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PROTECTIVE EFFECTS OF REGULAR EXERCISE COMBINED WITH CURCUMIN SUPPLEMENTATION AGAINST LEAD-INDUCED CEREBELLAR OXIDATIVE DAMAGE IN AN ANIMAL MODEL

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Lifestyle modifications, such as physical exercise and dietary supplements, were recommended as protective measures against some neurological disorders. We examined the effects of regular exercise combined with curcumin supplementation against lead-induced oxidative damage of the cerebellum in male rats. Experimental animals (n = 50) were randomly divided into five groups. Lead acetate (20 mg/kg) was i.p. administered to three groups (except sham and control ones), while the sham group received ethyloleate (30 mg/kg) three times per week. The curcumin and curcumin + exercise groups received curcumin (30 mg/kg) i.p. five times per week for eight weeks. The exercise program consisted of progressive running on a treadmill, speed from 15 to 22 m/min, 25 to 64 min per day, five times per week for eight weeks. Two days after the last application, the rats were euthanized, and their cerebellum was removed and homogenized to measure the levels of brain-derived neurotrophic factor (BDNF) and thiobarbituric acid-reactive substances (TBARSs). Chronic administration of lead significantly increased the cerebellar TBARS levels but did not alter considerably the BDNF levels. Curcumin and curcumin + exercise treatments significantly lowered the cerebellar TBARS levels; a significant increase in the BDNF level was observed in the cerebellum of rats treated with combined intervention. Thus, regular exercise combined with curcumin supplementation may exert a significant neuroprotective effect against lead-induced cerebellar injury by attenuating oxidative stress and improving the brain state through an increase in the BDNF amount.

Keywords: lead toxicity, brain-derived neurotrophic fact, cerebellar injury, oxidative stress, physical exercise, curcumin.

INTRODUCTION

Lead is a toxic heavy metal [1]; recognition of and prevention against its harmful effects are an important international public health priority [2]. Human lead exposure remains a serious occupational and public health problem in humans [3]. The half-life of lead in the blood is rather long [4], and its levels in the brain decrease very slowly [5]. Immediate effects of lead include alteractions in chemistry, physiology, and histology of the brain [6]. It has been reported that lead intoxication cause several abnormalities in the CNS, such as cognitive impairments, neurobehavioral disorders, and neurodegeneration [7]. Previous studies have indicated that lead can induce significant injuries in the cerebellum, to both histological characteristics and antioxidant capacity of this brain structure [1, 6].

Lead-induced oxidative stress has been known to play a crucial role in the pathogenesis of lead intoxication, so that significant amounts of reactive oxygen species (ROSs) generated due to lead exposure were detected in brain [8]. Because the brain is characterized by a high oxygen tension, low mitotic rate, high lipid content, and low antioxidant concentrations, it is very susceptible to oxidative damage [9]. Bennet et al. [10] indicated a region-specific oxidative stress in rat's brain exposed to lead so that high-intensity oxidative stress was observed in the hippocampus and cerebellum compared to other regions of the rat brain.

There is evidence that neurotrophins can play a significant protective role in the mammalian nervous sys-

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tem [11]. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, contributes to the survival, function, and plasticity of neurons in the adult brain [12]. Radak et al. [13] reported that accumulation of oxidative damage in the brain associated with impaired brain functions can interact with the function of the BDNF system [14].

Physical exercise can help to maintain a sufficient brain health and plasticity throughout life; it is an important public health goal [15]. Such exercise has a capability to alter the level of BDNF and the rate of oxidative damage in the brain of animals [16]. Seifert et al. [17] indicated that expression of BDNF in the rat hippocampus and release of BDNF in the human brain increased following endurance training. Besides, exercise effects and nutritional antioxidants (curcumin in particular) can protect the brain from oxidative stress [18, 19]. Curcumin (diferuloylmethane) is a polyphenol compound derived from the *Curcuma longa* plant and known as turmeric. It has been demonstrated that curcumin has a wide range of therapeutic properties, including antioxidant, analgesic, anti-inflammatory, and antiseptic activities [20]. Curcumin demonstrates a powerful scavenging activity against ROSs [21] and can protect neural cells from oxidative stress [20]. On the other hand, both ROSs and BDNF are common molecular targets for the action of the diet and exercise [22].

