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Antioxidant potential of fluoxetine in comparison to *Curcuma longa* in restraint-stressed rats

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Abstract

Stress plays a potential role in the onset and exacerbation of depression. Chronic restraint stress in rats, and psychosocial stress in humans, is implicated in the pathophysiology of mood and anxiety disorders. Oxidative damage is an established outcome of restraint stress, which has been suggested to induce many damaging processes contributing to the pathology of stress-induced depression. However, the modulatory role of clinically effective antidepressants, such as fluoxetine, in attenuating oxidative stress has not been well characterized. Therefore, the current study was designed to investigate the antioxidant effects of chronic treatment with fluoxetine in animals submitted to restraint stress. The antioxidant potential of the antidepressant fluoxetine was compared with that of turmeric, used as a standard since it integrates both antioxidant and antidepressant properties. Chronic fluoxetine administration to stressed animals for 21 days prevented restraint stress-induced oxidative damage with an efficacy similar to that of turmeric, as evidenced by significant enhancement of key endogenous antioxidant defense components, comprising the free-radical scavenging enzymes, superoxide:superoxide oxidoreductase (EC 1.15.1.1), hydrogen-peroxide:hydrogen-peroxide oxidoreductase (EC 1.11.1.6), glutathione *S*-transferase (EC 2.5.1.18) and glutathione:$NADP^+$oxidoreductase (EC 1.8.1.7), as well as non-enzymatic antioxidants, GSH, glucose and uric acid, which were severely depleted by restraint stress in animals receiving no treatment. Oxidative stress markers, (*S*)-lactate:NAD^+ oxidoreductase activity (EC 1.1.1.27), malondialdehyde levels (lipid peroxidation product) and protein carbonyl content were also significantly decreased following fluoxetine treatment. Both these drugs when given alone to non-stressed animals did not alter basal levels of antioxidant defense components and oxidative stress markers significantly. Our findings suggest that the therapeutic efficacy of fluoxetine may be mediated, at least partially, *via* reversal of oxidative damage as demonstrated by protective enhancement of antioxidant status following a stress-induced decline. In addition, this study demonstrates important implications for pharmacological interventions targeting cellular antioxidants as a promising strategy for protecting against oxidative insults in stress-induced depression.

Keywords: Fluoxetine; Antidepressant; Antioxidant; Restraint stress; Oxidative damage

1. Introduction

Stress exerts detrimental effects on several cell functions, through impairment of antioxidant defenses, leading to oxidative damage, which is central to many diseases (Torres et al., 2004). Free-radical damage by reactive oxygen species has been suggested to play a critical role in the pathophysiology of neurodegenerative diseases, neuropsychiatric disorders and stress-induced depression (Jenner, 1991; Sapolsky, 2000; Bilici et al., 2001). Although clinical depression, depressive symptoms and psychological stress should be distinguished, they are closely related with one another (Tsuboi et al., 2006) and play an important role in the development of affective disorders (Post, 1992). Repeated chronic stress has been associated with the development and manifestation of depression (Checkley, 1996). Restraint as a stress model combines both emotional and physical components of stress in addition to producing robust increases in basal oxidative stress (Zaidi and Banu, 2004; Zaidi et al., 2005; Walesiuk et al., 2006). Restraint has been used extensively to study the impact of stress on disease process and the effects of drugs in stress-related pathology in animals (Glavin et al., 1994).

Antidepressant drugs are widely used for the management/treatment of stress and stress-related depression and anxiety

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(Diamond and Rose, 1994). Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), effectively treats a wide spectrum of mood disorders (Wong et al., 1995) and also protects against the adverse effects of different types of stressors (Freire-Garabal et al., 1997; Ayelli Edgar et al., 2002). Fluoxetine has emerged as the treatment of choice for depression due to a better safety profile, fewer side effects and improved tolerability compared to the older tricyclic antidepressants (Wilde and Benfield, 1998; Anderson, 1999). However, the underlying mechanisms of its therapeutic efficacy remain unclear, particularly with reference to preventing oxidative routes of damage in stress disorders. Although restraint stress is widely employed to induce oxidative and neurotoxic damage, however, to the best of our knowledge, no study has as yet investigated the *in vivo* antioxidant modulating effects of antidepressants on restraint stress-induced oxidative damage.

Thus, the main objective of the present work is to probe the antioxidant potential of fluoxetine and its comparison with turmeric (*Curcuma longa* L.) in rats submitted to restraint stress. For the purpose of comparison, turmeric has been selected as the reference standard, as it effectively incorporates both antioxidant and antidepressant properties (Yu et al., 2002).

2. Materials and methods

2.1. Chemicals and test drugs

Fluoxetine hydrochloride was purchased from Cadilla, India. All other chemicals used were of analytical grade and purchased from commercial sources. Glucose estimation kit was purchased from Techno Pharmchem, Bahadurgarh, India and uric acid diagnostic reagent kit from Span Diagnostics Ltd., Surat, India. Turmeric (*C. longa* L.) rhizomes were purchased from the local market, washed extensively with sterilized water, air-dried and pulverized to obtain turmeric powder. It was stored at room temperature throughout the experiments.

