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Full Length Research Paper

Some medicinal plants counteract alterations of neuroendocrine stress response system, oxidative and nitrosative stress caused by repeated restraint in rats

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Stress affects neuroendocrine stress response system, oxidative and nitrosative stress. Some reports claim that antioxidants and antistressors could attenuate these alterations. This study was realized to investigate antistress and antioxidant effects of *Hypericum perforatum*, *Melissa officinalis*, *Valeriana officinalis* and *Passiflora incarnata* extracts on repeated restraint (RS) in rats. Thirty-six rats were equally divided into six groups: A (control), B (only RS treated), C (*H. perforatum* + RS treated), D (*M. officinalis* + RS treated), E (*V. officinalis* + RS treated), F (*P. incarnata* + RS treated). Cortisol, lipid peroxidation, protein oxidation and nitric oxide (NO) levels escalated dramatically in group B compared to control, whereas almost all of them diminished remarkably again in groups C, D, E and F. Besides slight alterations of reduced glutathione content and glutathione-s-transferase, catalase activities were recorded between groups. Among vitamins only depletion of vitamin E was significant in Group B comparing to control. Interestingly administration of each plant extract led to increments of vitamins A and E even when compared to control. The results revealed that the aforementioned plant extracts has remarkable potentials to counteract repeated RS caused alterations of cortisol as well as oxidative and nitrosative stress biomarkers probably through their antistress, antioxidant and free radical defusing effects.

Key words: Some medicinal plants, neuroendocrine stress response system, oxidative stress, nitrosative stress, restraint stress antioxidant.

INTRODUCTION

Physical and psychological stressors lead to release of glucocorticoids (GCs) from adrenal gland. These substances play an important role, activating energy to crucial tissues in the course of an emergency and repressing unessential anabolism. Cortisol is the most abundant glucocorticoid produced in human.

Although the exact mechanism of stress leading to brain damage has not been clarified, there is strong evidence claiming that, in the brain, high grades of GCs may generate harmful effects, including damage to synaptic plasticity, dendritic morphology and neurogenesis[1]. These events have been attributed to an escalated generation of reactive oxygen species (ROS) which cause oxidative stress (OS) (Mcintosh and Sapolsky, 1996). Brain is a very sensitive organ to OS owing to its high content of polyunsaturated fatty acids in the cell membranes and low antioxidant capacity (Metodiewa and Koska, 2000). Some previous studies have reported a relation between ROS and some nervous system disorders such as Parkinson's disease, Schizophrenia, and Alzheimer's disease (Smythies, 1999). ROS damage proteins, DNA and lipids of cells and thus impair all cellular functions. Besides formation of nitric oxide (NO) and inducible expression of NO synthase were informed to take place in the brain during stress (Olivenza et al., 2000).

Exposure to stress situations has been proposed to impair antioxidant defenses, leading to oxidative damage by changing the balance between oxidant and antioxidant factors (Mcintosh et al., 1988). Preventive role of antioxidants was reported in modifying the major

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Table 1. Extraction processes of used plants.

Plant name	Initiation weight of the plant (g)	Amount of added ethanol (ml)	Amount of added water (ml)	Amount of filtrate (ml)	Amount of filtrate remained after evaporation (ml)	Density of the extract (mg/ml)
Hypericum perforatum	50	250	50	200	50	90
Melissa officinalis	50	250	50	190	45	20
Valeriana officinalis	100	250	50	150	45	91
Passiflora incarnata *	-	-	-	-	-	-

*Since *Passiflora incarnata* obtained as liquid extract from a pharmacy, it was not subjected to foregoing processes.

diseases related to stress (Zafir and Banu, 2007). When ROS increase excessively, antioxidant deficiency occurs. This inadequacy may elevate through insufficient intake of both supplementary and dietary antioxidants (Zondervan et al., 1996). One of the materials being used as antioxidant source to eliminate free radicals emerging in RS exposure are plant extracts (Sharma et al., 2011).

It has been informed that repeated restraint (RS) is an easy and convenient model to induce both psychological (escape behavior) and physical stress (muscle fatigue) resulting in limited mobility as well as aggression (Singh et al., 1993; Ramanova et al., 1994). Among the stress types people encounter in daily life, the most harmful one is repetitive type. Unlike acute stress, the influences of repetitive RS on oxidative and nitrosative stress are less known. Yan et al. (2000) reported that cortisol level reaches the highest value at the end of the second hour. Owing to foregoing reasons we preferred repeated RS model through performing two hours daily during seven days.