Most investigations of lead-related damage were focused on the effects of lead on the histological structure of the cerebellum [1, 6]. It, however, remained unclear how a combination of physical exercise with curcumin treatment influences lead-induced oxidative stress in the rat cerebellum. Therefore, we studied the effect of regular long-lasting exercise (rather similar to human exercise training) combined with curcumin supplementation on lead-induced cerebellar oxidative damage in an animal model.

METHODS

Chemicals. Lead acetate, ethyloleate, curcumin, ketamine, xylazine, sodium chloride, Tris-HCl, NP40, glycerol, phenylmethylsulfonyl fluoride (PMSF), leupeptin, sodium vanadate, and 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (AEBSF) were obtained from Sigma-Aldrich (USA). TBARS and BDNF test kits were purchased from Cayman Chemical Co. (USA) and R&D Systems (USA).

Animals. The experiments were performed on male Wistar rats (250 ± 4 g, eight to ten weeks old) kept with free access to a standard pellet diet and water. All animals were obtained from the Pasture Institute of Iran and were housed in polycarbonate cages (five per cage) at a temperature of (22 ± 2°C) and humidity of (50 ± 5%), with the 12h/12h dark/light cycles.

Experimental Procedure. Fifty normal Wister rats matched for the age and body mass were randomly divided into five groups (10 animals each) and treated as follows. The control group received no treatment; the sham group received ethyloleate (i.p., 30 mg/kg body mass) for three days per week over eight weeks; the lead group received lead acetate (i.p., 20 mg/kg [23]) for three days per week over eight weeks; the curcumin group received the same lead doses, but plus curcumin (i.p., 30 mg/kg [23] for five days per week over eight weeks, and the exercise plus curcumin group received the same curcumin doses, but the animals performed aerobic exercises. The mode of the latter was the following; after one week of familiarization to a treadmill (KN-73, Natsume, Japan), exercise-group rats performed training at 15 m/min, 25 min per day with 0% grade for five days at the first week. Gradual increases in the training speed and duration were subsequently established such that the rats reached a speed of 22 m/min for 64 min per day at the end of the eighth week. This protocol was designed according to the maximum oxygen consumption. In every exercise session, 3 min-long run at the speed of 7 m/min was increased by 2 m/min for each minute until the desired speed was reached. A cool-down program was performed via a gradual decrease in the treadmill speed to approach the initial speed set at the beginning of the program [24].

Anesthesia and Tissues Collection. After the last applications (48 h) and overnight fasting (12 h), the animals were sacrificed under anesthesia by i.p. administration of a mixture of ketamine (60 mg/kg) and xylazine (5 mg/kg). The brains were removed, and cerebellum tissues were kept at –70ºC before biochemical examination.

Biochemical Analysis. Enzyme-linked immunosorbant assay [ELISA] was performed for BDNF according to the manufacturer's guidelines [25]. The level of TBARS, as a byproduct of lipid peroxidation, was measured by a TBARS kit (Cayman Chemicals, USA) in accordance with the method of Nabavi et al. [26, 27].

Statistical Analysis. Data are shown below as means ± s.e.m. Statistical analysis was performed with a one-way ANOVA and *post-hoc* testing by the Tukey's test. P values < 0.05 were considered indications of

statistically significant intergroup differences. All statistical tests were performed using a commercial software package (SPSS version 16.0 for Windows).

RESULTS

The results in Table 1 show that means of the body and brain masses of lead-treated rats decreased relative to the respective values in the sham (7.4 and 4.7%, respectively) and control groups (7.5% and 6.3%, respectively), but these shifts were statistically insignificant. The above changes in the body and brain masses were, however, noticeably smaller following administration of curcumin alone and that combined with regular exercise treatments ($P < 0.05$).

