Effect of *Celastrus paniculatus* seed oil (Jyothismati oil) on acute and chronic immobilization stress induced in swiss albino mice

George Lekha, Karthik Mohan[1], Irudhaya Arockia Samy[2]

Department of Botany, Mount Carmel College, Bangalore, Karnataka; [1]Departments of Biochemistry; [2]Plant Biology and Plant Biotechnology, St. Joseph's College (Autonomous), Trichy, Tamil Nadu, India

Submitted: 12-02-2010 Revised: 12-02-2010 Published: 19-07-2010

ABSTRACT

Stress alters the homeostasis and is produced by several factors. Immobilization stress induced due to reduced floor area provided for the mobility results in the imbalance of oxidant and antioxidant status. The modern computer savvy world decreases human mobility in the working environment, leading to the formation of oxygen free radicals and if left untreated might result in severe health problems like hypertension, cardiovascular disease, premature aging and brain dysfunction. Hence, modern medicines rely upon the medicinal plants for some drugs with zero side effects. In this context, Jyothismati oil (JO), extracted from *Celastrus paniculatus* seeds, was used to treat acute and chronic immobilization induced experimentally. *C. paniculatus* plant is considered to be rich in antioxidant content and so the seed oil extract's efficacy was tested against immobilization stress in albino mice. The animals were kept in a restrainer for short and long durations, grouped separately and fed with the drug. Animals were sacrificed and the samples were analyzed. The antioxidant enzyme levels of the animals regained and markedly increased in the acute and chronic immobilized groups, respectively. The results suggested that the extract of *C. paniculatus* seed was highly efficacious in reducing the stress induced by least mobility for hours.

Key words: Antioxidants, *Celastrus paniculatus*, free radicals, oxidative damage

INTRODUCTION

The word "stress" is a state involving demand on physical or mental energy; a condition or circumstance (not always adverse), which can disturb the normal physical and mental health of an individual. In medical parlance stress is defined as a perturbation of the body's homeostasis.[1] The pressures and demands that cause stress are known as stressors. The presence of a stressor does not automatically result in disabling stress symptoms. The degree to which any stressful situation or event impacts one's daily functioning. An acute stress is so usual that each and every one of us is exposed to it and it may not produce any notable physical and mental change unless or otherwise exposed to it for several days.[2] Chronic stress grinds away at the mental health, causing emotional damage in addition to physical ailments. Long-term stress can even rewire the brain, leaving the person more vulnerable to everyday pressures and less able to cope.[3]

Immobilization stress is produced when the animal's movements are restricted to a small floor area. Immobilization stress belongs to severe stressors and is known to activate several calcium transport systems.[4] Higher oxidative stress reportedly plays a key role in muscle damage caused by immobilization and subsequent remobilization.[5] Immobilization stress induces the formation of reactive oxygen species (ROS) and leads to the oxidative injury in various tissues.[6] The effects of immobilization stress on peripheral blood cell distribution, plasma level of thiobarbituric acid reactive substances (TBARS), and activities of antioxidant enzymes in erythrocytes were investigated in male wistar rats.[7] A significant increase in plasma TBARS was observed during and after the stress.[8] Dramatic increases in neutrophils and monocytes implied that ROS formation resulted from their activation.[9] Furthermore, the antioxidant activities of catalase (CAT) and superoxide dismutase (SOD) in erythrocytes were dramatically increased during and after the stress, while a large fall in erythrocyte number was observed.[10] These findings suggest that the activation

Address for correspondence:
Prof. Karthik Mohan, Department of Biochemistry, St. Joseph's College, Trichy, Tamil Nadu, India.
E-mail: km80_profsjc@yahoo.co.in

DOI: 10.4103/0974-8490.65512

of immune cells can be a source of the immobilization-induced ROS production, and that antioxidant enzymes in erythrocytes play an important role in preventing the ROS-induced injuries.[11,12]