Although *Hypericum perforatum* L*, Melissa officinalis* L*, Valeriana officinalis* L and *Passiflora incarnate* L have been informed to possess antioxidant features (Kumar et al., 2010; Hohmann et al., 1999; Sudati et al., 2009; Masteikova et al., 2008) and be valuable in treatment of many stress-mediated cases (Trofimiuk et al., 2005; Wheatly, 2005; Hattesohl et al., 2008; Akhondzadeh et al., 2001), protective effects of these plants against RS were not investigated up to current study. For this purpose levels of cortisol (as index of psychological and physical stress), malondialdehyde (MDA), protein carbonyl (PC) (as biomarkers lipid peroxidation and protein oxidation), nitric oxide (NO) (as a index of nitrosative stress), antioxidant defense systems including reduced glutathione (GSH), glutathione-s-transferase (GST), catalase (CAT), β-carotene, vitamins A, C, E in plasma or brain samples obtained from control and administrated rats were monitored.

MATERIALS AND METHODS

Plant materials and extraction procedure

H. perforatum samples were collected from Osmanbey campus area of Harran University (Sanliurfa, Turkey). *M. officinalis* and *V. officinalis* samples were obtained from a herbal store while the liquid extract of *P. incarnate* was purchased as “Passiflora” commercial name from a pharmacy in Sanliurfa. All plant samples were authenticated by Assist. Prof. Esat ÇETIN, PhD. Aerial parts of *H. perforatum*, leaves of *M. officinalis* and roots of *V. officinalis* were powdered carefully for extraction processes. After measuring the weights each was placed in separate bottles. As demonstrated in Table 1, ethanol and distilled water were added to these bottles in different ratios and kept 24 h at 40°C. After the filtration, evaporation process was realized with rotary evaporator (Heidolph, Laborata 4002). The plant extracts was concentrated under reduce pressure and lyophilized (Tel Star Cryodos) to obtain the dry residue. All extracts were prepared as 100 mg/kg by suspending in 1% gum arabica (GA) and given to rats by gavage. The doses of *H. perforatum*, *M. officinalis*, *V. officinalis* and *P. incarnate* (100 mg/kg body weight (bw)) used in this trial was adjusted according to some earlier studies (Kumar et al., 2010; Birdane et al., 2007; Circosta et al., 2007; Dhawan et al., 2001).

Animals

Seventy-two healthy male Wistar albino rats weighing 250 to 300 g were obtained from Experimental Animals Unite of Fırat University (Elazig, Turkey). They were housed in polypropylene cages and maintained under standard conditions (12/12 light/dark cycle at 22±2°C and 50 to 70% humidity). The rats were acclimatized to laboratory conditions for 20 days before beginning of the trial. Standard pellet feed and tap water were provided *ad libitum*. All animals received humane care in compliance with the European community guidelines on the care and use of laboratory animals.

Repeated restraint stress (RS) procedure

RS stress model was designed according to Torres et al. (2004). RS was realized by placing each animal in a plastic tube (4.5 cm in diameter and 12 cm in length), adjusted according to the size of the rat with plaster tape on the outside, so that the animal was unable to move. RS was performed 2 h daily between 09:00 to 11:00 a.m. during 7 days (Nadeem et al., 2005). Morning hours were preferred for restraint because the lowest plasma cortisol level was informed in these hours (Olivenza et al., 2000).

Experimental design

Rats were randomly assigned to six equal groups (n = 6). All animals except those of control group were exposed to RS for 7 days as follows: 1. Control group: rats were treated with 1 ml of normal saline (0.9% NaCl) during 7 days intraperitionally (i.p.); 2. RS (alone) group: rats were treated with 1 ml of normal saline i.p. and exposed to RS 1 h after saline treatment during 7 days; 3. RS+ *H. perforatum* group: rats were treated with 100 mg/kg bw of *H. perforatum* in 1 ml GA i.p. and exposed to RS one h after *H. perforatum* treatment during 7 days; 4. RS+ *M. officinalis* group: Rats were treated with 100 mg/kg bw of *M. officinalis* in 1 ml GA i.p. and exposed to RS 1 h after *M. officinalis* treatment during 7 days; 5. RS + *V. officinalis* group: Rats were treated with 100 mg/kg bw of *V. officinalis* in 1 ml GA i.p. and exposed to RS 1 h after *V. officinalis* treatment during 7 days; 6. RS+ *P. incarnate* group: rats were treated with 100 mg/kg bw of *P. incarnate* in 1 ml GA i.p. and exposed to RS 1 h after *P. incarnate* treatment during 7 days.