2.2. Experimental animals

Swiss Albino rats weighing 100–150 g were housed under standard laboratory conditions of temperature (25±5 °C) and natural 12-h light/dark cycle with free access to standard pellet chow (Ashirwad Industries, Chandigarh, India) and drinking water *ad libitum*. The experimental protocol strictly adhered to the prescribed animal ethical procedures according to the guidelines of the Institutional Research Committee.

2.3. Experimental protocol

Fluoxetine hydrochloride (20 mg/kg) dissolved in physiological saline and aqueous turmeric extract (50 mg/kg) was administered *via* oral route to animals according to the treatment schedule presented below. Pilot studies revealed that 50 mg/kg of turmeric extract was effective in restoring antioxidant status and normalizing other markers of oxidative stress, and this dose was selected for the present investigation (data not given). After 1 week of acclimatization, animals were randomized into six groups of six animals each. Group I animals served as non-stressed controls. Animals in groups II, III, IV, V and VI received drug and/or stress for 21 consecutive days as follows:

Group II: Oral administration of aqueous turmeric extract (50 mg/kg) (indicated as T in figures and table)
Group III: Oral administration of fluoxetine (20 mg/kg) (FL)
Group IV: Stress (4 h restraint stress daily) (S)
Group V: Stress, followed by oral administration of aqueous turmeric extract (50 mg/kg) (S+T)
Group VI: Stress, followed by oral administration of fluoxetine (20 mg/kg) (S+FL)

2.4. Restraint stress

All animals were exposed to stress for 4 h during the light phase of the cycle. Restraint stress was accomplished by immobilizing animals in snug body-size cages of wire mesh. This restrained all physical movement without subjection to pain. The animals were deprived of food and water during the entire period of exposure to stress. Subsequently the animals were released from their enclosure and provided access to water. 30 min post-release the animals received either food or the treatment under study, according to the experimental protocol. The stress regimen was followed daily during the experimental period of 21 days, at the end of which animals were sacrificed under light ether anesthesia for biochemical studies.

2.5. Collection of serum and preparation of tissue homogenates

Blood samples were collected under anesthesia for separation of serum. Brain and liver tissues were quickly removed and washed with ice-cold sterile physiological saline (0.9%). A 10% homogenate was prepared in 0.1 M sodium phosphate buffer, pH 7.4, centrifuged at 10,000 *g* (4 °C) for 15 min to remove cellular debris and the supernatant was used for further studies.

2.6. Antioxidant investigations

Brain and liver homogenates were used for the estimation of the following antioxidant enzymes: superoxide:superoxide oxidoreductase [superoxide dismutase: SOD; EC 1.15.1.1] (Marklund and Marklund, 1974), hydrogen-peroxide:hydrogen-peroxide oxidoreductase [catalase: CAT; EC 1.11.1.6] (Aebi, 1984), glutathione *S*-transferase [GST; EC 2.5.1.18] (Habig et al., 1974) and glutathione:$NADP^+$ oxidoreductase [glutathione reductase: GR; EC 1.8.1.7] (Carlberg and Mannervik, 1975). Lipid peroxidation [aldehydic product, malondialdehyde: MDA] (Beuge and Aust, 1978), glutathione content: GSH (Jollow et al., 1974) and protein carbonyl content (Levine et al., 1990) were determined by standard protocols. Serum was assayed for antioxidant enzyme activities and lipid peroxidation;

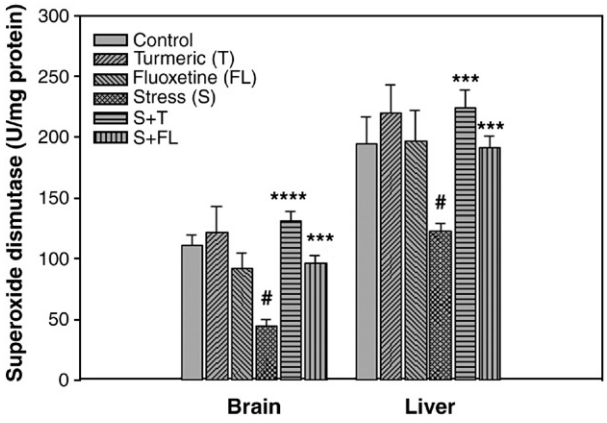

Fig. 1. Effect of chronic fluoxetine administration on superoxide dismutase activity in brain and liver. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.01$ in brain and $P<0.05$ in liver, compared with the control group by ANOVA.

