Curcumin and sertraline prevent the reduction of the number of neurons and glial cells and the volume of rats' medial prefrontal cortex induced by stress

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Chronic stress induces morphological changes in the neurons of several brain regions, including medial prefrontal cortex (mPFC). This region is involved in variety of behavioral tasks, including learning and memory. Our previous work showed that stress impaired function. The present work extends the earlier work to study mPFC in stressed and non-stressed rats with or without sertraline or curcumin treatments using stereological methods. Sertraline is a selective serotonin reuptake inhibitor and curcumin is the main ingredient of turmeric with neuroprotective effects. In this study, 42 male rats were randomly assigned to seven groups: stress + distilled water, stress + olive oil, stress + curcumin (100 mg/kg/day), stress + sertraline (10 mg/kg/day), curcumin, sertraline, and control groups. After 56 days, the right mPFC was removed. The volume of mPFC and its subdivisions and the total number of neurons and glia were estimated. The results showed ~8%, ~8%, and 24% decrease in the volume of the mPFC and its prelimbic and infralimbic subdivisions, respectively. However, the anterior cingulated cortex remained unchanged. Also, the total number of the neurons and glial cells was significantly reduced (11% and 5%, respectively) in stress (+distilled water or olive oil) group in comparison to the non-stressed rats ($P<0.01$). However, no significant reduction was observed in the volume of the mPFC and its subdivisions as well as the total number of the neurons and glial cells in stress + sertraline and stress + curcumin groups in comparison to the non-treated stressed rats ($P<0.01$). The result indicated that treatment of rats with curcumin and sertraline could prevent the stress-induced changes in mPFC.

Key words: sertraline, curcumin, stress, cortex, stereology

INTRODUCTION

Chronic stress is a risk factor for many psychopathological conditions, including depression and anxiety disorders (Kendler et al. 1995, Sapolsky 1996, Shelton 2004). Chronic Variable Stress (CVS) has significant effects on the cellular integrity and function of certain brain areas, most especially the prefrontal cortex and hippocampus of rats (Heim et al. 1997, Joels et al. 2007). The medial prefrontal cortex (mPFC) of the rats is mainly subdivided into three cytoarchitectonic subregions: prelimbic (PL), infralimbic (IL), and anterior cingulate cortex (ACC) (Krettek and Price 1977, Uylings et al. 2003). mPFC is an essential neural substrate for coordinating the cognitive-affective information and modulating the hypothalamo–pituitary–adrenal axis response to emotional stress (Diorio et al. 1993, MacDonald et al. 2000). Previous studies reported the significant role of rats' mPFC in stress. The study also demonstrated that chronic restraint stress changed the morphology of the neurons in the mPFC (Rajkowska et al. 1999, Akana et al. 2001). Furthermore, in clinical studies, structural brain modifications in depressed patients, such as decreased volume (Bremner et al. 2000) or neuronal density in hippocampus (Rajkowska et al. 1999), were similar to those found in

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the animal models of CVS (Cotter et al. 2001, Joels et al. 2004). Previous studies also showed that chronic stress exposure resulted in decreased neurogenesis in the hippocampal formation (Gould and Tanapat 1999, Czeh et al. 2001). Moreover, it has been agreed that repeated stressful experiences have intense effects on neuronal plasticity in different brain areas, particularly in the hippocampal formation, the prefrontal cortex, and the amygdale. CVS has been widely used for studying clinical depression as well as evaluating the antidepressant effects of diverse drugs in animal models (Willner 2005). Therefore, the first aim of the present study is to demonstrate the structural changes of the mPFC after CVS. This study also aims to evaluate and compare the therapeutic effects of curcumin and sertraline on CVS.

Curcumin is the principal curcuminoid of the popular Indian spice, turmeric. Curcumin has been reported to have protective effects in animal models of diseases, such as Alzheimer's, Parkinson's, and epilepsy (Xu et al. 2005, Kulkarni et al. 2008). Previous studies reported that the individuals consuming curcumin in daily life had better brain function and higher cognitive abilities (Kulkarni et al. 2009). In another study, curcumin was shown to protect the dopamine producing cells of the substantia nigra area of the brain in a rat model of Parkinson's disease (Kulkarni et al. 2009). Moreover, administration of curcumin completely reversed stress-induced serotonin and noradrenaline in the hippocampus and the frontal cortex (Xu et al. 2007).

Selective serotonin reuptake inhibitors, including sertraline, are primarily used to treat major depression in adult patients (Rush et al. 2006). The neuroprotective and antioxidant action of sertraline have also been reported in neurodegenerative diseases (Matar et al. 2006). In addition, various reports have suggested the beneficial effects of sertraline on animal model depression (Malberg et al. 2000, Santarelli et al. 2003).