Preparation of brain and plasma samples

At the end of the treatments, rats were prepared for experimental procedure under ether anesthesia and blood samples were taken from the animals into tubes with K_3EDTA by injectors from the heart. The plasma samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at 4°C. Whole brains were carefully removed and put in Petri dishes for washing with physiological saline. Brain tissues were homogenized in ice-cold phosphate buffer solution for 20 s using a homogenizer (Heidolph, Silent Crusher M) at 20000 rpm and then centrifuged at 6000 rpm for 10 min to obtain supertanatants. These supernatants kept at -80°C until analysis. All processes were carried out at 4°C. While protein concentration, some oxidant/antioxidant parameters and NO levels were determined in brain supernatants, the concentration of cortisol, β-carotene and vitamins A, C, E were assessed in plasma.

Biochemical analysis

The concentration of plasma cortisol were determined by an autoanalyser (Immulite, 2000) using the kits. CAT (EC 1.11.1.6) activity was analyzed by the method of Aebi (1974). The degradation speed of H_2O_2 by catalase was measured by reading the absorbance with a spectrophotometer set at 240 nm. GST (EC 2.5.1.18) was assayed spectrophotometrically by following the conjugation of GSH with CDNB at 340 nm as described by Mannervik and Guthenberg (1981). GSH concentration was determined by reacting with O-phthaldialdehyde (OPT, 10 mg/10 ml methanol) according to the modified method of Lee and Chung (1999). Pure reduced GSH was used as standard for calibration. GSH levels were measured by a spectrofluorimetry with excitation at 345 nm and emission at 425 nm. The levels of β-carotene at 425 nm and vitamin A (retinol) at 325 nm were detected after the reaction of plasma: ethanol: hexane at the ratio of 1:1:3 respectively by Suzuki and Katoh (1990). Plasma Vitamin C level was determined after derivatization with 2,4-dinitrophenylhydrazine by Omaye et al. (1979). Vitamin E (α-tocopherol) was analyzed colorimetrically with 2,4,6-tripridyl-s-triazin and $FeCl_3$ after the extraction with absolute ethanol and xylene by Martinek (1964). MDA level was estimated using the modified thiobarbituric acid-reactive substance (TBARS) method by Hegde et al. (2003). The plasma level of NO was determined using commercially available colorimetric assay kit (Roche; cat. no. 1756 281) (Green et al., 1982). Nitrogen monoxide is defined in plasma via nitrite. The plasma nitrate is converted to nitrite via nicotinamide adenine dinucleotide phosphate (NADPH) while nitrate reductase is available. The nitrite reacts with sulfanilamide as well as N-(1-napthyl)-ethylenediamine dihydrochloride and forms red-violet diazo dye. The diazo dye is assessed on the basis of its absorbance at 550 nm. MDA concentration was measured spectrofluorimetrically with excitation at 525 nm and emission at 560 nm. Calculations were performed using a linear regression from tetraethoxypropane for the MDA standard curve. PC level was assessed by Cayman's Protein Carbonyl Assay Kit (Cat. no 10005020) as carbonyl content in the samples. This kit bases on the principle which utilizes the 2,4-dinitrophenylhydrazine reaction to measure the protein carbonyl content in homogenate (Levine et al., 1990). The amount of protein-hydrozone produced is quantified spectrophotometrically at an absorbance 360 nm by 96-well plate reader (Spectra Max M5). The carbonyl content was standardized to protein concentration. The protein content in the samples was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Statistical analysis

The statistical analyses were realized using the Minitab 13 for windows package program. All data were presented as means ± standard deviation (SD) (n = 6). One-way analysis of variance (ANOVA) statistical test was used to determine the differences between means of the experimental groups accepting the significance level at $p< 0.05$.

