. Accordingly, Provalova et al have noted the lack of negative influence on granulocytopoesis during paradoxical sleep deprivation in humans supplemented with extracts from *Rhodiola rosea* [17].

Increase of cell death after hydro-alcoholic extract feeding is a rather unlikely explanation, as salidroside protect granulocytes from apoptosis process. [18]. Moreover, gallic acid, the main component of aqueous extract, has been

shown to induce apoptosis in the monocytic cell line and to inhibit lymphocyte proliferation [19]. Taking all these facts together, it may be supposed that the lowering of the number of granulocytes in the presented study was probably not dependent on the deleterious effect of hydro-alcohol extract on granulopoiesis, or on its pro-apoptotic activity.

A third possibility (enhanced by hydro-alcoholic and/or suppressed by aqueous extract migration of granulocytes to tissues and organs) would be the most probable explanation. Previously, we reported increased locomotor activity of splenocytes collected from mice fed with *R.rosea* hydro-alcoholic extract [20].

Components of the stress system, such as norepinephrine (NE) and glucocorticoids, appear to mediate a Th2 shift, while serotonin (5-HT) and melatonin might mediate a Th1 shift. Some anti-depressants would occur affecting these systems, acting on neurotransmitter balance (especially the 5-HT/NE balance) and expression levels of receptor subtypes, which in turn affect cytokine production and relative Th1/Th2 balance [21]. It has been shown that extract of *Rhodiola imbricata* stimulated Toll-like receptor 4, production of inflammatory mediators and Th1 cytokines [22]. This would induce an efflux in activated granulocytes from peripheral blood to the lungs and liver, decreasing their number and granularity in blood, and increasing respiratory burst. This should happen in our experiments with hydro-alcoholic extract, but has to be confirmed in the future.

Lack of this effect in experiments with aqueous extract might be connected with its high contents of gallic acid. Gallic acid and its derivative propyl gallate were shown to antagonize P-selectin mediated platelet-leukocyte interactions, and to suppress induced by TNF-alpha adherence of granulocytes to endothelial cells, avoiding their migration to the tissues [23]. The immune characteristics of some individual compounds from *R.rosea* hydro-alcoholic and aqueous extracts, selected by HPLC analysis, should be carried out in subsequent experiments. Such knowledge will contribute to the safe use of active compounds from herbs belonging to the genus *Rhodiola*.