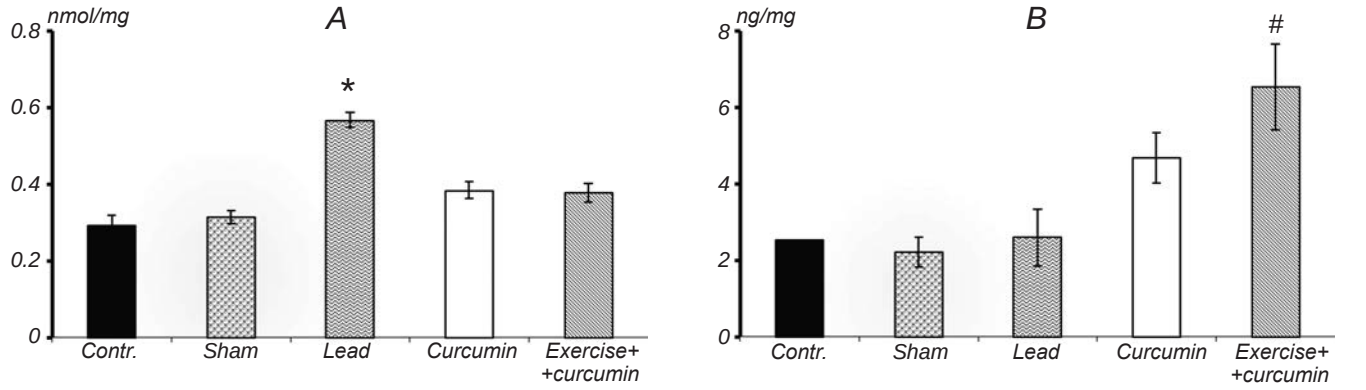
Cerebellar TBARS levels significantly increased in lead-administered rats, as compared with those in the sham and control groups (0.564 ± 0.045 vs. 0.315 ± 0.048 and 0.315 ± 0.075 nmol/mg MDA eq/g tissue, respectively, $P = 0.000$). Both curcumin supplementation alone and the combination of curcumin and regular exercise led to significant decreases in the cerebellar TBARS levels (0.384 ± ± 0.058 and 0.377 ± 0.066 nmol/mg MDA eq/g tissue respectively, $P = 0.000$). No significant difference was observed between the effects of both interventions on the intensity of lipid peroxidation in rat brain tissues (Fig. 1A). Furthermore, lead acetate administration exerted no significant effect on the cerebellar BDNF levels (2.59 ± 1.98 ng/mg protein) when compared with those in the sham and control groups (2.53 ± 2.03 and 2.21 ± 1.12 ng/mg protein, respectively). Eight week-long combined intervention significantly (more than two times) increased the cerebellar BDNF level (6.15 ± 1.73 ng/mg protein), compared with the respective values in the control ($P = 0.030$), sham ($P = 0.014$), and lead ($P = 0.044$) groups. Supplementation with curcumin provided a noticeable increase (4.68 ± 2.96 ng/mg protein) in the cerebellar BDNF level in lead-treated rats, but the diference did not reach the significance level (Fig. 1B).

Effect of regular physiological exercise combined with curcumin supplementation on the body and brain mass during chronic lead administration in rats

Вплив регулярних фізичних тренувань, поєднаних із споживанням куркуміну, на масу тіла та масу мозку щурів в умовах хронічної інтоксикації свинцем

Indices	Control	Sham	Lead acetate	Lead + + curcumin	Lead + curcumin+ + exercise
Body mass (g)	342.12 ±9.00	341.75 ±12.31	316.57± 9.14	322.00 ±8.54	343.38± 15.32
Brain mass (g)	1.73 ± 0.15	1.70 ± 0. 18	1.62 ± 0.29	1.72 ± 0.10	1.79 ± 0.07

Footnote: values are shown as means ± s.e.m. for 10 rats.