RESULTS

Influence on serum cortisol level

Results for plasma cortisol status of control and treated groups are demonstrated in Figure 1. While cortisol level elevated markedly in RS (alone) group comparing to that of control, it decreased significantly in all groups in which RS and plant extracts applied together.

Influence on MDA, PC and NO levels

MDA, PC and NO levels of groups are presented in Figures 2 to 4. While MDA, PC and NO concentrations elevated significantly in RS (alone) group comparing to control group, pretreatment with each plant extract gave rise to marked decreases of their levels. In addition when plant extract treated groups compared to control, significant escalations of foregoing parameters were observed with one accord only in RS+VA group.

Influence on antioxidant status

As shown in Table 2, slight alterations of cerebral GSH content and GST, CAT activities were recorded between groups. While CAT and GST activities increased slightly with pretreatment of each plant extract used in this study compared to RS (alone) group, there was a slight increase of GSH level only in *H. perforatum* and *M. officinalis* administrated groups. With regard to plasma β-carotene and antioxidant vitamins (Table 3), although their levels decreased in RS (alone) group comparing to control, only decline of vitamin E was remarkable. Supplementation of each plant extract (except *P. incarnate*) resulted in marked escalations of almost all vitamins and β-carotene. When plant extract administrated groups compared with that of control, marked high levels of vitamin A was detected in *M. officinalis* + RS while increased concentration of vitamin C was determined in *P. incarnate* + RS, however vitamin

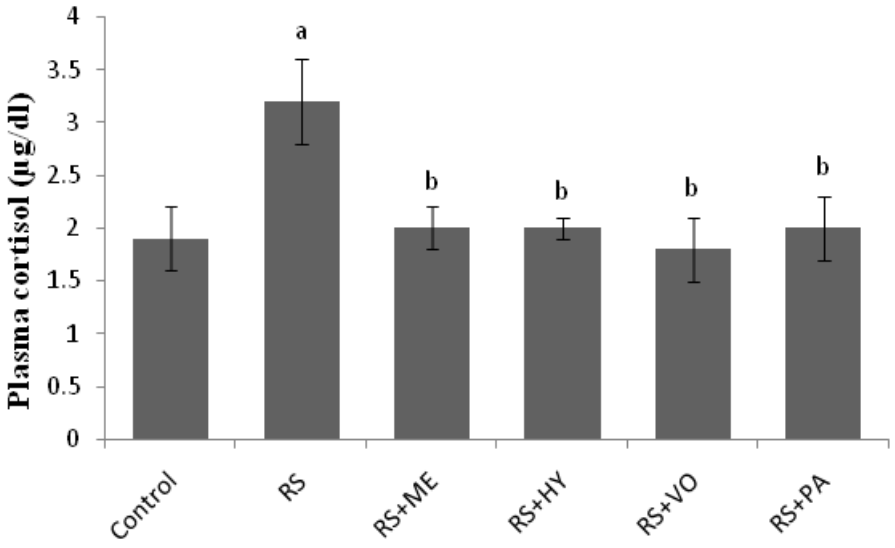

Figure 1. Protective effects *Hypericum perforatum* (HP), *Melissa officinalis* (MO), *Valeriana officinalis* (VO) and *Passiflora incarnata* (PI) extracts on cortisol level of rats exposed to repeated restraint stress (RS). Values are means, with standard deviations represented by vertical bars. Control, group in which only normal saline treated; RS (alone), group in which normal saline and RS treated together; RS + HP, group in which RS and HP treated together; RS + MO, group in which RS and MO treated together; RS + VO, group in which RS and VO treated together; RS + PI, group in which RS and PI treated together. [a] Mean values were significantly different from the control. [b] Mean values were significantly different from the RS (alone) group (P< 0.05, one-way ANOVA).