Celastrus paniculatus is a member of the Celastraceae family and is a large, woody climber (called a climbing shrub), with a yellow, corky bark.[13] It grows throughout India, and has been reported to grow as tall as almost 2000 m. The plants exhibit varying degrees of therapeutic values, some of which are its use in the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout, dyspepsia.[14] Oil from the seeds (called as Jyothismati oil, JO), which contains the alkaloids celastrine and paniculatin in varying amounts, is the most commonly used plant part.[15] Jyothismati is bitter in taste and hot in potency. The seeds and seed oil have great medicinal value. Externally, the seed oil is used for massage with great benefit, especially in vata diseases like sciatica, lumbago, paralysis, arthritis and facial palsy.[16-19] This has been in use in indigenous medicinal systems for centuries with known brain stimulating and antioxidant effects and hence its effect was tested against immobilization-induced stress in albino mice. The primary objective of this work was to determine the stress relieving effect of JO in immobilized animals so that this could be clinically tested in persons whose mobility is restricted to a limited space for a longer duration, viz., the professionals working in computer-related jobs remain immobilized for several hours a day, and bedridden patients might also develop stress due to their longer immobilization. In such conditions, the outcome of this work would pave a new way in treating this stress induced by immobilization.

MATERIALS AND METHODS

Swiss albino mice of both sexes were used for the experimental study. The average body weights of the animals were 25–30 g. The animals were housed in polypropylene cages maintained in a well-ventilated room with 12 hours day and night cycle. The animals were fed with standard pelleted diet and water *ad libitum*. The experiments were carried out in accordance with the guidelines of the institutional animal ethical committee. The animals were grouped into eight groups of six in each group, after acclimatization for 2 days in the laboratory conditions. Group I animals served as the control, in group II acute immobilization stress was induced, and in group III chronic immobilization stress was induced. Group IV, V, VI, VII and VII animals were pretreated with the oil. Animals of groups V and VI were exposed to acute immobilization stress and groups VII and VII were subjected to chronic immobilization stress.

Drug preparation

Exactly 0.5 ml of *C. paniculatus* seed oil (JO) was solubilized in 1% Tween 20 and 5% dimethyl sulfoxide (DMSO) was the solvent. One milliliter of DMSO and 0.2 ml Tween 20 made up to 20 ml was used as the vehicle. Five milliliters of this solution would contain 0.5 ml of the oil and 4.5 ml of the vehicle such that the concentration of 0.5 ml of oil was about 500 mg.

Induction of immobilization stress

The animals were adapted to the experimental conditions by daily handling for 7 days to avoid manipulative stress. On the day of experiment, the cages containing the mice were moved to a laboratory and left there for at least 2 hours to acclimatize to the experimental environment. The mice were restrained on a small transparent plastic cage of standard dimension 3" × 3" × 3" with holes for breathing. For acute immobilization stress, they were kept isolated for 6 hours in the cage after pre-treatment and for chronic immobilization the same process was continued for 7 days. They were then released and returned to their home cages. The animals belonging to group IV were pre-treated with the oil (with the maximal dose used) from day 1 after 7 days of acclimatization for another 7 days. The animals belonging to groups V and VII were treated with the oil (200 mg/kg of body weight) and the mode of administration was through intraperitoneal injections (i.p.). The animals belonging to groups VI and VIII were treated with JO oil 400 mg/kg of body weight. The animals of groups II, V and VI were sacrificed on day 9 after acute immobilization induction. Serum was collected and the brain was dissected out, blotted dry and preserved for analysis. The animals belonging to groups III, VII and VIII were pretreated with the respective concentration of the oil for 7 days and immobilization was induced on 8th day and was continued for another 7 days till day 14, along the drug treatment as well and sacrificed on day 15. Brain tissue was dissected out and stored for analyses. Serum was utilized for biochemical analysis. To determine the activity of antioxidant enzymes, tissue homogenates (brain) were centrifuged at 10,000 *g* for 15 minutes (4°C) to sediment mitochondria. The supernatant was recentrifuged for 30 minutes. The resulting supernatant was used for measuring cytosolic enzymes. The activities of the enzymes SOD,[20] CAT,[21] glutathione peroxidase (GPx),[22] glutathione reductase (GR),[23] glutathione *S*-transferase (GST)[24] and lipid peroxides[25] were studied using spectrophotometer. The protein concentrations present in the extracts were determined by Lowry's method[26] initially. The activities of the enzymes studied are expressed as units per milligram of protein by Lowry procedure. The results were statistically analyzed for significance by student's '*t*' test.[27]