F i g 1. Mean cerebellar TBARS (A) and BDNF (B) levels in groups of the rats after eight weeks of treatment. $^{*}P < 0.001$ vs. the control, sham, curcumin, and exercise +curcumin groups; $^{\#}P < 0.05$ vs. the control, sham, and lead groups. Values are shown as means ± s.e.m. for ten rats; TBARS: thiobarbituric acid-reactive substances; BDNF: brain-derived neurotrophic factor.

Р и с. 1. Середні рівні TBARS (*A*) та BDNF (*B*) у групах щурів через вісім тижнів після початку експерименту.

DISCUSSION

In our study, lead was i.p. administered to male Wistar rats at a dose of 20 mg/kg body mass for eight weeks. At the end of the treatment period, decreases in the body and brain masses of the treated rats were observed when compared with the respective indices in sham and control animals, but these changes did not reach the significance level. Comparable observations of the lead effects were described by other authors [6, 28]. Sidhu and Nehru [29] reported that the mass of both cerebrum and cerebellum decreased in lead-intoxicated rats, and such shifts in the experiments of these authors were significant. The normal arrangement of three layers in the cerebellum was modified enough to disrupt the normal arrangement of the molecular layer, Purkinje cell layer, and granular cell layer of the cerebellum after lead treatment [6]. Lead toxicity can intensely damage normal histological structure of the brain, modify morphological characteristics of the nerve cells and ganglia in the CNS, and alter normal functions performed by them [7]. These shifts perhaps explain why lead exposure affected somewhat the brain mass in our study.

We found that chronic lead administration in rats significantly increased the TBARS level in the cerebellum, but this pathophysiological change was not accompanied by significant changes in the cerebellar BDNF level (Fig. 1B). Earlier studies [6, 29, 30] have established that exposure of rats to lead caused an increase in the intensity of lipid peroxidation and decreased the activity of antioxidant enzymes in the cerebellum, compared with the controls. Lead neurotoxicity in the brain is mediated by oxidative stress to a considerable extent, which forwardly implies to depletion of antioxidant enzyme stores. Lead acts as a nonspecific enzyme inhibitor due to its high affinity for sulfhydryl groups in protein molecules [6, 31]. In addition, lead accumulation in tissues increases the level of δ-aminolevulinic acid, and this intensifies oxidative stress and impairs the membranes, DNA, and proteins [31]. However, increased oxidative stress observed in our study exerted no dramatic influence on the cerebellar BDNF level, and we failed to detect the respective effect. In contrast, Hosseinzadeh et al. [32] reported a significant decrease in the hippocampal BDNF level, which was accompanied by an increase (while insignificant) in the intensity of lipid peroxidation in rats exposed to lead acetate. It seems that the effects of lead on the BDNF levels in various regions of the brain do not appear to be uniform.

As shown in Fig. 1A, both curcumin supplementation alone and such treatment combined with regular physical exercise led to comparable significant decreases in the cerebellar TBARS levels in lead-intoxicated rats. These positive changes were accompanied by elevations in the cerebellar BDNF levels. Combined regular exercise and curcumin treatment synergistically induced a higher BDNF level than either treatment alone. This protective effect of curcumin agrees with findings of Shukla et al. [29] who demonstrated a significant increase in the antioxidant defense and a decreased intensity of lipid peroxidation in the cerebellum, *corpus striatum*, hippocampus, and frontal cortex in rats simultaneously treated with curcumin and lead. Similar effects were found in the hippocampus of male rats after eight weeks of combination of continuous exercise training and curcumin treatment in another study [32]. Although the exact mechanisms by which exercise or curcumin treatment counteracts lead-induced cerebellar injuries are mostly unclear, it should be mentioned that we observed a synergistic effect of these influences on the cerebellar BDNF level following combined intervention. It seems that both curcumin and regular physical exercise cause parallel changes in the oxidative stress and promote the respective repair mechanisms in the brain. Curcumin as a powerful antioxidant agent (known to be a scavenger of superoxide anions, hydroxyl radicals, and nitrogen dioxide, and also as a hydrogen donor) exhibits the antioxidant activity directly and also indirectly protects the brain against various oxidative stressors [33].