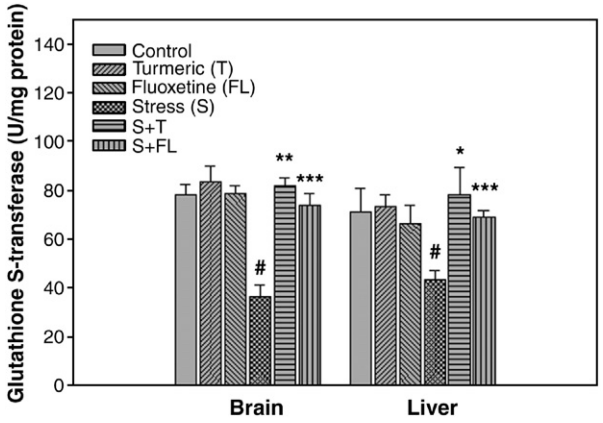

Fig. 3. Effect of chronic fluoxetine administration on glutathione *S*-transferase activity in brain and liver. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.01$ in brain and $P<0.05$ in liver, compared with the control group by ANOVA.

non-enzymatic circulatory antioxidants glucose and uric acid were also determined using commercial kits. (*S*)-lactate:NAD^+ oxidoreductase activity [lactate dehydrogenase: LDH; EC 1.1.1.27] was evaluated as an oxidative stress marker and index of membrane integrity (Wroblewski and La Due, 1955). Protein concentration was estimated using BSA as standard (Lowry et al., 1951).

2.7. Statistical evaluation

Data was expressed as mean±S.E.M. of six values and analyzed by one-way ANOVA. Differences among controls and treatment groups were determined using Student's *t* test. *P* values less than 0.05 were considered statistically significant. All comparisons were made with non-stressed, untreated controls. Stress+T/FL (groups V and VI) were also compared with stress alone treatment (group IV).

3. Results

3.1. Effect of fluoxetine on restraint stress-induced decline in antioxidant enzyme activities

Figs. 1–4 depict levels of superoxide dismutase, catalase, glutathione *S*-transferase and glutathione reductase, respectively, in brain and liver tissues of experimental animals, while Table 1 summarizes the circulating levels of these enzymes, which reflect *in vivo* cellular antioxidant status.

From the results depicted, stress was found to elicit a significant decrease in the levels of all antioxidant enzymes in

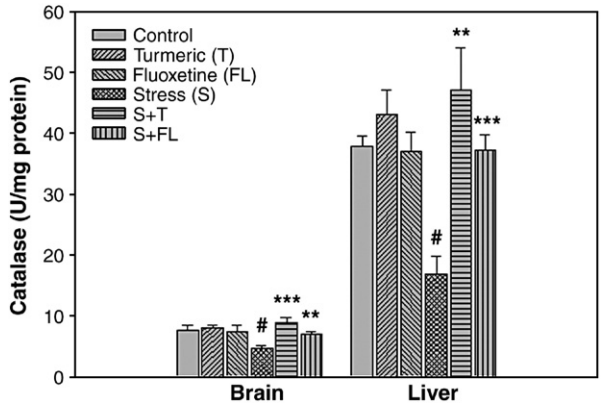

Fig. 2. Effect of chronic fluoxetine administration on catalase activity in brain and liver. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.01$ in brain and liver, compared with the control group by ANOVA.

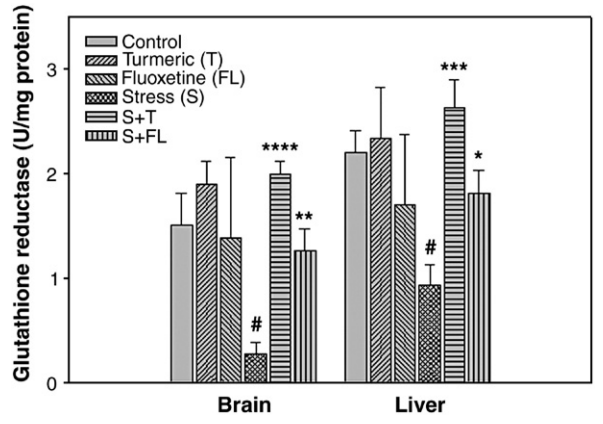

Fig. 4. Effect of chronic fluoxetine administration on glutathione reductase activity in brain and liver. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.02$ in brain and liver, compared with the control group by ANOVA.

Table 1
Modulatory effects of chronic fluoxetine treatment on restraint stress-induced alterations in circulating levels of antioxidant defenses and oxidative stress markers