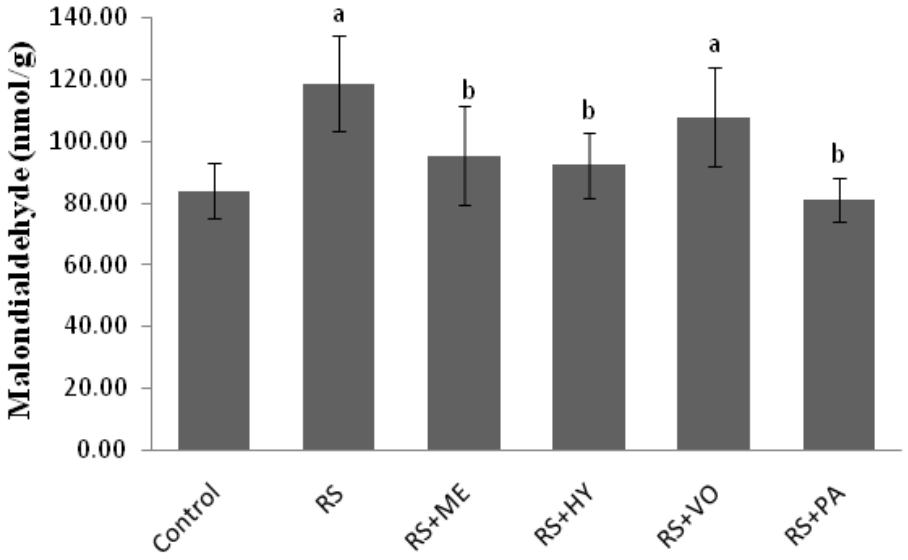

Figure 2. Protective effects *Hypericum perforatum* (HP), *Melissa officinalis* (MO), *Valeriana officinalis* (VO) and *Passiflora incarnata* (PI) extracts on malondialdehyde level of rats exposed to repeated restraint stress (RS). Values are means, with standard deviations represented by vertical bars. Control, group in which only normal saline treated; RS (alone), group in which normal saline and RS treated together; RS + HP, group in which RS and HP treated together; RS + MO, group in which RS and MO treated together; RS + VO, group in which RS and VO treated together; RS + PI, group in which RS and PI treated together. [a] Mean values were significantly different from the control. [b] Mean values were significantly different from the RS (alone) group (P< 0.05, one-way ANOVA).

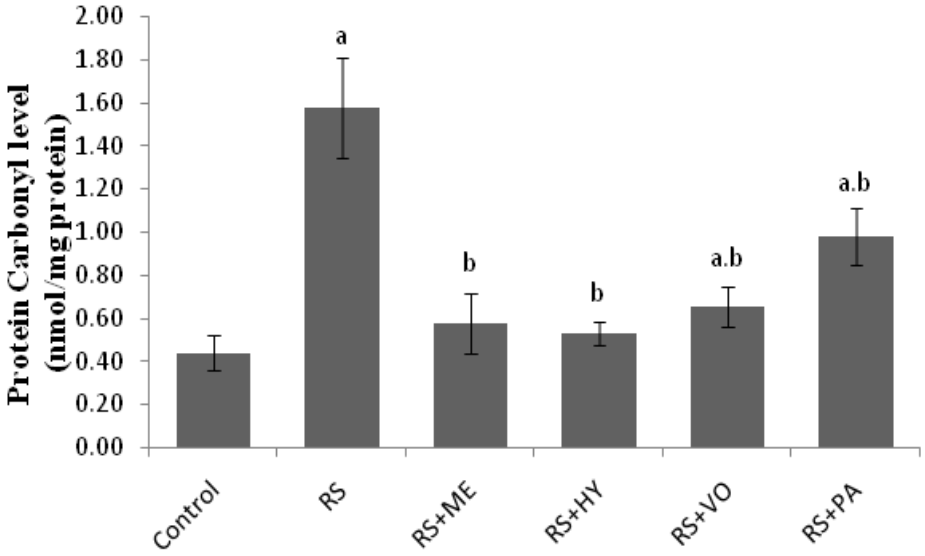

Figure 3. Protective effects *Hypericum perforatum* (HP), *Melissa officinalis* (MO), *Valeriana officinalis* (VO) and *Passiflora incarnata* (PI) extracts on protein carbonyl level of rats exposed to repeated restraint stress (RS). Values are means, with standard deviations represented by vertical bars. Control, group in which only normal saline treated; RS (alone), group in which normal saline and RS treated together; RS + HP, group in which RS and HP treated together; RS + MO, group in which RS and MO treated together; RS + VO, group in which RS and VO treated together; RS + PI, group in which RS and PI treated together. [a] Mean values were significantly different from the control. [b] Mean values were significantly different from the RS (alone) group ($P< 0.05$, one-way ANOVA).