The human studies have been reported that prefrontal cortex shows alterations in the cerebral structures in depressed patients (Lorenzetti et al. 2009). Our previous study on the animal model of stress showed that stress affected the behavioral tests, such as spatial learning and memory, anxiety, and anhedonia (Noorafshan et al. 2013a). The present study extends the earlier work to evaluate the histological parameters of the mPFC of the rats submitted to CVS using stereological methods. The possible protective effects of curcumin and sertraline on the structural parameters are evaluated, as well. Briefly, the study aims to find responses to the following questions:

How much does the volume of the mPFC and its subdivisions change after CVS? How many neurons and glial cells of the mPFC are lost after CVS? Do sertraline and curcumin protect the mPFC structure after CVS? Which drug is more effective?

METHODS

Animals

In this study, 42 adult male Sprague-Dawley rats (260±20 g) were obtained from the laboratory animal center of Shiraz University of Medical Sciences, Shiraz, Iran. The Ethics Committee of the University approved the animal experiment (Approval No. 91-6124). The male rats were randomly assigned to experimental and control groups. Each group included 6 rats that were housed under standard conditions, room temperature (22–24°C), and a 12:12 h light-dark schedule and had free access to water and food. The animals were divided into seven groups: (I) stress +
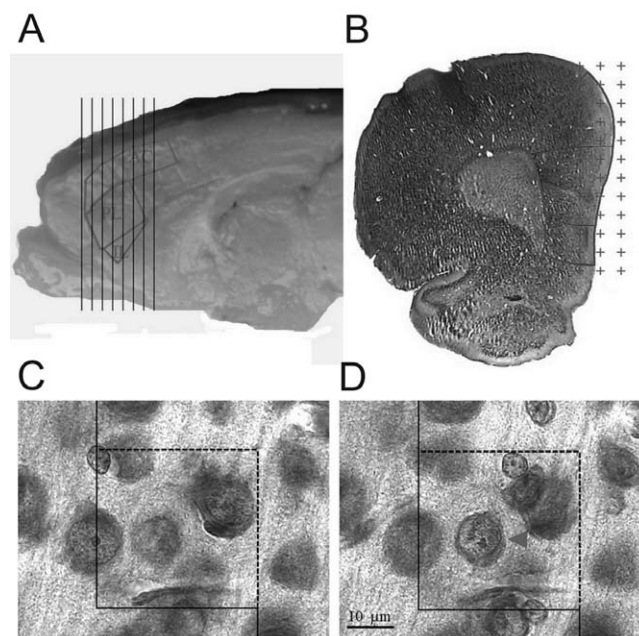

Fig. 1. [A] mPFC and its prelimbic (PL), infralimbic (IL), and anterior cingulate cortex (ACC) subdivisions. [B] Point counting method. [C], [D] Optical disector method. The cells whose nuclei came into focus during scanning the disector height and did not touch the left and lower borders of the frame were counted (arrow head).

distilled water group daily receiving stress and distilled water, (II) stress + olive oil group daily receiving stress and olive oil, (III) stress + curcumin group daily receiving stress and curcumin (100 mg/kg/day) solved in olive oil (Noorafshan et al. 2013a,b), (IV) stress + sertraline group daily receiving stress and sertraline (10 mg/kg/day) solved in distilled water (Yildirim et al. 2012), (V) control group, (VI) curcumin group receiving curcumin (100 mg/kg/day) solved in olive oil (Noorafshan et al. 2013a,b), and (VII) sertraline group receiving sertraline (10 mg/kg/day) solved in distilled water (Yildirim et al. 2012).

Stress model

The animals were submitted to a CVS regime over a 56-day period or remained in their home cages without stress manipulation (Tagliari et al. 2010). The induction of CVS is described in Table I. The following stressors were applied: (1) 24 hours of water proscription, (2) 1 to 3 hours of restraint, as described below, (3) 1.5 to 2 hours of restraint at 4°C, (4) flashing light during 120 to 210 minutes, (5) isolation (2 to 3 days), (6) tilting of the home cages at a 45° angle for 4–6 hours, and (7) moist bedding (pouring 300 ml water onto the bedding during 1.5 to 2 hours). Restraint was applied by placing the animals in a 25×7 cm plastic cylinder with the holes for breathing. In addition, exposure to flashing light was completed by placing

the rats in a 50 cm-high, 40×60 cm open field made of brown floor with a frontal glass wall. A 40 W lamp was flashed at a frequency of 60 flashes per minute (Tagliari et al. 2010).

Tissue preparation

The rats were deeply anesthetized and decapitated. Then, their brains were exposed by an incision along the midline of the skull. A small amount of fixative was poured on the exposed brain immediately. The brains were removed and mPFC was identified according to atlas of Paxinos and Watson (Paxinos and Watson 2007) (Fig. 1). The right prefrontal cortex was immersed in neutral formaldehyde for one week and then embedded in paraffin block. Then, complete serial coronal sections (26 μm thick) (T) were made at 4.70–2.70 mm ventral and 4.70–2.70 mm dorsal to the bregma using a microtome (Akana et al. 2001). Every 10 section was sampled starting with a random number between 1 and 10. After all, about 8–10 sections in each animal were selected in a systematic random manner. The sections were stained with cresyl violet (0.1% in distilled water) in order to estimate the volume of the region as well as the total number of the neurons and glial cells of the mPFC. It should be mentioned that the glial cells were distinguished from neurons by their smaller size and lack of a nucleolus and stained cytoplasm.