RESULTS AND DISCUSSION

From the earliest times, herbs have been prized for their pain-relieving and healing abilities and today we still rely largely on the curative properties of plants. Over the centuries, societies around the world have developed their own traditions to make sense of medicinal plants and their uses. Some of those traditions and medicinal practices may seem strange and magical, others appear rational and sensible, but all of them are attempts to overcome illness and suffering, with an aim to enhance the quality of life. The brain is the most complex organ of our body and hence no wonder that only a very few drugs have been approved by regulatory authorities for treating multifactorial ailments alike Alzheimer's disease. The oriental system of medicine like "Ayurveda" which is as old as 5000 years, had classified selected plants under "medhya rasayanas". In Sanskrit, "medhya" means intellect/cognition and "rasayana" means "rejuvenation". These are used both in herbal and conventional medicine and offer benefits that pharmaceutical drugs lack, helping to combat illness and support the body's efforts to regain good health and intellect. The experimental plant of our interest *C. paniculatus* is widely used in the indigenous medicinal systems from ancient times for neurophyschological disorders.[28] Hence, the plant's seed oil (JO) was selected for scientifically validating its curative effect in immobilization stress induced mice, produced by virtue of its antioxidant effect.

Stress is defined as a disruption of homeostasis,[29] and stimuli that challenge homeostasis are designated as stressors. Stressors can be divided into three general categories:[30-32] 1) physical (for example, restraint, foot shock, and exercise); 2) psychosocial, including isolation**,** anxiety, fear, or mental frustration; and 3) metabolic, including upright tilt, heat exposure, hypoglycemia, and hemorrhage. Stress has been further subdivided based on duration as acute (single, intermittent, and time-limited exposures) and chronic (intermittent and prolonged or continuous exposures). Stressors used in research are often of a mixed type. For example, immobilization stress is a mixture of physical and psychological stressors, restricting movement and isolating the individual from its group.[33] In adult mice, IMO stress induced a sharp rise in serum marker enzymes and fall in tissue antioxidant levels relative to unstressed controls. It is likely, therefore, that IMO stress, as an acute stressor, does not decrease the levels of the marker and antioxidant enzymes only to a lesser extent, which agrees with data from other laboratories.[34-37] This contrasts with chronic stress where there is evidence for decreases in the marker enzyme concentration and increase in antioxidant enzymes.[38] Stress induced by immobilization (restraint stress) is particularly effective because it combines physical stress (i.e., increased muscular work) and emotional stress (i.e., enhanced flight reaction). Here, we used this well-established model of stress to investigate for the first time the effects of stress induced by immobilization at a definite number of immobilization sessions (i.e., 1 (acute) and 7 (chronic) immobilization sessions).

The tissue (brain) total protein was analyzed by Lowry *et al*'s method. The protein value was found to be decreased in the tissue of animals belonging to acute and chronic immobilization stress induced groups and it was more pronounced in chronic stress induced animals. On treatment with the JO, the reversal of the concentration was identified and it was dose dependent, increase in dosage increased the protein concentration. The protein levels in animals treated with the oil alone was near normal. Significant increase was noted in chronic stress induced animals treated with 400 mg of the drug. This decrease in the total protein content might be attributed to the degeneration of tissue protein in the brain.[39] The degree of decrease was directly proportional to the exposure of animals to stressed condition.[40] Hence, the decrease was profound in chronic immobilization than in acute immobilization [Table 1]. The pretreatment (acute and chronic) and cotreatment (chronic) to immobilized animals significantly increased the protein level which is an indication of lesser tissue degeneration or necrosis.[41]