Parameter	Groups					
	Control	Turmeric (T)	Fluoxetine (FL)	Stress (S)	S+T	S+FL
Superoxide dismutase (U/mg protein)	22.80±0.23	23.34±4.29	19.74±4.03	$13.83^{\#c}$±1.87	23.89^{*b}±1.53	20.79^{*a}±0.85
Catalase (U/mg protein)	1.52±0.14	1.74±0.57	1.12±0.33	$0.59^{\#a}$±0.26	2.03^{*a}±0.40	1.27^{*a}±0.15
Glutathione *S*-transferase (U/mg protein)	0.30±0.06	0.41±0.10	0.26±0.06	$0.12^{\#a}$±0.02	0.39^{*d}±0.02	0.24^{*a}±0.03
MDA (nmol/mg protein)	0.090±0.015	0.052±0.008	0.070±0.006	$0.17^{\#a}$±0.016	0.084^{*c}±0.017	0.096^{*a}±0.017
Glucose (mg/dl)	110.83±4.99	109.89±10.36	115.00±12.93	$78.83^{\#c}$±4.23	118.50^{*c}±7.72	108.5^{*c}±6.77
Uric acid (mg/dl)	3.76±0.39	4.73±0.55	3.17±0.53	$1.31^{\#c}$±0.17	4.13^{*c}±0.48	3.46^{*d}±0.20

Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Results are expressed as mean±S.E.M. obtained from six observations.
a, b, c, d: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$; #: compared with the control group; *: compared with stress alone by ANOVA.

comparison to controls (superoxide dismutase: $P<0.01$ in brain, <0.05 in liver, <0.01 in serum; catalase: $P<0.01$ in brain, <0.01 in liver, <0.05 in serum; glutathione *S*-transferase: $P<0.01$ in brain, <0.05 in liver, <0.05 in serum; glutathione reductase: $P<0.02$ in brain, <0.02 in liver). However, compared to stressed animals, a significant repletion in enzymatic antioxidant status was observed by chronic fluoxetine administration following the stress paradigm (superoxide dismutase: $P<0.01$ in brain, <0.01 in liver, <0.05 in serum; catalase: $P<0.02$ in brain, <0.01 in liver, <0.05 in serum; glutathione *S*-transferase: $P<0.01$ in brain, <0.01 in liver, <0.05 in serum; glutathione reductase: $P<0.02$ in brain, <0.05 in liver). The used reference antioxidant turmeric also produced comparable augmentation in enzyme activities (superoxide dismutase: $P<0.001$ in brain, <0.01 in liver, <0.02 in serum; catalase: $P<0.01$ in brain, <0.02 in liver, <0.05 in serum; glutathione *S*-transferase: $P<0.02$ in brain, <0.05 in liver, <0.001 in serum; glutathione reductase: $P<0.001$ in brain, <0.01 in liver). In non-stressed animals, neither fluoxetine nor turmeric caused any significant alteration of basal antioxidant enzyme activities.

3.2. Effect of fluoxetine on restraint stress-induced decline in non-enzymatic antioxidant levels

Fig. 5 shows the modulation of GSH by stress and drug intervention. In stressed animals, there was a significant reduction in the GSH content from controls ($P<0.001$ in brain, <0.01 in liver). Following exposure to stress, treatment with fluoxetine significantly restored the basal levels of GSH ($P<0.02$ in brain, <0.01 in liver). Turmeric induced a similar increase in comparison to stressed animals ($P<0.001$ in brain, <0.01 in liver).

Circulating levels of glucose (Table 1) were significantly decreased following restraint stress ($P<0.01$). Both fluoxetine and turmeric caused significant reversal of the stress-induced decline in serum glucose concentration ($P<0.01$) toward control values.

Restraint stress also caused a significant decline in serum uric acid (Table 1: $P<0.01$). In comparison to animals exposed to stress alone, treatment with fluoxetine was effective in significantly preventing stress-induced decrease of uric acid ($P<0.001$). Turmeric extract had the same protective effect ($P<0.01$).

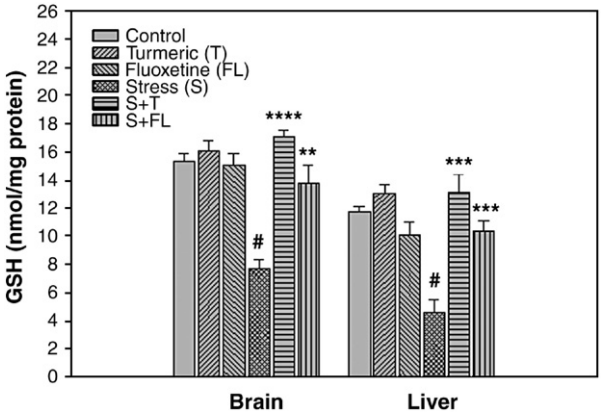

Fig. 5. Effect of chronic fluoxetine administration on GSH levels in brain and liver. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.001$ in brain and $P<0.01$ in liver, compared with the control group by ANOVA.