Estimation of the volume of mPFC and its subdivisions (PL, IL, ACC)

Using a projecting microscope, the live image of each brain section was evaluated according to the rat brain atlas (Gundersen et al. 1988a,b). The boundaries of the mPFC were considered from the most anterior section where the underlying white matter appeared and continued on every mounted section up to the appearance of the genu of the corpus callosum where decussating of the fibers could be observed. Following this procedure, 8–10 sections were obtained and analyzed for each mPFC. The volumes were calculated using the Cavalieri method (Gundersen et al. 1988a,b). Besides, the image of each section was evaluated according to the rat brain atlas at the final magnification of 25× (Paxinos and Watson 2007). Using the stereological software designed at Histomorphometry and Stereology
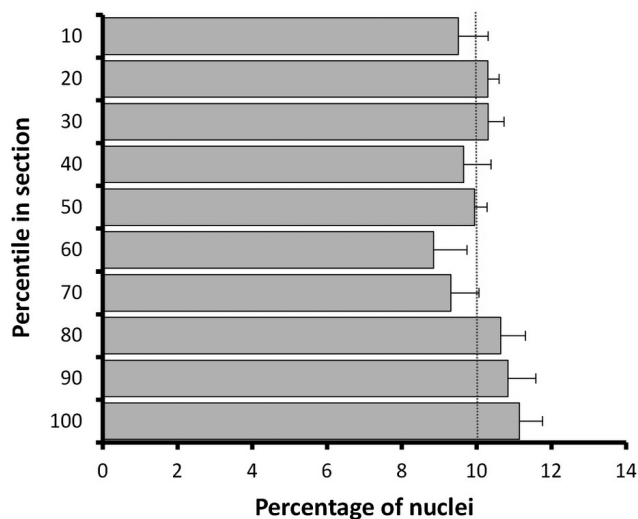

Fig. 2. Z-axis distribution of the nuclei. There are ten histograms. Each histogram represents the percent of the counted nuclei in 10 percent of the section thickness from the top to the bottom of the section.

Research Center (Shiraz University of Medical Sciences), a grid of points was superimposed on the images (Fig. 1). The volume of the mPFC was estimated by the following formula:

$$V(mPC) = \sum P \times \left(\frac{a}{p} \right) \times d \; ,$$

where "$\sum P$" was the total points hitting the mPFC sections (here 450–500 points per animal), "a/p" was the area associated with each point (here was 0.04 mm²), and "d" was the distance between the sampled sections.

Estimation of the number of neurons and glia

The total number of neurons and glia lcells was determined using the optical disector method at the final magnification of 2 800× (Gundersen et al. 1988a,b). The position of the microscopic fields was selected by systematic uniform random sampling, with moving the stage in equal distances in x- and y-directions. An oil immersion objective lens (60×, numerical aperture: 1.4) was used, as well. An unbiased counting frame with inclusion and exclusion borders was superimposed on the images (Fig. 1). This frame avoids the "edge effect" and biased counting of the cells. The focal plane was moved downwards in z-direction. Then, a microcator was attached to the stage of the microscope to measure the z-axis traveling (depth). The upper and lower guard zones were used to avoid cutting artifacts that occurred at the upper and lower surfaces of the tissue sections. The height of the disector was defined as the section thickness excluding the 4 μm thick guard zone at the top and bottom of each section. Any nucleus coming into maximal focus within the next focal sampling plane was selected if it lay completely or partly inside the counting frame and did not touch the exclusion line (Fig. 1).

The suitable guard zone was determined after estimating the percent of nuclei in the ten columns of the z-axis thickness. Each column presented 10 percent of the section thickness. According to the histogram, the upper and lower 20% was considered as the guard zone. The remaining columns were considered as the height of the disector (Fig. 2). The numerical density (N_v) was estimated using the following formula (Dorph-Petersen et al. 2001):

$$Nv\left(\frac{Cells}{mPFC} \right) = \left[\frac{\sum Q^-}{\sum P \times \frac{a}{f} \times h} \times \frac{t}{BA} \right] ,$$

where „ΣQ^-" was the number of the nuclei coming into focus and counted (on the average, 340–450 neurons and 120–260 glial cells were counted per mPFC), "ΣP" was the total number of the counting frames in all fields, a/f was the area per frame (35×35 μm²), "h" was the height of the disector, "t" was the real section thickness measured using the microcator when the Q^- was counted (here ~20 μm on the average), and BA was the block advance of the microtome which was set at 26 μm. The total number of the neurons was estimated by multiplying the numerical density (Nv) by the V (mPFC) (Gundersen et al. 1988a,b).