SOD, CAT, and GR are known to be inactivated *in vitro* by H_2O_2, $O_2^{-\bullet}$ and $OH^{\bullet}$, respectively. SOD and CAT are major antioxidant defence components that primarily catalyze the conversion of superoxide radical $O_2^{-\bullet}$ to H_2O_2 (SOD) and decomposition of H_2O_2 to H_2O (CAT). H_2O_2 is normally detoxified in cells by either CAT and/or GPx. GPx catalyzes the reduction of H_2O_2 by reduced glutathione (GSH). GSH is readily oxidised to glutathione disulfide (GSSG) by the GPx reaction. GSSG can be reduced by NADPH-dependant reaction catalyzed by GR.[42] The immobilization stress induced by both acute and chronic immobilization resulted in the decrease of the enzymes levels of SOD (Cu-Zn SOD), CAT, GPx, GST and GSH.[43] On treatment with the *C. paniculatus* seed oil, the levels increased significantly and the effect was greater in chronic immobilization stress induced animals which received nearly 14 days of drug treatment than the acute immobilized mice. The decrease in the levels from normal was drastic in chronic immobilization than in acute type and increase in levels of these antioxidant enzymes in the treated group was significant [Table 1].

The free radicals generated as a result of immobilization stress induction propagate a chain reaction, leading to lipid peroxidation in cellular membranes, destruction of Ca^{2+} homeostasis, induction of neuronal cell injury and finally resulting in cell death.[44] In this study, the TBARS

Table 1: Antioxidant enzyme levels in acute and chronic immobilization stress induced albino mice and those treated with JO

Groups	Tissue protein (mg/g of tissue)	CAT (IU/ mg of Protein)	IU / mg of Protein	GPx (nmol NADPH consumed/ minute/ mg of protein)	GST (IU/mg of protein)	GR(IU/mg of protein)	Lipid peroxide (nmol malondialdehyde/ Mg of protein / 30 minutes)
Group I	201. 2 ± 0.14	0.55 ± 0.08	230.4 ± 10.2	13.2 ±0.4	70.2 ± 10.3	16.2 ± 0.4	3.2 ± 0.4
Group II	164.3 ± 0.32	0.41 ± 0.15	177.2 ± 11.3	11.4 ± 0.8	63.5 ± 9.7	15.4 ± 0.2	4.4 ± 0.8
Group III	104.1 ± 0.98	0.29 ± 0.94	165.3 ± 9.6	10.2 ± 0.3	60.1 ± 10.5	15.1 ± 0.1	4.8 ± 0.6
Group IV	199.2 ± 0.87	0.54 ± 0.12	233.2 ± 5.2	13.1 ± 0.6	71.4 ± 11.2	16.0 ± 0.5	3.1 ± 0.32
Group V	170.3 ± 1.02**	0.44 ± 0.05**	193.7 ± 10.5**	11.9 ± 1.2*	64.8 ± 2.3*	15.4 ± 0.6*	3.8 ± 0.1*
Group VI	183.5 ± 0.68**	0. 49 ± 0.18**	210.3 ± 9.6**	12.2 ± 0.8**	68.6 ± 1.9*	15.5 ± 0.2*	4.0 ± 1.2**
Group VII	149.3 ±0.55**	0. 36 ± 0.23**	187.4 ± 13.6**	10.7 ± 0.3*	62.5 ± 0.7**	15.3 ± 0.2*	3.2 ± 0.3*
Group VIII	188.6 ± 0.82**	0. 47 ± 0.32**	219.8 ± 12.1**	11.9 ± 0.6**	67.9 ± 1.2**	15.8 ± 0.3*	3.9 ± 0.1**