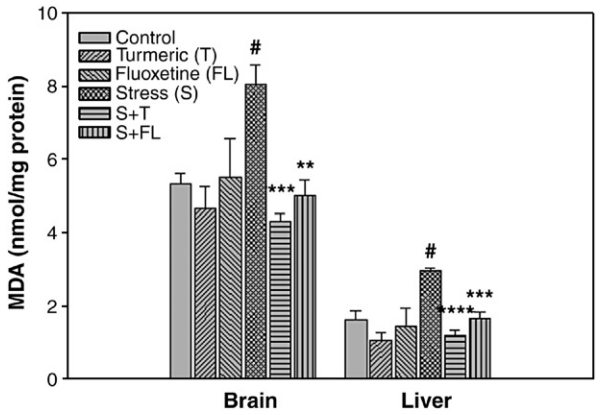

Fig. 6. Inhibition of lipid peroxidation in brain and liver by chronic fluoxetine administration. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.05$ in brain and $P<0.01$ in liver, compared with the control group by ANOVA.

Both fluoxetine and turmeric alone treatments to non-stressed animals did not alter significantly any of the assessed non-enzymatic antioxidants.

3.3. Effect of fluoxetine on other oxidative stress markers

3.3.1. Lipid peroxidation

The previously described depletion of antioxidant defenses in brain, liver and circulation induced by restraint stress was paralleled by an increase in lipid peroxidation, as demonstrated (Fig. 6) by the accumulation of the aldehydic product of lipid peroxidation, malondialdehyde (MDA) in stressed animals ($P<0.05$ in brain, <0.01 in liver, <0.05 in serum). Pharmacological intervention by daily dosing of fluoxetine significantly prevented the accumulation of MDA after stress ($P<0.02$ in brain, <0.01 in liver, <0.05 in serum). Treatment with the positive control, turmeric extract, also caused a similar decrease in comparison to stress ($P<0.01$ in brain, <0.001 in liver, <0.01 in serum). In comparison to controls, fluoxetine alone and turmeric alone treatments did not cause any significant change in lipid peroxidation.

3.3.2. Lactate dehydrogenase activity

Due to its intracellular location, lactate dehydrogenase activity was evaluated as an index of membrane damage exerted by free radical production in response to stress. As depicted in Fig. 7, restraint stress resulted in a significant elevation in lactate dehydrogenase ($P<0.02$). A significant reversion of lactate dehydrogenase levels to control values was caused by fluoxetine ($P<0.02$) as well as turmeric extract ($P<0.02$) following stress, in comparison to the effect of stress alone, while both these drugs when given alone did not alter the enzyme activity from controls.

3.3.3. Protein carbonyl production

Oxidative stress is known to introduce carbonyl groups into the amino acid side chains of proteins (Levine et al., 1990).

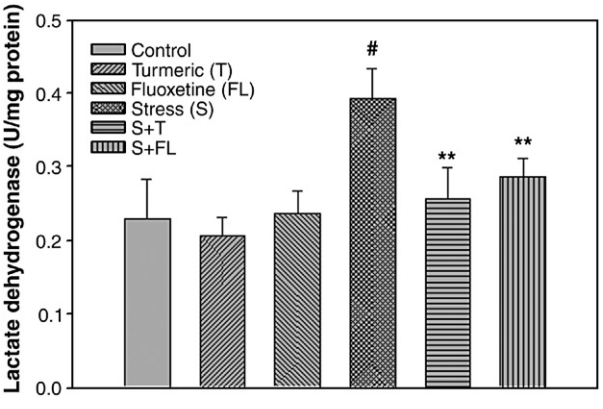

Fig. 7. Effect of chronic fluoxetine administration on lactate dehydrogenase activity. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.02$, compared with the control group by ANOVA.

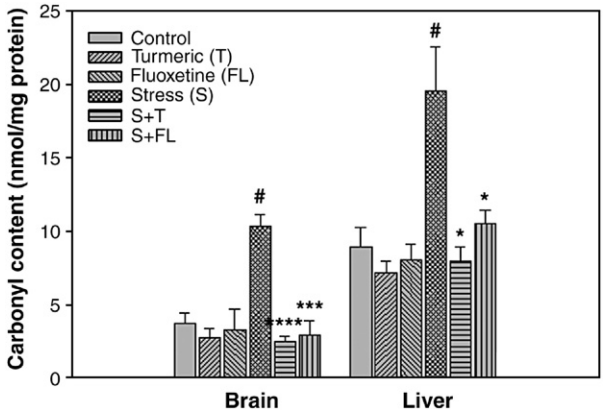

Fig. 8. Inhibition of restraint stress-induced protein carbonyl production in brain and liver by chronic fluoxetine administration. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Values shown for each group are the mean±S.E.M. obtained from six observations. *, **, ***, ****: $P<0.05$, $P<0.02$, $P<0.01$, $P<0.001$ compared with stress alone by ANOVA; #: $P<0.01$ in brain and $P<0.05$ in liver, compared with the control group by ANOVA.

Restraint stress elicited a significant increase of carbonyl groups compared to controls (Fig. 8; $P<0.01$ in brain, <0.05 in liver). A significant effect on the inhibition of protein oxidation due to stress was exerted by fluoxetine ($P<0.01$ in brain, <0.05 in liver). Turmeric extract yielded similar results ($P<0.001$ in brain, <0.05 in liver). Administration of both drugs alone did not change carbonyl content from controls.