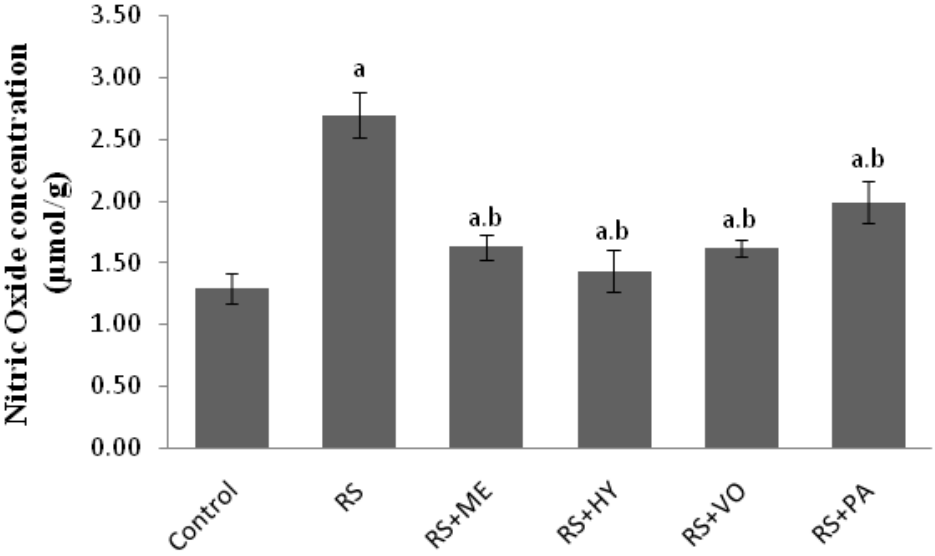

Figure 4. Protective effects *Hypericum perforatum* (HP), *Melissa officinalis* (MO), *Valeriana officinalis* (VO) and *Passiflora incarnata* (PI) extracts on nitric oxide level of rats exposed to repeated restraint stress (RS). Values are means, with standard deviations represented by vertical bars. Control, group in which only normal saline treated; RS (alone), group in which normal saline and RS treated together; RS + HP, group in which RS and HP treated together; RS + MO, group in which RS and MO treated together; RS + VO, group in which RS and VO treated together; RS + PI, group in which RS and PI treated together. [a] Mean values were significantly different from the control. [b] Mean values were significantly different from the RS (alone) group ($P< 0.05$, one-way ANOVA).

Table 2. Protective effects *Hypericum perforatum* (HP), *Melissa officinalis* (MO), *Valeriana officinalis* (VO) and *Passiflora incarnata* (PI) extracts on GSH level and CAT, GST activities of rats exposed to repeated restraint stress (RS).

Groups	GSH (nmol/g)	GST (EU/g)	CAT (EU/g)
Control	229.20±26.79	10.37±1.91	2.22±0.62
RS (alone)	214.83±32.13	8.37±2.78	2.14±0.62
RS + HY	221.77±27.17	10.20±2.39	2.36±0.41
RS + MO	219.87±36.26	10.02±2.58	2.41±0.46
RS + VO	202.68±32.70	10.93±2.59	2.01±0.43
RS + PI	197.70±42.3	8.88±1.48	2.18±0.20

Values are given as the means ± SD. Control, group in which only normal saline treated; RS (alone), group in which normal saline and RS treated together; RS + HP, group in which RS and HP treated together; RS + MO, group in which RS and MO treated together; RS + VO, group in which RS and VO treated together; RS + PI, group in which RS and PI treated together. No statistically significant value was detected between groups ($P > 0.05$, one-way ANOVA).

Table 3. Protective effects *Hypericum perforatum* (HP), *Melissa officinalis* (MO), *Valeriana officinalis* (VO) and *Passiflora incarnata* (PI) extracts on β-carotene and and antioxidant vitamin levels of rats exposed to repeated restraint stress (RS).