Group I – normal control, group II – acute immobilization stress induced animals (ACI), group III – chronic immobilization stress induced animals (CHI), group IV – JO administered animals (pretreated for 7 days), group V – acute immobilization stress induced to JO pretreated animals (200 mg/kg of body weight), group VI – acute immobilization stress induced to JO pretreated animals (400 mg/kg of body weight), group VII – chronic immobilization stress induced to JO pretreated animals and the treatment was continued during the stress induction period (200 mg/kg of body weight), group VIII – chronic immobilization stress induced to JO pretreated animals and the treatment was continued during the stress induction period (400 mg/kg of body weight); Values are expressed as mean ± standard deviation for six animals in each group. *Significant when compared to group II;** not significant when compared to group II ($P \leq 0.05$)

level was significantly increased during stress condition and was found to be decreased on treatment with oil; this effect was also dose dependent [Table 1]. The oil caused a decrease in TBARS level in enzymatic assay in immobilization stress induced animals. The enzymatic NADPH-dependant Lipid Peroxide(LPO) is catalyzed by the NADPH-cytochrome P450 reductase and propagated by cytochrome P450 with generation of free radicals, i.e, $O_2^{-\bullet}$ and $ROO^{\bullet}$. The oil of *C. paniculatus* might have inhibited the activity of NADPH-dependant LPO due to its association with its free radical scavenging ability [Table 1]. Elevations in the levels of products of free radicals like TBARS in brain of acute and chronic stress induced group again support the low antioxidant enzyme activity that elevates the lipid peroxidation while TBARS is the product of lipid peroxidation. Another possibility for such an elevation in TBARS may be due to ischemia-reperfusion phenomenon[45,46] or due to high rate of catecholamine secretion that generates free radicals either through auto-oxidation or through metal ion or superoxide-catalyzed oxidation[47] [Table 1].

Immobilization-induced oxidative stress in mice brain has been established here by noting the low activities of SOD, CAT, GST, important antioxidant enzymes, which is consistent with the observation of others.[48] The decrease in antioxidant enzyme activities due to immobilization might be due to their use against the free radicals destruction and/or their inhibition by free radical species.[49] It is well established that SOD activity is inhibited by hydrogen peroxide that reduced Cu^{+2} to Cu^{+1} in SOD.[50] The reduced Cu^{+1} can act as a promoter of hydroxyl by Haber-Weis reaction. Low antioxidant enzyme activities further facilitate the increased susceptibility to lipid peroxidation.[51] The reduction of hydrogen peroxide is catalyzed by CAT that protects the tissues from highly reactive hydroxyl radicals.[52] Reduction of hydrogen peroxide and hydroperoxides to non-toxic products is catalyzed by GST and peroxidase. Hence, JO was efficient in combating the free radicals produced due to the immobilization stress induced with the help of restrainers. The key findings of this research are that the antioxidant nature of this oil would probably relieve stress in individuals who remain immobilized either due to their job profile or physical conditions.

CONCLUSION

The antioxidant rich *C. paniculatus* seed oil, JO, was found to be efficient against immobilization stress induced in the groups of animals. It was more pronounced in case of the animals receiving chronic immobilization stress. In acute stress, the reversal of the conditions was recorded within 24 hours and the effects were dose dependant, increase in the dosage increased the activity. Several works need to be carried out to ascertain the molecular mechanism of stress release in the case of immobilization stress and develop a new therapeutic molecule using JO oil.

REFERENCES

1. Selye H. Diseases of adaptation. Wis Med J 1950;49:515-6.
2. McEwen BS. Physiology and neurobiology of stress and adaptation: Central role of the brain. Physiol Rev 2007;87:873-904.
3. Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, *et al*. Chronic behavioural stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. Neuroscience 2004;125:1-6.
4. Nadeem A, Masood A, Masood N, Gilani RA, Shah ZA. Immobilization stress causes extra-cellular oxidant-antioxidant imbalance in rats: Restora. Eur Neuropsychopharmacol

2006;16:260-7.