3.4. Effect of fluoxetine on restraint stress-induced decline in body weight

Fig. 9 depicts influence of drug treatment on body weights of animals measured every 7 days beginning from the day of experimental manipulations. Restraint stress induced a decrease

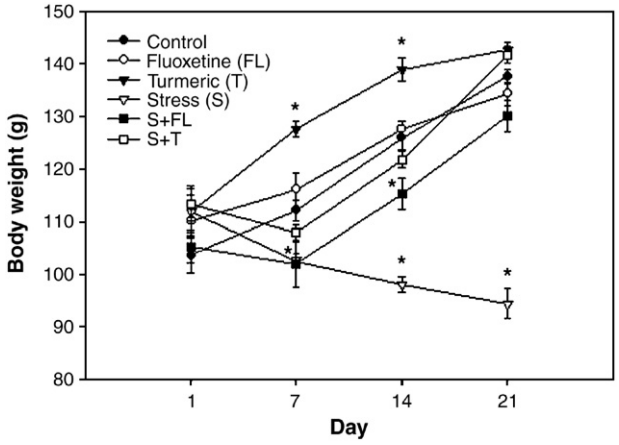

Fig. 9. Influence of chronic fluoxetine administration on weight gain of rats. Animals in treatment groups received turmeric extract (T) or fluoxetine (FL) p.o. for 21 consecutive days, either alone or following 4 h of daily restraint stress (S+T, S+FL). Body weight was measured every 7 days. Values shown for each group are the mean<0.05) in body weight compared with the control group by ANOVA.

in body weight evident from the first week onward and remained significantly decreased throughout exposure to the stress paradigm (day 7: $P<0.02$; day 14: <0.001; day 21: <0.001). Following exposure to stress, treatment with both fluoxetine and turmeric was effective in preventing restraint stress-induced losses in body weight, which was particularly significant ($P<0.05$) on day 14 for fluoxetine. Similar to controls, both drug alone treatments markedly increased weight gain over the treatment period of 21 days, with significant increases observed by turmeric alone on day 7 ($P<0.01$) and 14 ($P<0.02$).

4. Discussion

To the best of our knowledge, the data presented in this study demonstrates for the first time the *in vivo* antioxidant action of fluoxetine in restraint stress-treated rats. Similar evidence corroborating this finding comes from a clinical study (Bilici et al., 2001) which showed that antioxidant enzymes may be state markers of depression and useful in monitoring SSRI effects, as well as from certain *in vitro* studies (Kolla et al., 2005).

Reactive oxygen species generated by a severe stressor (restraint stress) significantly compromises the *in vivo* antioxidant defenses of animals submitted to restraint. Chronic fluoxetine administration to stressed animals for 21 days, which is the time course of therapeutic action of the antidepressant, produced a protective enhancement of the antioxidant status. This modulation of antioxidant parameters to basal levels (as in controls) was strikingly at par with the powerful antioxidant efficacy of turmeric, a natural antioxidant and indigenous food component implicated for the significantly lower prevalence of certain neurodegenerative diseases in India (Ganguli et al., 2000). Thus our results are also significant from the point of view of neuroprotection.

Exposure to chronic restraint stress in rats, and psychosocial stress in humans, is implicated in the pathophysiology of mood and anxiety disorders (Walesiuk et al., 2006). Previous work in our laboratory has established that restraint stress causes robust increases in the production of reactive oxygen species, and consequent oxidative damage, with a concomitant decline in *in vivo* antioxidant defenses (Zaidi and Banu, 2004; Zaidi et al., 2005). Oxidative stress induces many damaging processes in stress disorders such as mitochondrial dysfunction, dysregulation of calcium homeostasis (Amoroso et al., 2000), disruption of energy pathways (Papadopoulos et al., 1997), damage to neuronal precursors, impairment of neurogenesis (Kroemer, 1997), induction of signalling events in apoptotic cell death (Cregan et al., 2002), ultimately leading to atrophy and morphological changes in the brain characteristic in stress-induced depression (Bremner, 1999; Sapolsky, 2000). Moreover, immobilization stress in mice has been shown to cause neuronal death in the cerebral cortex by apoptosis, which was effectively prevented by antioxidant pretreatment with an associated decrease in reactive oxygen species production (Lee et al., 2006). Recent *in vitro* studies on the underlying mechanisms of stress-induced neuronal damage have demonstrated that corticosterone released from the adrenal cortex during stress either induces the formation of reactive oxygen species (Lin et al., 2004) or decreases antioxidant enzyme activity, resulting in increased neurotoxicity in cortical cultures (Brooke et al., 2002). Other *in vitro* studies have also shown that upregulation of superoxide dismutase and similar antioxidant enzymes may protect astrocytes from apoptosis or necrosis upon exposure to a range of neurotoxicants (Kolla et al., 2005). Superoxide dismutase is extensively distributed in the central nervous system, including regions purported to be atrophied in depression, such as the hippocampus (Jeste et al., 1988). Thus, if episodes of clinical depression are accompanied by progressive hippocampal atrophy throughout the duration of the disease, antidepressant therapy or other forms of treatment that upregulate superoxide dismutase1 gene expression may prevent worsening of affective symptoms that are either directly or indirectly related to hippocampal degeneration (Li et al., 2000). In this context, the use of pharmacological agents targetting cellular antioxidants is a promising strategy for protecting against oxidative insults in depression. Accordingly, our study was undertaken to clarify and characterize the modulation of restraint stress-induced oxidative damage by fluoxetine. The brain is a target of stress along with the metabolic systems of the body, of which the liver is the central organ (McEwen, 2000). Hence, our antioxidant investigations were made on these two tissues.