REFERENCES

1. Furmanowa M, Skopińska-Różewska E, Rogala E, Hartwich M. *Rhodiola rosea* in *vitro* culture- phytochemical analysis and anti-oxidant action. Acta Societat Botan Pol. 1998; 67(1): 69–73.
2. Panossian A, Wikman G, Sarris J. Rosenroot (*Rhodiola rosea*): traditional use, chemical composition, pharmacology and clinical efficacy. Phytomedicine. 2010; 17(7): 481–493.
3. Skopińska-Różewska E, Malinowski M, Wasiutyński A, Sommer E, Furmanowa M, Mazurkiewicz M et al. The influence of *Rhodiola quadrifida* 50% hydro-alcoholic extract and salidroside on tumor-induced angiogenesis in mice. Pol J Vet Sci. 2008; 11(2): 97–104.
4. Skopińska-Różewska E, Hartwich M, Siwicki AK, Wasiutyński A, Sommer E, Mazurkiewicz M et al. The influence of *Rhodiola rosea* extracts and rosavin on cutaneous angiogenesis induced in mice after grafting of syngeneic tumor cells. Centr Eur J Immunol. 2008; 33(3): 102–107.
5. Skopińska-Różewska E, Wasiutyński A, Sommer E, Mielcarek S, Mścisz A, Krajewska-Patan A et.al. The influence of *Rhodiola rosea*, *Rhodiola kirilowii* and *Rhodiola quadrifida* extracts on cutaneous angiogenesis induced in mice after grafting of human kidney cancer tissue. Centr Eur J Immunol. 2008; 33(4): 185–189.
6. Siwicki AK, Skopińska-Różewska E, Hartwich M, Wójcik R, Bakuła T, Furmanowa M et al. The influence of *Rhodiola rosea* extracts on non-specific and specific cellular immunity in pigs, rats and mice. Centr Eur J Immunol. 2007; 32(2): 84–91.
7. Allen RC, Stjenholm RL, Steel RH. Evidence for the generation of an electronic excitation state in human polymorphonuclear leucocytes and its participation in bactericidal activity. Biochem Biophys Res Commun.1972; 47(4): 679–684.
8. Easmon CSF, Cole PJ, Williams AJ, Hastings M. The measurement of opsonic and phagocytic function by luminol-dependent chemiluminescence. Immunology. 1980; 41: 67–74.
9. Tono-Oka T, Ueno N, Matsumoto T, Ohkawa M, Matsumoto S. Chemiluminescence of whole blood. 1. A simple and rapid method for the estimation of phagocytic function of granulocytes and opsonic activity in whole blood. Clin Immunol Immunopathol. 1983; 26(1): 66–75.
10. Skopińska-Różewska E, Wójcik R, Siwicki AK, Sommer E, Wasiutyński A, Furmanowa M et al.The effect of *Rhodiola quadrifida* extracts on cellular immunity in mice and rats. Pol J Vet Sci. 2008; 11(2): 105–111.
11. Skopinska-Różewska E, Sokolnicka I, Siwicki AK, Stankiewicz W, Dąbrowski MP, Buchwald W et al. Dose-dependent in vivo effect of *Rhodiola* and *Echinacea* on the mitogen-induced lymphocyte proliferation in mice. Pol J Vet Sci. 2011; 14(2): 265–272.
12. Kucinskaite A, Pobłocka-Olech L, Krauze-Baranowska M, Sznitowska M, Savickas A, Briedis V. Evaluation of biologically active compounds in roots and rhizomes of *Rhodiola rosea* L. cultivated in Lithuania. Medicina (Kaunas). 2007; 43(6): 487–494.
13. Hohtola A. Bioactive compounds from northern plants. Adv Exp Med Biol. 2010; 698: 99–109.
14. Perfumi M, Mattioli L. Adaptogenic and central nervous system effects of single doses of 3% rosavin and 1% salidroside *Rhodiola rosea* L. extract in mice. Phytother Res. 2007; 21(1): 37–43.
15. Guan S, Xiong Y, Song B, Song Y, Wang D, Chu X et al. Protective effects of salidroside from *Rhodiola rosea* on LPS-induced acute lung injury in mice. Immunopharmacol Immunotoxicol. 2012; 34(4): 667–672.
16. Zhang X, Zhu B, Jin S, Yan S, Chen Z. Effects of salidroside on bone marrow matrix metalloproteinases of bone marrow depressed anemic mice. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. 2006; 23(6): 1314–1319.
17. Provalova NV, Skurikhin EG, Suslov NI, Dygai AM, Gol'dberg ED. Effects of adaptogens on granulocytopoiesis during paradoxical sleep deprivation. Bull Exp Biol Med. 2002; 133(3): 261–264.
18. Sun L, Isaak CK, Zhou Y, Petkau JC, O K, Liu Y, Siow YL. Salidroside and tyrosol from *Rhodiola* protect H9c2 cells from ischemia/reperfusion-induced apoptosis. Life Sci. 2012; 91(5–6): 151–158.
19. Kim NS, Jeong SI, Hwang BS, Lee YE, Kang SH, Lee HC et al. Gallic acid inhibits cell viability and induces apoptosis in human monocytic cell line U937. J Med Food. 2011; 14(3): 240–246.
20. Skopińska-Różewska E, Bychawska M, Białas-Chromiec B, Sommer E. The *in vivo* effect of *Rhodiola rosea* and *Rhodiola quadrifida* hydro-alcoholic extracts on chemokinetic activity of spleen lymphocytes in mice. Centr Eur J Immunol. 2009; 34(1): 42–45.
21. Martino M, Rocchi G, EscelsiorA, Fornaro M. Immunomodulation mechanism of antidepressants: interactions between serotonin-norepinephrine balance and Th1/Th2 balance. Curr Neuropharmacol. 2012; 10(2): 97–123.
22. Mishra KP, Ganju L, Chanda S, Karan D, Sawhney RC. Aqueous extract of *Rhodiola imbricata* rhizome stimulates Toll-like receptor 4, granzyme-B and Th1 cytokines *in vitro.* Immunobiology. 2009; 214(1): 27–31.
23. Jiang YR, Chen KJ, Xu YG, Yang XH, Yin HJ. Effects of propyl gallate on adhesion of polymorphonuclear leukocytes to human endothelial cells induced by tumor necrosis factor alpha. Chin J Integr Med. 2009; 15(1): 47–53.