Curcumin ameliorates reserpine-induced pain–depression dyad: Behavioural, biochemical, neurochemical and molecular evidences

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AlloDynia; Biogenic amines; Pain–depression dyad; Substance P

Summary An apparent clinical relationship between pain and depression has long been recognized. Depression and pain are often diagnosed in the same patients. The emerging concept for pain–depression pathogenesis is the dysfunction of biogenic amine-mediated pain–depression control and the possible involvement of nitroductive stress-induced neurogenic inflammation. The present study was designed to investigate the effect of curcumin on reserpine-induced pain–depression dyad in rats. Administration of reserpine (1 mg/kg subcutaneous daily for three consecutive days) led to a significant decrease in nociceptive threshold as evident from reduced paw withdrawal threshold in Randall Sellitto and von-Frey hair test as well as significant increase in immobility time in forced swim test. This behavioural deficit was integrated with decrease in the biogenic amine (dopamine, norepinephrine and serotonin) levels along with increased substance P concentration, nitroductive stress, inflammatory cytokines, NF-κβ and caspase-3 levels in different brain regions (cortex and hippocampus) of the reserpinised rats. Curcumin (100, 200, 300 mg/kg; ip) dose dependently ameliorated the behavioural deficits associated with pain and depression by restoring behavioural, biochemical, neurochemical and molecular alterations against reserpine-induced pain–depression dyad in rats.

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1. Introduction

Several epidemiological studies demonstrate that pain and depression frequently co-exist in up to 70% of chronic pain cases (Bair et al., 2003; Arnow et al., 2006). Depression has been shown to result in decreased pain threshold and increased analgesic requirement (Jackson and Onge, 2003). It is estimated that the occurrence of depression in patients with chronic pain is higher, ranging from 30% to 54%, than that (about 17%) in the general population (Ferrer-Garcia et al., 2006). In a World Health Organization Collaborative Study of Psychological Disorders in Primary Care, International Classification of Diseases-10, persistent somatoform pain disorder...
was found in 32.4% of patients with depression and in 8.6% of primary care patients without depression (Sartorius et al., 1993). Recently, Miller and Cano (2009) reported that prevalence of chronic pain due to any cause was 21.9% and approximately 35% of participants with chronic pain also had comorbid depression. This relationship between pain and depression gave rise to the theories that depression might increase pain perception or that depression is a common consequence of pain symptoms (Fishbain et al., 1997; Landi et al., 2005). This complex interaction is often labeled as Depression—Pain syndrome or Pain—Depression dyad (Lindsay and Wyckoff, 1981; Bair et al., 2004; Goldbergen, 2010), and implying that the conditions often coexist and respond to similar treatments, exacerbate one another, and share common biological pathways. Although this intricate relationship between pain and depression has attracted increasing attention in all areas of research, but the mechanisms underlying the association of depression and pain are however not clear (Dersh et al., 2002).

A combination of interactions between neurotransmitters (Russell et al., 1992; Elhwuegi, 2004), neuropeptides (Malmberg and Yaksh, 1992; Kramer et al., 1998) nitrooxidative stress (Bagis et al., 2005; Maes et al., 2010) and cytokines (Wallace, 2006; Dowlati et al., 2010) are thought to take part in pathogenesis of pain—depression dyad.

The biochemical theory of depression—pain dyad posits a neurochemical imbalance or a functional deficiency of key neurotransmitters, the monoamines: serotonin, norepinephrine, and dopamine (Fishbain et al., 1997; Stahl, 2002; Campbell et al., 2003). As with major depressive disorder (MDD) patients, studies in patients with chronic pain have consistently found lower serotonin levels and/or reduced reuptake and lower plasma and cerebrospinal fluid tryptophan levels compared to controls (Moldofsky, 1982; Russell et al., 1992). Furthermore, both conditions involve increased plasma and cerebrospinal fluid substance P concentrations. Similarly, as in MDD, elevated concentrations of substance P in cerebrospinal fluid have been found in patients with chronic pain (Larson et al., 2000) and substance P is also thought to play a role in the development and treatment of MDD (Blier et al., 2004). Neurotransmission of substance P is negatively modulated by efferent serotonergic neurons (Naranjo et al., 1989). It has also been observed that increased levels of substance P in brain increases 5-HT levels in spinal cord, while 5-HT decreases the release of substance P into the spinal cord (Moldofsky, 1982). Finally, since substance P is involved in pain control as well as depression, there is a need to investigate the role of substance P along with the biogenic amines, particularly because empirical data suggest that substance P and biogenic amines are involved in both the etiopathogenesis and treatment response of pain and depression (Blier et al., 2004; Staud and Spaeth, 2008).