The biological function of BDNF is exerted through binding to its receptor, tropomyosin-related kinase B (TrkB), which initiates multiple signaling cascades [34]. Curcumin produces neuroprotective effects, in particular via activating brain-derived neurotrophic factor/TrkB-dependent MAPK and PI-3K cascades [35]. Also, it was reported that both moderate treadmill running and wheel running in animal experiments up-regulate the BDNF-TrkB pathway in the hippocampus [29]. Exercise exerts its neuroprotective effects on the brain through several mechanisms. In fact, it is clear that voluntary exercise can increase the levels of BDNF and other growth factors, stimulate neurogenesis, and increase the resistance to brain insult [15]. Thus, generation of cerebellar BDNF may form a considerable protective response to lead-induced oxidative stress, because BDNF acts not only via protection of the existing neurons, but also via intensification of neurogenesis and improvement of synaptic plasticity [36].

In conclusion, regular physical exercise combined with curcumin supplementation may provide rather significant neuroprotective effects against lead-induced cerebellar injury by attenuating oxidative stress; these influences improve brain health in particular through an increase in the BDNF level.

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The experimental protocol followed the US National Institutes of Health guidelines for the care and use of laboratory animals and was approved by the Institutional Animal Care and Use Committee (Approval number: No. 03-19-5/15819 1390 IAU).

The authors, M. Habibian, S. J. Moosavi, and P. Farzanegi, confirm that they have no conflict of interest with any organization or person that may be related to this study; there was also no conflict of interest in interrelations between the authors.

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ПРОТЕКТИВНИЙ ВПЛИВ КОМБІНАЦІЇ ФІЗИЧНОГО ТРЕНУВАННЯ ТА ВВЕДЕННЯ КУРКУМІНУ НА ІНДУКОВАНЕ СВИНЦЕВОЮ ІНТОКСИКАЦІЄЮ ОКСИДАТИВНЕ УШКОДЖЕННЯ МОЗОЧКА В ЕКСПЕРИМЕНТАЛЬНІЙ МОДЕЛІ

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Р е з ю м е

Модифікації стилю життя, такі як фізичні тренування та вживання добавок до дієти, рекомендуються як протективні заходи щодо низки неврологічних розладів. Ми дослідили впливи регулярних тренувань, поєднаних з уведенням куркуміну, на індуковане свинцевою інтоксикацією оксидативне ушкодження мозочка у самців щурів. Експериментальні тварини (n = 50) були рандомізовано поділені на п’ять груп. Ацетат свинцю (20 мг/кг) уводився тваринам трьох груп (дві слугували контролем). Одна з контрольних груп отримувала етилолеат (30 мг/кг) тричі на тиждень. У двох інтоксикованих групах тварини отримували 30 мг/кг куркуміну п’ять разів на тиждень протягом восьми тижнів. Програма тренувань включала в себе прогресивно збільшувані епізоди бігу на тредбані (від 15 до 25 м/хв, від 25 до 64 хв/добу, п’ять разів на тиждень протягом восьми тижнів). Через дві доби після закінчення курсів щурів піддавали евтаназії, мозочок видаляли та гомогенізували для вимірювання рівнів мозкового нейротрофічного фактора (BDNF) та речовин, реактивних щодо тіобарбітурової кислоти (TBARSs). Хронічне введення свинцю викликало вірогідне збільшення рівнів TBARS, але не змінювало істотно рівнів BDNF. У групах «куркумін» та «куркумін+тренування» спостерігалися значно нижчі рівні TBARS; вірогідно більший рівень BDNF у мозочку відмічався в групі з комбінацією заходів. Отже, регулярні фізичні тренування, поєднані з уведенням куркуміну, можуть забезпечити істотний протекторний ефект щодо індукованого свинцем ушкодження мозочка, опосередкований зменшенням оксидативного стресу та поліпшенням стану мозку завдяки певному збільшенню кількості BDNF.

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