A simultaneous decline in the activities of free-radical scavenging enzymes, superoxide dismutase and catalase, as evidenced by the data obtained in this study following chronic restraint stress, may be due to inactivation caused by excess reactive oxygen species production. This damages the first line of enzymatic defense against superoxide anion and hydrogen-peroxide. Consistent with our experimental findings, clinical studies on patients with affective disorders have also revealed lower levels of superoxide dismutase (Bilici et al., 2001) and catalase (Ozcan et al., 2004). Moreover, we also observed a significant depletion of GSH, glutathione *S*-transferase and glutathione reductase in brain and liver of restraint-stressed rats, indicating damage to the second line of antioxidant defense. This probably further exacerbates oxidative damage by adversely affecting critical GSH-related processes such as free-radical scavenging, detoxification of electrophilic compounds, modulation of cellular redox status and thiol-disulphide status of proteins, and regulation of cell signalling and repair pathways. A similar depletion of brain glutathione has been reported earlier in mice under stress-induced depression (Pal and Dandiya, 1994). Therefore, a coordinate decline in GSH and related enzymes by restraint stress suggests a severely compromised *in vivo* antioxidant status. This oxidative route of damage may act as a potential trigger to a plethora of damaging events observed in stress and depression. In our findings, we show that fluoxetine could effectively limit/reverse the above-indicated stress-induced antioxidant deficits in brain and liver tissues as well as in circulation in all considered parameters. The simultaneous elevation of key endogenous antioxidants, superoxide dismutase, catalase, glutathione *S*-transferase, glutathione reductase and GSH levels by fluoxetine treatment may thus largely contribute to the increased resistance of chronically stressed rats to

in vivo oxidative damage, in comparison to stressed animals receiving no treatment. By restoring the activity of glutathione reductase, fluoxetine may act to increase cellular levels of GSH, the predominant thiol antioxidant in the brain, which is controlled in part by glutathione reductase. Glucose and uric acid, among the major circulating antioxidant molecules, were also severely depleted by chronic stress. Glucose is a scavenger of hydroxyl radicals, having a rate constant comparable with mannitol, while the antioxidant role of uric acid is important in directly scavenging oxidizing species and thus also inhibiting lipid peroxidation (Halliwell and Gutteridge, 1990). Therefore, fluoxetine-induced elevation in their levels demonstrates a direct ability to protect against highly damaging hydroxyl radicals that react with and damage most cellular targets including lipids, proteins and DNA (Warner et al., 2004). This may be another method by which fluoxetine prevents stress-induced oxidative damage. Since increased oxidative stress or impaired antioxidant activity are implicated in major depression (Tsuboi et al., 2006), the therapeutic efficacy demonstrated clinically by fluoxetine may be mediated, at least in part, *via* reversal of oxidative damage as evidenced by our data.

Lipid peroxidation is considered a critical mechanism of injury occurring in cells during oxidative stress (Halliwell and Gutteridge, 1989). An initial formation of large amounts of reactive oxygen species during stress may also initiate lipid peroxidation as demonstrated to occur in brain (Liu et al., 1996) and liver (Hu et al., 2000), in agreement to our present findings. Psychological stress, which accompanies severe depression, may enhance lipid peroxidation (Hibbeln and Salem, 1995) and recent clinical studies have directly demonstrated higher levels of MDA in patients with affective disorders (Ozcan et al., 2004). Our study showed that treatment with fluoxetine effectively prevented membrane lipid peroxidation. Since lipid peroxidation disrupts membrane integrity, high levels of lactate dehydrogenase activity in stressed animals may be interpreted as a progression of cell injury because of its intracellular localization (Campo et al., 2005). The decrease in lactate dehydrogenase after treatment with fluoxetine may be a consequence of decreased oxidative stress and the concomitant prevention of cell membrane damage. Protein oxidation, measured as an increase in carbonyl groups has been shown to be an early event in oxidative stress (Pacifici and Davies, 1990). Treatment with fluoxetine inhibited protein carbonyl production in stressed animals, indicating that fluoxetine not only prevents the downstream cascade of oxidative damage comprising lipid peroxidation, but also targets early oxidative events as evidenced by a significant reduction in protein oxidation.