The second facet of the pain—depression dyad is the involvement of nitrooxidative stress-induced neurogenic inflammation. Human studies have reported a number of oxidative disturbances in patients with major depression suggested by the elevated lipid peroxidation products (Bilici et al., 2001; Khanzode et al., 2003; Sarandol et al., 2007), findings of altered antioxidant enzyme with reduced levels of superoxide dismutase (Herken et al., 2007). Moreover, a significant positive correlation was found between oxidative stress index and the Hamilton depression rating scale (Yanik et al., 2004). Similarly higher serum levels of pentosidine and malondialdehyde along with reduced serum superoxide dismutase (a marker of antioxidant capacity) were found in patients with chronic pain compared with normal controls (Hein and Franke, 2002; Bagis et al., 2005). Cordero et al. (2009) found higher levels of ROS production in mononuclear cells from fibromyalgia patients again suggesting enhanced oxidative stress. This enhanced oxidative stress can stimulate the production of NF-kB and can lead to increase in the levels of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-8, IFN-γ (Pall, 2007). Depressed patients and patients with pain disorders often display enhanced cytokine levels including interleukin-6 (IL-6), C-reactive protein, interleukin-1-beta (IL-1β), and tumor necrosis factor alpha (TNF-α) (Raison et al., 2006; Omoigui, 2007; Dowlati et al., 2010) Recently, Kadetoff et al. (2009) found higher mRNA levels of TNF-α in patients of fibromyalgia. Thus, enhanced ROS along with generation of pro-inflammatory cytokines (TNF-α and IL-1β) may modulate NF-kB signaling and caspase-3 pathway which may be responsible for the development and perpetuation of pain in depression or depression in pain (Joseph and Levine, 2004).

Thus, an animal model of pain depression dyad ideally should include widespread pain as well as the depression. Previously published study from our lab (Kulkarni and Robert, 1982) and recently published reserpine-mediated animal model of fibromyalgia implicates reserpine-induced dysfunction of biogenic amines in mediation of CNS pain control (Nagakura et al., 2009). Reserpine is a monoamine depletor that exerts a blockade on the vesicular monoamine transporter for neuronal transmission or storage, promoting dopamine-autodestruction and oxidative catabolism by monoamine oxidase may result in oxidative stress (Lohr et al., 2003). This dual action of reserpine (monoamine depletion and oxidative stress) makes it a prominent to address both facets involved in the pathophysiology of the pain depression dyad.

Curcumin, a polyphenol found in turmeric, is a yellow curry spice with a long history of use in traditional Indian diets and herbal medicine. Curcumin (dicaffeoyl methane) has many pharmacological activities including replenishment of the monoamines (Xu et al., 2005) anti-inflammatory properties (Jain et al., 2009) and powerful antioxidant activity, studies have shown that curcumin is a powerful scavenger of the superoxide anion, the hydroxyl radical, and nitrogen dioxide (Unnikrishnan and Rao, 1995). Curcumin exerts anti-inflammatory and growth inhibitory effects in TNF-α treated HaCaT cells through inhibition of NF-κB and mitogen activated protein kinase pathways (Cho et al., 2007). Curcumin is also known to exhibit anti-hyperalgesic (Sharma et al., 2006) and antidepressant effects in wide variety of animal models (Xu et al., 2005).