Groups	β-carotene (µg/dl)	Vitamin A (µg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Control	96.89±28.46	171.40±32.60	0.59±0.06	1.44±0.19
RS (alone)	77.75±18.21	134.19±30.54	0.48±0.12	1.07±0.26[a]
RS + HY	95.06±18.33	211.26±53.98[b]	0.67±0.09[b]	2.10±0.29[a.b]
RS + MO	106.78±24.9[b]	239.46±58.87[a.b]	0.62±0.08[b]	2.09±0.31[a.b]
RS + VO	110.61±22.05[b]	217.52±63.34[b]	0.55±0.11	1.77±0.25[a.b]
RS + PI	80.54±20.49	185.11±55.00	0.70±0.09[a.b]	1.76±0.20[a.b]

Values are given as the means ± SD. Control, group in which only normal saline treated; RS (alone), group in which normal saline and RS treated together; RS + HP, group in which RS and HP treated together; RS + MO, group in which RS and MO treated together; RS + VO, group in which RS and VO treated together; RS + PI, group in which RS and PI treated together. [a] Mean values were significantly different from the control. [b] Mean values were significantly different from the RS (alone) group ($P< 0.05$, one-way ANOVA).

E concentration elevated significantly in all groups.

DISCUSSION

Today as stress has become one of the main problems of our age, efforts are needed to provide effective protection against its detrimental influences. Some experimental trials have investigated the effect of a number of plant extracts in this regard. The aim of this study was to seek for the first time whether *H. perforatum*, *M. officinalis*, *V. officinalis* and *P. incarnate* extracts could prevent the damage caused by RS, such as decrease the levels of plasma cortisol, cerebral MDA, PC, NO and increase cerebral antioxidant defense systems (GSH, GST, CAT) and plasma β-carotene and antioxidant vitamin levels in rats.

It has been put forward that stress elevates mRNA levels of corticotropin releasing factor (CRF) in the hypothalamus (Marti et al., 1999). Escalated CRF release leads to damages in regulation of corticosterone secretion. Stress also gives rise to an increase in the secreting of adrenocorticotropic hormone (ACTH) and thus, an augmentation in plasma cortisol. Since cortisol is known to be an important stress hormone, its plasma concentration can be index of body's stress intensity. It may be concluded that in current study repeated RS activated the hypothalamic-pituitary-adrenal axis that caused elevation of cortisol in aforementioned way. Pretreatment with each plant extract during repeated RS exposure have prevented stress-mediated hypercortisolemia. These results indicate that plant extracts used in current experiment may have act as antistressor and diminished the neuropathological influence of RS as evidenced from ameliorated cortisol level. Dramatic elevation of cortical in RS (alone) group indicated that animal model of psychological stress was achieved successfully (Figure 1). This result is in agreement with some earlier studies (De Boer et al., 1988; Marzatico et al., 1988).

All plant extracts used in current study performed antioxidative role against RS in rats (Table 2). This was obvious from our observation that almost in all groups where RS and plant extracts administrated together, the concentration of cerebral MDA and PC decreased significantly compared with RS (alone) group. High MDA and PC contents of RS (alone) group may have been occurred from elevation of ROS as a result of RS exposure (Liu et al., 1996; Sahin et al., 2007). It has been suggested that the generation of free radicals in the brain is owing to catecholamine metabolism, for example, dopamine and norepinephrine, and increased catecholamine levels might undergo autooxidation, where electrons produced can in turn generate ROS (Carpagnano et al., 2003).

In our study escalations in concentration of plasma cortisol and cerebral nitric oxide (NO) in restrained rats might also be showed among reasons for the stress-mediated enhancement of free radicals as stated previously (Mccann, 1997; Sahin and Gumuslu, 2007). Furthermore, Bao et al. (2008) reported increment of xanthine oxidase (XOD) activity in restrained rats and suggested that another reason for escalations of ROS which led to oxidation of unsaturated fatty acids and protein structures of cells might be resulted from increased XOD activity, because XOD has a key role in ROS production through catalyzing the oxidation of hypoxanthine to xanthine and generates free radicals, leading to cellular integrity damage and functional diabolisms. Pretreatment with *H. perforatum, M. officinalis, V. officinalis* and *P. incarnate* extracts might have blocked the inducing effect of RS on lipid and protein oxidation through their antistress and free radical quenching features. Although there are some studies suggesting similar reducing effects of these plants on lipid peroxidation, except current trial did not coincide with any study investigating their *in vivo* influences on protein oxidation.