Estimation of the coefficient of error (CE)

The CE for the estimate of the volume; i.e., CE(V), was calculated by the following formula:

$$CE(v) = \left(\sum P \right)^{-1} \times \left[\frac{1}{240} \left(3 \times \sum PiPi + \sum PiPi_{+2} - 4 \sum PiPi_{+1} \right) + \right.$$
$$\left. + 0.0724 \times \frac{B}{\sqrt{A}} \times \left(\sqrt{n \sum Pi} \right) \right]^{\frac{1}{2}} ,$$

where "B" and "A" represented the mean section boundary length and mean sectional area, respectively. The CE for the estimate of the total neuron and glial cell number, CE(N), was derived from CE(V) and CE(Nv) using the following formula (Gundersen and Jensen 1987, Braendgaard et al. 1990, Gundersen et al. 1999):

$$CE(N) = \left[\sqrt{CE^2(Nv) + CE^2(V)} \right]$$

$$CE(Nv) = \left[\left(\frac{n}{n-1} \right) \times \left[\frac{\left(\sum Q^- \right)^2}{\sum Q^- \sum Q^-} + \frac{\left(\sum P \right)^2}{\sum P \sum P} - \frac{2 \sum \left(Q^- P \right)}{\sum Q^- \sum P} \right] \right]^{\frac{1}{2}} .$$

Statistical analysis

The data were analyzed using Kruskall-Wallis, and Mann-Whitney *U*-test with adjusted alpha level. $P \leq 0.05$ was considered as statistically significant.

Table I

The protocol for induction of the chronic variable stress (CVS) in 56 days for the rat model

Day	Stressor applied	Day	Stressor applied
1	Cold restraint (1.5 h)	29	Damp bedding (2 h)
2	Inclination of home cages (4 h)	30	No stressor applied
3	Flashing light (2 h)	31	Water deprivation (24 h)
4	Restraint (2 h)	32	Inclination of home cages (6 h)
5	Isolation	33	Flashing light (2 h)
6	Isolation	34	Cold restraint (2 h)
7	Isolation	35	Isolation
8	Damp bedding (2 h)	36	Isolation
9	Inclination of home cages (6 h)	37	Isolation
10	No stressor applied	38	Flashing light (3 h)
11	Flashing light (2 h)	39	Damp bedding (2 h)
12	Water deprivation (24 h)	40	Restraint (3 h)
13	Restraint (3 h)	41	Cold restraint (1.5 h)
14	Damp bedding (3 h)	42	Inclination of home cages (4 h)
15	Inclination of home cages (4 h)	43	Flashing light (2 h)
16	Cold restraint (2 h)	44	Restraint (2 h)
17	Flashing light (3 h)	45	Isolation
18	Restraint (2.5 h)	46	Isolation
19	Damp bedding (3 h)	47	Isolation
20	Isolation	48	Damp bedding (2 h)
21	Isolation	49	Inclination of home cages (6 h)
22	Isolation	50	No stressor applied
23	Cold restraint (1.5 h)	51	Flashing light (2 h)
24	Water deprivation (24 h)	52	Water deprivation (24 h)
25	Inclination of home cages (4 h)	53	Restraint (3 h)
26	Restraint (3 h)	54	Damp bedding (3 h)
27	Flashing light (3 h)	55	Inclination of home cages (4 h)
28	Restraint (1 h)	56	Cold restraint (2 h)

Table II

Coefficients of error (CE) for total volume, neuronal and glial numbers as well as neuronal and glial densities in the medial prefrontal cortex