Thus, the aim of the present study was two-fold, first to investigate the protective effects of curcumin on the reserpine-induced pain, tactile allodynia and accompanied depression in rats and second to investigate the protective potential of curcumin against the reserpine induced biogenic amine depletion and nitrooxidative stress mediated inflammatory cascade and apoptotic signalling pathway.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (200—220 g) bred in Central Animal House facility of Panjab University were used. The animals
were housed under standard laboratory conditions, maintained on a 12:12 h light: dark cycle and had free access to food (Ashirwad Industries, Mohali, India) and water. Animals were acclimatized to laboratory conditions before the tests. All experiments were carried out between 09:00 and 17:00 h. The experimental protocols were approved by the Institutional Animal Ethics Committee of Panjab University and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals, Government of India on animal experimentation.

2.2. Drugs

Reserpine and curcumin were purchased from Sigma (St. Louis, MO, USA). TNF-α, IL-1β, substance P ELISA kit was purchased from R&D Systems (USA). While NF-κB and caspase-3 ELISA kits were procured from Imegenex, San Diego, USA and Biovision, USA respectively. All other chemicals used for biochemical estimations were of analytical grade.

2.3. Experimental design

Pain and depression were induced by administration of reserpine (1 mg/kg subcutaneous daily) for three consecutive days (Nagakura et al., 2009). The animals were randomly divided into six experimental groups with 8 animals in each. Group I comprised control animals receiving 1 ml/kg vehicle subcutaneously; Group II animals were administered reserpine (1 mg/kg; subcutaneously) for three consecutive days (i.e. day 1, 2 and 3); Group III, IV, V consisted of reserpinised rats receiving curcumin (100, 200 and 300 mg/kg; ip) for two days after reserpine (i.e. day 4 and 5); Group VI consisted of control animals receiving curcumin (300 mg/kg; intraperitoneally) (Fig. 1). Dose of curcumin was selected on the basis of previous studies stating CNS effects of curcumin (Dohare et al., 2008; Mehta et al., 2010) and from the study done by Mittal et al. (2009) who shows the antinociceptive effect of curcumin at the selected dose range. Hyperalgesia (thermal and mechanical) and allodynia were assessed 48 h after the last reserpine injection. After behavioural assessment, rats were sacrificed under deep anaesthesia and different brain regions were isolated and stored at −80 °C for biochemical estimations. Reserpine was dissolved in glacial acetic acid, diluted to a final concentration of 0.5% acetic acid with distilled water. Curcumin was injected intraperitoneally as absorption seems to be higher by this route than after oral administration, as gavage results in very low levels into the blood. Curcumin was prepared by using a conventional pharmacologically acceptable carrier using a mixture of 1% sodium carboxy methylcellulose and Tween 80 as these agents possess absorption enhancing capacity in formulations. The animals were sacrificed under deep anaesthesia on the fifth day immediately after behavioural assessment and brain samples were rapidly removed and placed on dry ice for isolation of cerebral cortex and hippocampus. Brain samples were incubated with 1 ml of ice cold 1× hypotonic buffer supplemented with 1 mM dithiothreitol and 1% detergent solution for 30 min on ice. After incubation, the samples were centrifuged for 10 min at 10,000 rpm at 4 °C. The supernatant (Cytoplasmic Fraction) was transferred into a separate tube and stored at 4 °C. The nuclear pellet was re-suspended in 100 μl nuclear lysis buffer by pipetting up and down. The samples were vortexed vigorously and suspension was incubated at 4 °C for 30 min. The suspension was vortexed again for 30 s and centrifuged at 14,000 rpm for 10 min at 4 °C in a microcentrifuge. The supernatant was transferred (Nuclear Fraction) into a pre-chilled microcentrifuge tube. The cytoplasmic fractions were separated from the brain homogenate for the biochemical estimations and for quantification of TNF-α, IL-1β and caspase-3 while nuclear fraction was used only for estimation of NF-κB levels.