5. Liu MJ, Li JX, Lee KM, Qin L, Chan KM. Oxidative stress after muscle damage from immobilization and remobilization occurs locally. Clin Orthop Relat Res 2005;246-50.
6. Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. FASEB J 1996;10:1532-8.
7. Liu J, Wang X, Mori A. Immobilization stress-induced antioxidant defense changes in rat plasma; effect of treatment with GSH. Int J Biochem 1994;26:511-7.
8. Selektor Y, Parker RB, Sun Y, Zhao W, Bhattacharya SK, Weber KT. Tissue 65Zinc Translocation in a rat model of chronic aldosteronism. J Cardiovasc Pharmacol 2008;51:359-64.
9. Oishi K, Yokoi M, Maekawa S, Sodeyama C, Shiraishi T, Kondo R, *et al*. Oxidative stress and haematological changes in immobilized rats. Acta Physiol Scand 1999;165:65-9.
10. Bar-Shai M, Carmeli E, Ljubuncic P, Reznick AZ. Exercise and immobilization in aging animals: The involvement of oxidative stress and NF-k. Free Radic Biol Med 2008;44:202-14.
11. Kuriyama T, Oishi K, Kakazu H, Machida K. Changes of physiological functions in rats induced by immobilization stress. Nippon Eiseigaku Zasshi 1998;52:647-53.
12. Meerson FZ, Dolgikh VT, Smolentseva VN, Batrachenko ER. Prevention of impairment of myocardium metabolism and function in immobilization stress. Vopr Med Khim 1985;31:41-5.
13. Chatterjee A, Prakashi SC. The Treatise on Indian Medicinal Plants. Vol. 3. New Delhi: 2003. p. 160.
14. Nadkarni K.M, Nadkarni A.K. Indian materia medica, vol 1. In: Nadkarni KM. (7th Popular Bombay Prakashan Pvt. Ltd.1976) p 296.
15. Singh U, Wadhwani AM, Johri BM. Dictionary of Economical Plants of India. New Delhi: 1996. p. 46.
16. Nalina K, Aroor AR, Kumar KB, Anjali Rao. Studies on biogenic amines and their metabolites in mentally retarded children in Celastrus oil therapy. Alternat Med 1986;4:355-60.
17. Thakur RS, Puri HS, Akhtar H. Major Medicinal Plants of India. 1989. p. 240 CIMAP, Lucknow, India..
18. Rajkumar R, Kumar EP, Sudha S, Suresh B. Evaluation of anxiolytic potential of Celastrus oil in rat models of behaviour. Fitoterapia 2007;78:120-4.
19. Godkar PB, Gordon RK, Ravindran A, Doctor BP. Celastrus paniculatus seed water soluble extracts protect against glutamate toxicity in neuronal cultures from rat forebrain. J Ethnopharmacol 2004;93:213-9.
20. Suttle NF. Analysis of super oxide dismutase. Vet Rec 1986;119:519-22.
21. Catalases LH. In: Bregmeyer HU, editor. Methods of Enzymatic Analysis. Vol. 2. Academic Press; New York: 1974. p. 885.
22. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as component of glutathione peroxidase. Science 1973;179:588-90.
23. Kinoshita C, Saze KI, Kumata S, Mastuki T, Homma S. A simplified method for estimation of glutathione reductase. J Dairy Sci 1995;79:1543-8.
24. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase: The first enzymatic step in. mercapturic acid formation. J Biol Chem 1974;249:7130-9.
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagaent. J Biol Chem 1951;193:265-75.
27. Bennett CA, Franklin NL. Statistical analysis in Chemistry and chemical industry. New York: John Willey and sons, Inc; 1967. p. 133.
28. Godkar PB, Gordon RK, Ravindran A, Doctor BP. *Celastrus paniculatus* seed water soluble extracts protect against glutamate toxicity in neuronal cultures from rat forebrain. J Ethnopharmacol 2004;93:213-9.
29. Rivier C, Rivest S. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: Peripheral and central mechanisms. Biol Reprod 1991;45:523-32.
30. Pacak K, Palkovits M, Yadid G, Kvetnansky R, Kopin IJ, Goldstein DS. Heterogeneous neurochemical responses to different stressors: A test of Selye's doctrine of nonspecificity. Am J Physiol 1998;275:1247-55.