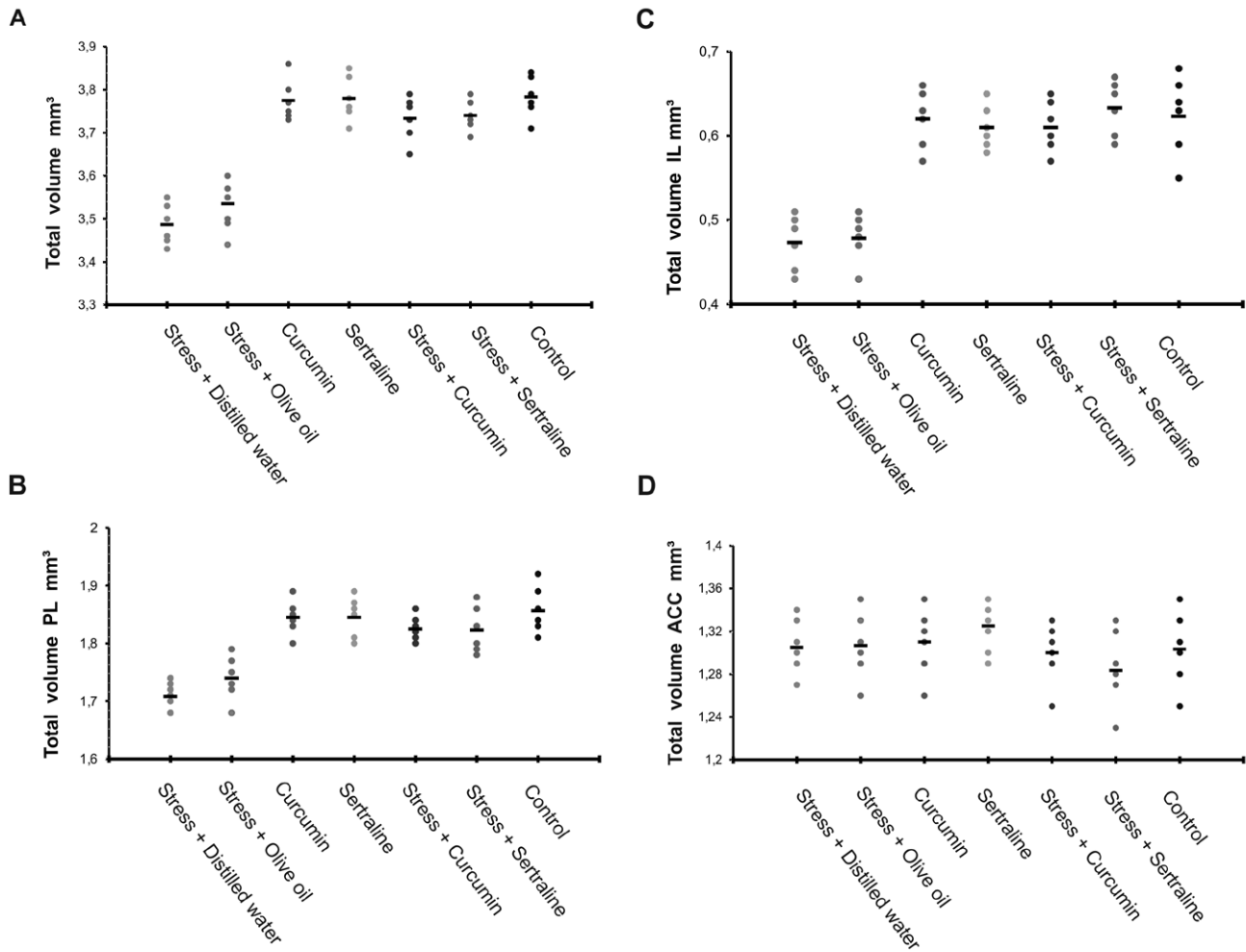| Groups	Volume	Neuron density	Neuron number	Glia density	Glia number
Distilled Water	0.03	0.05	0.05	0.03	0.07
Olive Oil	0.04	0.04	0.04	0.04	0.05
Curcumin	0.04	0.04	0.06	0.03	0.05
Sertraline	0.03	0.03	0.05	0.03	0.06
Stress + curcumin	0.05	0.05	0.05	0.04	0.05
Stress + Sertraline	0.03	0.03	0.04	0.03	0.06
Control	0.04	0.04	0.06	0.04	0.06

Fig. 3. Dot plots showing the total volume of the mPFC and its subdivisions, including prelimbic (PL), infralimbic (IL), and anterior cingulate cortex (ACC). Each dot represents an animal in stressed or non-stressed group with or without curcumin or sertraline treatment.
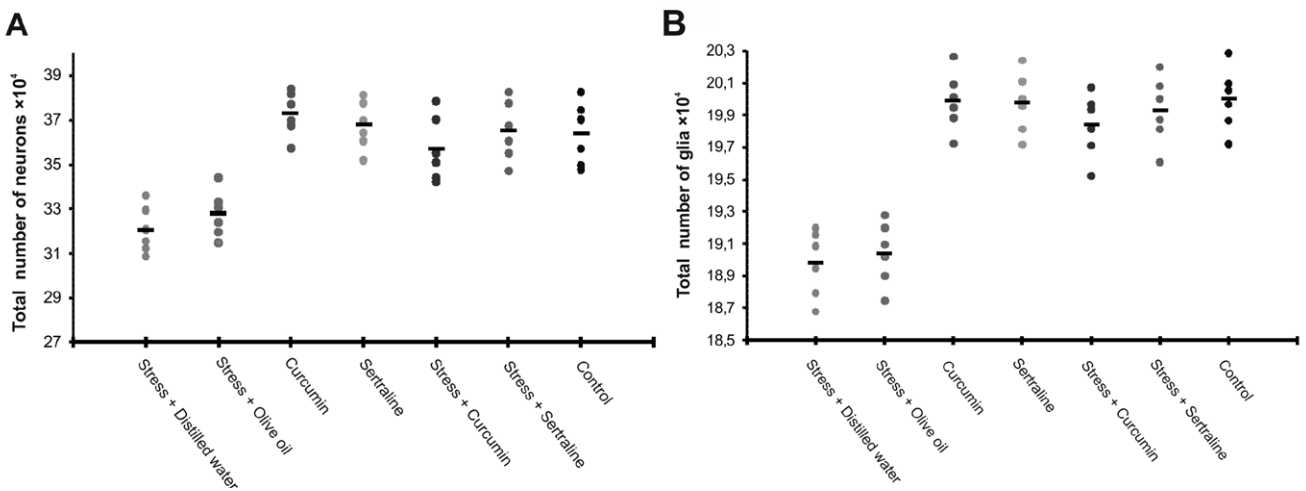

Fig. 4. Dot plots showing the total number of the neurons and the total number of the glial cell in the mPFC. Each dot represents an animal in stressed or non-stressed group with or without curcumin or sertraline treatment.