2.4. Behavioural tests

Thermal hyperalgesia was assessed in a water bath maintained at 42 °C (a temperature that is normally innocuous in naive rats until tail withdrawal or signs of struggle were observed (cut-off time: 15 s) (Chopra et al., 2010). Mechanical

![Figure 1](image-url)  
**Figure 1**  Pain and depression was induced by administration of reserpine (1 mg/kg subcutaneous daily) for three consecutive days followed by intraperitoneal administration of curcumin 100, 200 and 300 mg/kg for two days after the last reserpine injection and all behaviour experiments were conducted 30 min after curcumin administration. Brain samples were harvested immediately after the measurement of the behaviour paradigms and the samples were stored at −80 °C for biochemical estimations on next day.
hyperalgesia: the nociceptive flexion reflex was quantified using the Randall Selitto paw pressure device (IIITC, Woodland Hills, USA), which applies a linearly increasing mechanical force (in g) to the dorsum of the rat's hindpaw (Chopra et al., 2010). Mechanical allodynia: rats were placed individually on an elevated mesh (1 cm² perforations) in a clear plastic cage and adapted to the testing environment for at least 15 min. von-Frey hairs (IIITC, Woodland Hills, USA) with calibrated bending forces (in g) of different intensities were used to deliver punctuated mechanical stimuli of varying intensity. Starting with the lowest filament force, von-Frey hairs were applied from below the mesh floor to the plantar surface of the hindpaw, with sufficient force to cause slight bending against the paw, and held for 1 s (Chopra et al., 2010). Each stimulation was applied 5 times with an inter-stimulus interval of 4–5 s. Care was taken to stimulate random locations on the plantar surface. A positive response was noted if the paw was robustly and immediately withdrawn. Paw-withdrawal threshold was defined as the minimum pressure required to elicit a withdrawal reflex of the paw, at least one time on the five trials. Voluntary movement associated with locomotion was not considered as a withdrawal response. Mechanical allodynia was defined as a significant decrease in withdrawal thresholds to von-Frey hair application. All the pain measurement studies were done by the experimenter who was blind to the drug treatment.

Immobility period: the forced swim test was performed based on the original method described by Porsolt et al. (1977). One day prior to the test, a rat was placed for conditioning in a clear plastic tank (45 cm × 35 cm × 60 cm) containing 30 cm of water (24 ± 0.5 °C) for 15 min (pre-test session). Twenty-four hours later (test session), the total duration of immobility within a 5-min session was recorded as immobility scores (in s). A rat was judged to be immobile when its hind legs were no longer moving and the rat was hunched forward (a floating position). The immobility time was recorded manually by an observer who was blind to the drug treatment.

2.5. Biochemical estimations

2.5.1. Estimation of lipid peroxidation
The malondialdehyde content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances by the method of Wills (1965). Briefly, 0.5 ml of cytosolic fraction of both brain regions and 0.5 ml of Tris–HCl were incubated at 37 °C for 2 h. After incubation 1 ml of 10% trichloroacetic acid was added and centrifuged at 1000 × g for 10 min. Then 1 ml of 0.67% thiobarbituric acid was added to 1 ml of supernatant and the tubes were kept in boiling water for 10 min. After cooling, 1 ml double-distilled water was added and absorbance was measured at 532 nm. Thio-barbituric acid-reactive substances were quantified using an extinction coefficient of 1.56 × 105 M⁻¹ cm⁻¹ and expressed as nmol of malondialdehyde per mg protein. Tissue protein was estimated using the Biuret method and the malondialdehyde content expressed as nmol/mg protein.

2.5.2. Estimation of non protein thiols
Non protein thiols were assayed by the method of Jollow et al. (1974). Briefly, 1.0 ml of cytosolic fraction of both brain regions was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4 °C for at least 1 h and then subjected to centrifugation at 1200 × g for 15 min at 4 °C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 5,5-dithiobis-(2-nitrobenzoic acid) (Ellman’s reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm and the reduced glutathione levels were expressed as μmol/mg protein.

2.5.3. Estimation of superoxide dismutase
Superoxide dismutase activity was assayed by the method of Kono (1978). The assay system consisted of 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM of nitro-blue tetrazolium (NBT). In a cuvette, 2 ml of the above mixture was taken and 0.05 ml of cytosolic fraction of both brain regions and 0.05 ml of hydroxylamine hydrochloride (adjusted to pH 6.0 with NaOH) were added to it. The auto-oxidation of hydroxyamine was observed by measuring the change in optical density at 560 nm for 2 min at 30/60-s intervals.

2.5.4. Estimation of catalase
Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml cytosolic fraction of both brain regions in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of k min⁻¹ and expressed as mean ± S.E.M.