31. Van de Kar LD, Blair ML. Forebrain pathways mediating stress-induced hormone secretion. Front Neuroendocrinol 1999;20: 1-48.
32. Tilbrook AJ, Turner AI, Clarke IJ. Effects of stress on reproduction in non-rodent mammals: The role of glucocorticoids and sex differences. Rev Reprod 2000;5:105-13.
33. Pacák K, Palkovits M. Stressor specificity of central neuroendocrine responses: Implications for stress-related disorders. Endocr Rev 2001;22:502-48.
34. Charpenet G, Tache Y, Forest MG, Haour F, Saez JM, Bernier M, *et al*. Effects of chronic intermittent immobilization stress on rat testicular androgenic function. Endocrinology 1981;109:1254-8.
35. Mann DR, Orr TE. Effect of restraint stress on gonadal proopiomelanocortin peptides and the pituitary-testicular axis in rats. Life Sci 1990;46:1601-9.
36. Srivastava RK, Taylor MF, Mann DR. Effect of immobilization stress on plasma luteinizing hormone, testosterone, and corticosterone concentrations and on 3ß-hydroxysteroid dehydrogenase activity in the testes of adult rats. Proc Soc Exp Biol Med 1993;204:231-5.
37. Orr TE, Taylor MF, Bhattacharyya AK, Collins DC, Mann DR. Acute immobilization stress disrupts testicular steroidogenesis in adult male rats by inhibiting the activities of 17 beta-hydroxylase and 17,20-lyase without affecting the binding of LH/hCG receptors. J Androl 1994;15:302-8.
38. Veldhuis JD. Male hypothalamic-pituitary-gonadal axis. In: Lipschultz LI, Howards SS, editors. Infertility in the Male. St Louis, Mo: Mosby; 1997. p. 47-8.
39. Adlard PA, Cotman CW. Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. Neuroscience 2004;124:985-92.
40. Adlard PA, Perreau VM, Cotman CW. Chronic immobilization stress differentially affects the expression of BDNF mRNA and protein in the mouse hippocampus. Stress Health 2004;20:175-80.
41. Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. FASEB J 1996;10:1532-8.
42. Kuriyama T, Oishi K, Kakazu H, Machida K. Changes of physiological functions in rats induced by immobilization stress. Nippon Eiseigaku Zasshi 1998;52:647-53.
43. Lee YJ, Choi B, Lee EH, Choi KS, Sohn S. Immobilization stress induces cell death through production of reactive oxygen species in mice. Neurosci Lett 2006;392:27-31.
44. Emel M, Saadet S. Stress-dependent induction of protein oxidation, lipid peroxidation and anti-Oxidants in Wister rats. Clin Exp Pharmacol Physiol 2007;34:425-31.
45. Oishi K, Yokoi M, Maekawa S, Sodeyama C, Shiraishi T, Kondo R, *et al*. Oxidative stress and haematological changes in immobilized rats. Acta Physiol Scand 1999;165:65-9.

46. Freeman BA, Crapo JD. Biology of disease: Free radicals and tissue injury. Lab Invest 1982;47:412-26.
47. Jewett SL, Eddy LJ, Hochstein P. Is the antioxidation of catecholamines involved in ischaemic reperfusion injury. Free Radic Biol Med 1989;6:185-8.
48. Dillard CJ, Litov RE, Tappel AL. Effects of dietary vitamin-E, selenium and polyunsaturated fats on *in vivo* lipid peroxidation in the rats as measured by pentane production. Lipids 1978;13:396-402.
49. Venkateswaran S, Pari L. Effect of *Coccinia indica* leaves on antioxidant status in streptozotocin-induced diabetic rats. J Ethnopharmacol 2003;84:163-8.
50. Debnath D, Mandal TK. Study of quinalphos (an environmental oestrogenic insecticide) formulation (Ekalux 25 E.C.)-induced damage of the testicular tissues and antioxidant defence systems in Sprague-Dawley albino rats. J Appl Toxicol 2000;20:197-204.
51. Marklund S, Marklund G. Involvement of superoxide anion radical in autoxidation of pyragallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
52. Ji LL. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 1999;222:283-92.

Source of Support: Nil, **Conflict of Interest:** None declared.