RESULTS

Volume of the mPFC

The study results revealed ~8% decrease in the volume of the mPFC in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3). However, the total volume of the mPFC reminded unchanged in the stressed rats treated with sertraline or curcumin ($P<0.01$).

Volume of the PL

The study findings showed ~8% reduction in the volume of the prelimbic region in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3). Nonetheless, the volume of the prelimbic region reminded unchanged in the stressed rats treated with sertraline or curcumin ($P<0.01$).

Volume of the IL

The study results revealed ~24% decrease in the volume of the infralimbic region in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3). Nonetheless, the volume of the infralimbic region remained unchanged in the stressed rats treated with sertraline or curcumin ($P<0.01$).

Volume of the ACC

According to the findings of the current study, the volume of the anterior cingulated cortex remained unchanged in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3).

Total number of the neurons

The results showed that the total number of the neurons in the mPFC was significantly reduced by 11% in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats ($P<0.01$). However, the total number of the neurons reminded unchanged in the stressed rats treated with sertraline or curcumin ($P<0.01$) (Fig. 4).

Total number of the glial cells

The results showed that the total number of the cells in the mPFC was significantly reduced by 5% in the stress + olive oil group in comparison to the stress + curcumin group. Further analysis also revealed 5% decrease in the stress + distilled water group in comparison to the stress + sertraline group ($P<0.01$) (Fig. 4).

DISCUSSION

The present study investigated the changes in the total volume and total number of the neurons and glial cells in mPFC resulting from CVS using stereological methods. The advantage of using stereological studies is obtaining unbiased and accurate estimations. The volume of the mPFC and different regions of the PFC was evaluated in advance. The volume reduction which was found in this study is in accordance with other studies that explained the structural changes followed by stress. Holmes and Wellman (2009) explained that exposure to even short periods of severe stress was sufficient to cause major structural remodeling of the main projection neurons within the rodents' PFC. In addition, Garrett and Wellman (2009) showed that stress could decrease the apical dendritic branch number and length in male rats, but could increase the apical dendritic length in female rats. According to these preclinical findings, chronic stress reduced the grey matter volume in the mPFC (Frodl et al. 2008). This study demonstrated that chronic exposure to stress reduced the volume of the mPFC as well as the PFC sub-regions (PL and IL), whereas the volume of the ACC region remained unchanged. Therefore, there are functional and anatomical differences among these three PFC sub-regions. It has been stated that in comparison to the ACC region, neurons in the prelimbic and infralimbic regions of the mPFC are more susceptible to stress (Helmeke et al. 2008). The present study also indicated that chronic exposure to stress reduced the number of the neurons in the mPFC, which is in agreement with the results of another study conducted on the issue (Bachis et al. 2008). These researchers found an increase in caspase-3 positive neurons in the cerebral cortex, suggesting apoptosis as a result of chronic mild stress. Furthermore, in clinical studies, structural brain modifications, such as decreased neuronal density in mPFC of the depressed patients, were

similar to those found in the animal models of the present study (Rajkowska et al. 1999). The previous works reporting a decrease in cytogenesis in the rat mPFC following chronic stress (Czeh et al. 2007, 2008). Czeh and coworkers (2007) showed that chronic stress repressed cell proliferation both in the cerebral hemispheres of adult rats. They also showed that the survival rate of the newborn cells was also inhibited by chronic stress in both hemispheres. In the present study, the number of glial cells in the mPFC was also quantified using stereological techniques and the results showed reductions in the number of the glial cells in the mPFC after chronic stress. Other studies have also indicated a significant reduction in the number of the glial cells in the mPFC in major depressive disorder (Bowley et al. 2002). Ongur and colleagues (1998) and Cotter and others (2001) also reported a significant decrease in the glial cell number in the mPFC after major depressive disorder.