2.5.5. Nitrite estimation
Nitrite was estimated in the cytosolic fraction of different brain regions using the Greiss reagent and served as an indicator of nitric oxide production. A measure of 500 ml of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylamine diamine dihydrochloric acid in water) was added to 100 ml of post-mitochondrial supernatant and absorbance was measured at 546 nm (Green et al., 1982). Nitrite concentration was calculated using a standard curve for sodium nitrite and nitrite levels were expressed as mg/ml. Although, the Griess spectrophotometric assay is not a leading methodology for the quantification of nitric oxide, it employs an indirect measure of nitric oxide content.

2.6. Neurotransmitters estimation
Biogenic amines (dopamine, serotonin and norepinephrine) were estimated by HPLC with electrochemical detector. Waters standard system consisting of a high pressure isocratic pump, a 20 μl sample injector valve, C18 reverse phase column and electrochemical detector were used. Data was recorded and analyzed with the help of empower software. Mobile phase consisting of sodium citrate buffer (pH 4.5)–acetonitrile (87:13, v/v). Sodium citrate buffer consist of 10 mM citric, 25 mM NaH2PO4, 25 mM EDTA, and 2 mM of 1-hexane sulphonic acid (Patel et al., 2005). Electrochemical conditions for the experiment were +0.75 V, sensitivity ranges from 5 to 50 nA. Separation was carried out at a flow rate of 0.8 ml/min. Samples (20 μl) were injected manually. On the day of experiment frozen brain samples were thawed.
and they were homogenized in homogenizing solution containing 0.2 M perchloric acid. After that samples were centrifuged at 12000 \( \times g \) for 5 min. The supernatant was further filtered through 0.22 \( \mu \)m nylon filters before injecting in the HPLC injection pump. Data was recorded and analyzed with the help of empower software.

2.7. TNF-\( \alpha \), IL-1\( \beta \) and substance P ELISA

The quantifications of TNF-\( \alpha \), IL-1\( \beta \) and substance P were done with the help and instructions provided by R&D Systems Quantikine Rat TNF-\( \alpha \), IL-1\( \beta \) and substance P immunoassay kit.

2.8. Quantification of NF-\( \kappa \)\( \beta \) p65 unit

The nuclear levels of p65 may correlate positively with the activation of NF-\( \kappa \)\( \beta \) pathway. The NF-\( \kappa \)\( \beta \)/p65 ActivELISA (Imgenex, San Diego, USA) kit was used to measure NF-\( \kappa \)\( \beta \) free p65 in the nuclear lysate. The NF-\( \kappa \)\( \beta \) ActivELISA is a sandwich ELISA. Free p65 was captured by anti-p65 antibody coated plates and the amount of bound p65 was detected by adding a second anti-p65 antibody followed by alkaline phosphatase (AKP)-conjugated secondary antibody using colorimetric detection in an ELISA plate reader at 405 nm.

2.9. Caspase-3 colorimetric assay

Caspase-3, also known as CPP-32, Yama or Apopain, is an intracellular cysteine protease that exists as a pro-enzyme, becoming activated during the cascade of events associated with apoptosis. The tissue lysates/homogenates can then be tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the color reporter molecule p-nitroaniline (pNA). The cleavage of the peptide by the caspase releases the chromophore pNA, which can be quantitated spectrophotometrically at a wavelength of 405 nm. The level of caspase enzymatic activity in the cell lysate/homogenate is directly proportional to the color reaction. The enzymatic reaction for caspase activity was carried out using R&D systems caspase-3 colorimetric kit.

2.10. Statistical analysis

Results were expressed as means \( \pm \) S.E.M. The intergroup variation was measured by one-way analysis of variance (ANOVA) followed by Tukey’s test. Statistical significance was considered at \( p < 0.05 \). The statistical analysis was done using the SPSS Statistical Software version 16 (SPSS Inc. 233 South Wacker Drive, 11th Floor Chicago, IL 60606-6412).