The body needs enzymatic (SOD, CAT, GST and GSH-Px) and non-enzymatic (GSH and some vitamins) antioxidants in order to scavenge ROS. Glutathione has an important role in the detoxification of ROS in brain. In many studies, it is informed that stress decreases GSH. In the current study, GSH content and activities of GST and CAT were found to be decreased slightly in response to repeated RS.

There are several conflicting reports concerning the cerebral CAT activity of restrained rats. A marked increase of this enzyme activity in brain has been stated after RS (3 h daily for 15 days) (Sahin and Gumuslu, 2007). Conversely, no alteration in CAT activity in hippocampus after repeated RS (1 h/day for 40 days) has also been informed (Fontella et al., 2005). In another study it has been reported that CAT activity deescalates in plasma in response to RS (Kashif et al., 2003). Diminished CAT activity could be related to increased H_2O_2 formation caused by RS.

Production of superoxide ($O_2^{\cdot-}$) and H_2O_2 is known to be increased after stress. One of the glutathione-dependent enzymes GST undertakes a considerable role in the detoxification of detrimental compounds. Slightly lower activity of GST in RS (alone) group may have been resulted from oxidative inactivation of this enzyme and depleted levels of its substrate, GSH. To the best of our knowledge influence of RS on plasma antioxidant vitamin levels (β-carotene and vitamins A, C, E) was evaluated in this study for the first time. Although levels of all vitamins decreased in RS (alone) group compared with control, the statistically significant drop was noted only in Vitamin E level.

These decrements in vitamin contents might be attributed to their depletion due to increased oxidant status of restrained rats as evidenced from enhanced cerebral MDA, PC level and decreased GSH content and CAT, GST activities. This result is in line with our previous studies which reported influence of augmented OS on serum vitamin levels (Ozkol et al., 2011; Tuluce et al., 2011). Elevations of β-carotene and antioxidant vitamin levels were observed in each plant extract pretreated group even when compared with control group. This result may be associated with mitigating effects of plant extracts on OS as stated previously (Kumar et al., 2010; Hohmann et al., 1999; Sudati et al., 2009; Masteikova et al., 2008).

The short-lived free radical gas NO is synthesized from L-arginine by NO synthase (NOS), either in peripheral tissues or in central nervous system (CNS). NO is currently realized to be an intracellular messenger in CNS. It has been accepted as a neurotransmitter/neuromodulator (Zhang et al., 1995). Although NO has been informed to be necessary in some physiological functions, it has also been reported to take part in various pathological states. In present study determination of nitrite level revealed a significant increase of cerebral NO content in restrained animals. This increment is in accordance with the results of Akpinar et al. (2008).

Lipid peroxidative influence of NO might have been performed primarily by peroxynitrite ($ONOO^-$) which is a durable oxidant (Siu et al., 1999). $ONOO^-$ (in pure form) harms to DNA, protein, carbohydrate, lipid, organelles and cell systems (Rubbo et al., 1994). Data on NO levels obtained from this experiment clearly indicated that treatment with each plant extract significantly reduces its generation.

This decrease might be owing to direct interactions with NO, to diminished activation of NO synthase, or to elevated Vitamin E concentration. In an earlier experimental study performed on rats, Vitamin E was reported to be an efficient antioxidant in reducing NO induced lipid peroxidation (Siu et al., 1999). Our results are in accordance with Uzbay et al. (2007) and Dastmalchi et al. (2008) in which the reducing influences of *H. perforatum* and *M. officinalis* on NO level were

reported respectively. With respect to *V. officinalis* and *P. incarnate* we did not find any study in the literature informing the effects of these plants on NO concentration.

Conclusion

The results presented here led us to conclude that exposure to repeated RS resulted in increased levels of plasma cortisol, cerebral lipid peroxidation, protein oxidation, NO, and decreased some cerebral antioxidant defense systems (GSH, GST, CAT) and plasma β-carotene, antioxidant vitamin levels in rats. Pretreatment with each of *H. perforatum, M. officinalis V. officinalis* and *P. incarnate* extracts has a significant potential to counteract RS induced foregoing alterations. These plant extracts may probably neutralize RS induced damage by the way of their antistress, antioxidant and free radical defusing effects.

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