The findings of animal studies have suggested that many of these changes can be reversed by antidepressant treatment (Sapolsky 2000, Henn and Vollmayr 2004, Joels et al. 2004). In rats, antidepressants reverse hippocampus and prefrontal cortex atrophy, compartmental changes, and spatial memory dysfunction (Rocher et al. 2004). In this study, we evaluated the protective effect of chronic administration of a natural compound (curcumin) and a synthetic compound (sertraline) on the histological parameters altered by stress. Our results demonstrated that both curcumin and sertraline administered in stress model were able to prevent the reduction in the number of the neurons and glial cells and the total volume of the mPFC with no preferences. In general, sertraline is used to treat major depression and many other neurodegenerative disorders (Matar et al. 2006, Rush et al. 2006). Selective serotonin reuptake inhibitors are the most widely prescribed antidepressant class today and exert their antidepressant-like effects by inhibiting the neuronal reuptake of serotonin and increasing the synaptic concentrations of serotonin (Matar et al. 2006, Rush et al. 2006). Studies have reported that antidepressants administration prevented both hipocampal atrophy and altered neurogenesis in dentate gyrus induced by social stress (Gould and Tanapat 1999). Czeh and coworkers (2007) also showed that concomitant fluoxetine treatment counteracted the stress-induced effect on cytogenesis.

Curcumin can be a future drug for treatment of various neurological disorders, such as major depression, tardive dyskinesia, and other related neurodegenerative disorders (Kulkarni et al. 2008). Various studies have explained different mechanisms for curcumin. Curcumin possesses antidepressant activity and modulates the levels of norepinephrine, dopamine, and serotonin in the brain (Kulkarni et al. 2009). There are also some evidence suggesting curcumin to have the ability to increase the levels of brain derived neurotrophic factor (Kulkarni et al. 2009). Moreover, curcumin is an inhibitor of monoamine oxidase enzyme. By inhibiting the activity of monoamine oxidases enzyme, curcumin increases the concentration of these neurotransmitters in the synapse (Kulkarni et al. 2009). Furthermore, there is evidence that curcumin may work better than other antidepressants for some subtypes of depression because its side effects are much less pronounced (Kulkarni et al. 2008, 2009).

CONCLUSION

In conclusion, our result indicated that treatment of rats with curcumin and sertraline prevented the reduction of the number of neurons and glial cells and the volume of the mPFC induced by stress.

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REFERENCES

Akana SF, Chu A, Soriano L, Dallman MF (2001) Corticosterone exerts site-specific and state-dependent effects in prefrontal cortex and amygdala on regulation of adrenocorticotropic hormone, insulin and fat depots. J Neuroendocrinol 13: 625–637.

Bachis A, Cruz MI, Nosheny RL, Mocchetti I (2008) Chronic unpredictable stress promotes neuronal apoptosis in the cerebral cortex. Neurosci Lett 442: 104–108.

Bowley MP, Drevets WC, Ongür D, Price JL (2002) Low glial numbers in the amygdala in major depressive disorder. Biol Psychiatry 52: 404–412.

Braendgaard H, Evans SM, Howard CV, Gundersen HJ (1990) The total number of neurons in the human neocortexunbiasedly estimated using optical disectors. J Microsc 157: 285–304.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000) Hippocampal volume reduction in major depression. Am J Psychiatry 157: 115–118.

Cotter D, Mackay D, Landau S, Kerwin R, Everall I (2001) Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. Arch Gen Psychiatry 58: 545–553.

Czéh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, Bartolomucci A, Fuchs E (2001) Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad SciU S A 98: 12796–12801.

Czéh B, Müller-Keuker JI, Rygula R, Abumaria N, Hiemke C, Domenici E, Fuchs E (2007) Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. Neuropsychopharmacology 32: 1490–1503.

Czeh B, Perez-Cruz C, Fuchs E, Flügge G (2008) Chronic stress-induced cellular changes in the medial prefrontal cortex and their potential clinical implications: does hemisphere location matter? Behav Brain Res 190: 1–13.

Diorio D, Viau V, Meaney MJ (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J Neurosci 13: 3839–3847.

Dorph-Petersen KA, Nyengaard JR, Gundersen HJ (2001) Tissue shrinkage and unbiased stereological estimation of particle number and size. J Microsc 204: 232–246.

Frodl TS, Koutsouleris N, Bottlender R, Born C, Jäger M, Scupin I, Reiser M, Möller HJ, Meisenzahl EM (2008) Depression-related variation in brain morphology over 3 years: effects of stress? Arch Gen Psychiatry 65: 1156–1165.

Garrett JE,Wellman CL (2009). Chronic stress effects on dendritic morphology in medial prefrontal cortex: sex differences and estrogen dependence. Neuroscience 162: 195–207.

Gould E, Tanapat P (1999) Stress and hippocampal neurogenesis. Biol Psychiatry 46: 1472–1479.

Gundersen HJ, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B (1988a) The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. APMIS 96: 857–881.

Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A (1988b) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96: 379–394.

Gundersen HJ, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. J Microsc 147: 229–263.

Gundersen HJ, Jensen EB, Kiêu K, Nielsen J (1999) The efficiency of systematic sampling in stereology--reconsidered. J Microsc 193: 199–211.

Heim C, Owens MJ, Plotsky PM, Nemeroff CB (1997) The role of early adverse life events in the etiology of depression and posttraumatic stress disorder. Focus on corticotropin-releasing factor. Ann N Y Acad Sci 821: 194–207.