The molecular mechanisms underlying the fluoxetine-mediated elevation of antioxidant defenses, and concomitant reduction of oxidative stress markers, remain to be investigated. It has been suggested that the *in vitro* neuroprotective actions of some antidepressants include the upregulation of superoxide dismutase activity, with superoxide dismutase1 gene expression as a potential target of antidepressant regulation (Li et al., 2000; Kolla et al., 2005). Several antidepressants with different mechanisms of action were demonstrated to increase superoxide dismutase1 gene expression, for e.g., amitryptyline (a classic tricyclic antidepressant), bupropion (a second generation antidepressant), doxepin (a norepinephrine reuptake inhibitor), venlafaxine (a new 5HT/norepinephrine reuptake inhibitor) and L-deprenyl (a selective MAO-B inhibitor). Thus, although a common mechanism of action of antidepressants has eluded researchers for years, and since antidepressants act on many different neurotransmitter systems and receptors, it is proposed that one of the shared mechanisms of action of antidepressants is the upregulation of antioxidant enzymes such as superoxide dismutase1 (Li et al., 1998; Li et al., 2000). Our study provides evidence for this effect *in vivo* for the first time in terms of enhanced activity, not only of superoxide dismutase, but also of other key antioxidant enzymes, by fluoxetine (a selective serotonin reuptake inhibitor) following stress exposure. Chronic antidepressant treatment has been demonstrated to upregulate cAMP-response element-mediated gene expression in rat cortex and hippocampus (Thome et al., 2000), and to upregulate the expression of cAMP-response element-binding protein (CREB) in both rodents and humans. Upregulation of superoxide dismutase1 may occur by an induction of cAMP and CREB (Nibuya et al., 1996; Dowlatshahi et al., 1998). Regarding other antioxidant enzymes, it may be suggested that the enhanced activity demonstrated by us, may be through a mechanism similar to that for superoxide dismutase described above. Thus, the increased activities of not only superoxide dismutase, but also catalase and glutathione reductase, which act in concert with superoxide dismutase, by fluoxetine treatment in our study, may be *via* a genomic action leading to enhanced gene expression of these critical antioxidant enzymes. Thus, our study not only confirms, but also extends previous findings, implicating the importance of antioxidant status in stress disorders and the consequent need to evaluate antidepressants in terms of their antioxidant/prooxidant ability. Although depression is currently considered a heterogeneous disease (Kolla et al., 2005), stress has been implicated in the origin and exacerbation of this disease. Thus, in patients whose depression is caused, or accompanied by stress, therapeutic strategies aimed at maintaining or increasing levels of neuroprotective enzymes such as superoxide dismutase may prove extremely beneficial (Kolla et al., 2005). In addition, it is possible that upregulation of this enzyme by antidepressants may prevent further free radical-mediated neurotoxicity in depression caused by stress (Li et al., 2000). Therefore, the data presented here indicating the elevation of not only superoxide dismutase, but a wide range of key components of the antioxidant defense system by fluoxetine may hold great potential in preventing further clinical deterioration in depression.

Another major finding of our study is that in all antioxidant parameters evaluated, the protective efficacy of the antidepressant fluoxetine was strikingly comparable to that observed for the powerful natural antioxidant turmeric (Sreejayan Rao, 1994), used as a reference standard. Turmeric powder by itself, and its major bioactive component curcumin, is known to protect against oxidative stress (Cohly et al., 1998) by typical radical-trapping ability as a chain-breaking antioxidant (Sreejayan Rao, 1994). Turmeric also contains other antioxidant principles besides curcumin, such as demethoxycurcumin, bisdemothoxycurcumin

and a number of polypeptides with antioxidant activity, such as turmerin (Ramirez-Tortosa et al., 1999). Dietary turmeric is known to lower lipid peroxidation by enhancing the activities of antioxidant enzymes (Pulla Reddy and Lokesh, 1994). The similar antioxidant efficacies of turmeric and fluoxetine demonstrated in our study may suggest a common mechanism of action. With the view that antioxidant defenses are also compromised in stress-induced depression, it may be hypothesized that classical stress drugs such as fluoxetine which can reduce stress symptomatology, may probably also affect and act *via* modulation of endogenous antioxidant capacity. Since endogenous antioxidants play a critical role in maintaining the integrity of the cell, their enhancement by fluoxetine with an efficacy similar to turmeric probably also accounts for the low side effects of fluoxetine observed clinically (Anderson, 1999).

In conclusion, the present study indicates that the antioxidant potential of fluoxetine probably contributes to its therapeutic actions. As treatment with fluoxetine ameliorates stress-induced oxidative damage, our study thus demonstrates that enhancement of *in vivo* antioxidant defenses and improvement in cellular antioxidant status may be an important mechanism underlying the protective pharmacological effects of fluoxetine observed clinically in the treatment of various stress disorders.

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