Helmeke C, Ovtscharoff W Jr, Poeggel G, Braun K (2008) Imbalance of immunohistochemically characterized interneuron populations in the adolescent and adult rodent medial prefrontal cortex after repeated exposure to neonatal separation stress. Neuroscience 152: 18–28.

Henn FA, Vollmayr B (2004) Neurogenesis and depression: etiology or epiphenomenon? Biol Psychiatry 56: 146–150.

Holmes A, Wellman CL (2009) Stress-induced prefrontal reorganization and executive dysfunction in rodents. Neurosci Biobehav Rev 33: 773–783.

Joels M, Karst H, Alfarez D, Heine VM, Qin Y, van Riel E, Verkuyl M, Lucassen PJ, Krugers HJ (2004) Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus. Stress 7: 221–231.

Joels M, Karst H, Krugers HJ, Lucassen PJ (2007) Chronic stress: implications for neuronal morphology, function and neurogenesis. Front Neuroendocrinol 28: 72–96.

Kendler KS, Kessler RC, Walters EE, MacLean C, Neale MC, Heath AC, Eaves LJ (1995) Stressful life events, genetic liability, and onset of an episode of major depression in women. Am J Psychiatry 152: 833–842.

Krettek JE, Price JL (1977) The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J Comp Neurol 171: 157–191.

Kulkarni S, Dhir A, Akula KK (2009) Potentials of curcumin as an antidepressant. Scientific World Journal 9: 1233–1241.

Kulkarni SK, Bhutani MK, Bishnoi M (2008) Antidepressant activity of curcumin: involvement of serotonin and dopamine system. Psychopharmacology (Berl) 201: 435–442.

Lorenzetti V, Allen NB, Fornito A, Yücel M (2009) Structural brain abnormalities in major depressive disorder: a selective review of recent MRI studies. J Affect Disord 117: 1–17.

MacDonald AW, Cohen JD, Stenger VA, Carter CS (2000) Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science 288: 1835–1838.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20: 9104–9110.

Matar MA, Cohen H, Kaplan Z, Zohar J (2006) The effect of early poststressor intervention with sertraline on behavioral responses in an animal model of post-traumatic stress disorder. Neuropsychopharmacology 31: 2610–2618.

Noorafshan A, Abdollahifar MA, Karbalay-Doust S, Asadi-Golshan R, Rashidian-Rashidabadi A (2013a) Protective effects of curcumin and sertraline on the behavioral changes in chronic variable stress-induced rats. Exp Neurobiol 22: 96–106.

Noorafshan A, Asadi-Golshan R, Karbalay-Doust S, Abdollahifar MA, Rashidiani-Rashidabadi A (2013b) Curcumin, the main part of turmeric, prevents learning and memory changes induced by sodium metabisulfite, a preservative agent, in rats. ExpNeurobiol 22: 23–30.

Ongur D, Drevets WC, Price JL (1998) Glial reduction in the subgenual prefrontal cortex in mood disorders. Proc Natl Acad Sci U S A 95: 13290–13295.

Paxinos G, Watson C (2007) The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 45: 1085–1098.

Rocher C, Spedding M, Munoz C, Jay TM (2004) Acute stress-induced changes in hippocampal/prefrontal circuits in rats: effects of antidepressants. Cereb Cortex 14: 224–229.

Rush AJ, Trivedi MH, Wisniewski SR, Stewart JW, Nierenberg AA, Thase ME, Ritz L, Biggs MM, Warden D, Luther JF, Shores-Wilson K, Niederehe G, Fava M, Team SDS (2006) Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. N Engl J Med 354: 1231–1242.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301: 805–809.

Sapolsky RM (1996) Why stress is bad for your brain. Science 273: 749–750.

Sapolsky RM (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57: 925–935.

Shelton CI (2004) Diagnosis and management of anxiety disorders. J Am Osteopath Assoc 104: S2–5.

Tagliari B, Noschang CG, Ferreira AG, Ferrari OA, Feksa LR, Wannmacher CM, Dalmaz C, Wyse AT (2010) Chronic variable stress impairs energy metabolism in prefrontal cortex and hippocampus of rats: prevention by chronic antioxidant treatment. Metab Brain Dis 25: 169–176.

Uylings HB, Groenewegen HJ, Kolb B (2003) Do rats have a prefrontal cortex? Behav Brain Res 146: 3–17.

Willner P (2005) Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52: 90–110.

Xu Y, Ku B, Cui L, Li X, Barish PA, Foster TC, Ogle WO (2007) Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Brain Res 1162: 9–18.

Xu Y, Ku BS, Yao HY, Lin YH, Ma X, Zhang YH, Li XJ (2005) The effects of curcumin on depressive-like behaviors in mice. Eur J Pharmacol 518: 40–46.

Yildirim E, Erol K, Ulupinar E (2012) Effects of sertraline on behavioral alterations caused by environmental enrichment and social isolation. Pharmacol Biochem Behav 101: 278–287.