3. Results

3.1. Effect of curcumin on behavioural paradigms

3.1.1. Modulation of thermal hyperalgesia

Reserpine produced a significant decrease in tail flick latency (4.40 \( \pm \) 0.34 s, \( p < 0.05 \)) as compared to control group (6.73 \( \pm \) 0.88 s). Curcumin (100, 200 and 300 mg/kg) significantly and dose-dependently increased the shortened tail flick latency in reserpine rats (4.87 \( \pm \) 0.39, 5.93 \( \pm \) 0.24 and 6.53 \( \pm \) 0.25) respectively \([F(6,29) = 6.179 \ (p < 0.001)]\). However, there was no significant change in the mean tail flick latency in per se group.

3.1.2. Modulation of mechanical hyperalgesia

Reserpine produced a significant decrease in paw-withdrawal threshold (60.77 \( \pm \) 3.99 g, \( p < 0.05 \)) as compared to control group (136.17 \( \pm \) 3.63 g) (Fig. 2). Curcumin (100, 200 and 300 mg/kg), significantly and dose-dependently increased the paw-withdrawal threshold \([F(6,29) = 84.68 \ (p < 0.01)]\) in reserpine-treated rats. However, there was no significant change in the mean paw-withdrawal threshold in per se group.

3.1.3. Effect on mechanical allodynia

In von-Frey hair test, reserpinised rats showed significant increase in pain sensitivity to non-noxious stimulus

![Figure 2](image-url) Data are expressed as mean \( \pm \) S.E.M. \({ }^*} (p < 0.05)\) different from control group; \({ }^{{ }^2}\) (\( p < 0.05 \)) different from reserpine-administered group; \({ }^{{ }^3}\) (\( p < 0.05 \)) different from one another. Ctrl, control; R, reserpine (1 mg/kg) C1, curcumin (100 mg/kg); C2, curcumin (200 mg/kg); C3, curcumin (300 mg/kg).
Figure 3  Data are expressed as mean ± S.E.M. *(p < 0.05) different from control group; **(p < 0.05) different from reserpine-administered group; *** (p < 0.05) different from one another. Ctrl, control; R, reserpine (1 mg/kg) C1, curcumin (100 mg/kg); C2, curcumin (200 mg/kg); C3, curcumin (300 mg/kg).

(23.67 ± 2.70 g, p < 0.05) as compared to control rats (63.00 ± 0.85 g, p < 0.05) (Fig. 2). Curcumin (100, 200 and 300 mg/kg) produced significant and dose-dependent increase in paw-withdrawal threshold in response to von-Frey hair stimulation [F(6,29) = 88.70 (p < 0.01)]. However, there was no significant change in the mean paw-withdrawal threshold in per se group.

3.2. Effect on immobility period in Forced swim test

The mean immobility period (Fig. 3) of reserpine treated rats (86.60 ± 4.73 s, p < 0.05) was significantly increased as compared to control group (66.60 ± 5.43 s, p < 0.05). Treatment with curcumin (100, 200 and 300 mg/kg) significantly and dose-dependently decreased immobility time in reserpine-treated rats [F(6,29) = 29.06 (p < 0.01)]. However, there was no significant change in the mean immobility time in per se group.

3.3. Effect of curcumin on neurotransmitter levels

Chronic administration of reserpine resulted into decreased levels of dopamine, norepinephrine and serotonin in both cortex and hippocampal region (Table 1) which was dose dependently replenished by curcumin (100, 200 and 300 mg/kg). Curcumin 300 mg/kg produced a significant increase in the NE [2.26-fold], DA [2.62-fold] and 5-HT [2.86-fold] in the cortex region and similar increase in the levels of NE [1.84-fold], DA [1.87-fold] and 5-HT [3.72-fold] in the hippocampus of reserpine administered rats. Curcumin (300 mg/kg) per se did not cause any significant change in dopamine, norepinephrine and serotonin concentration as compared to control.

3.4. Effect of curcumin on substance P levels

There was significant increase in substance P levels in the cerebral cortex [1.87-fold] and hippocampus [2.02-fold] (Fig. 4) of reserpine administered rats respectively. Curcumin (100, 200 and 300 mg/kg) produced a significant reduction in substance P levels in a dose-dependent manner in both cerebral cortex [F(6, 29) = 2223 (p < 0.001)] and hippocampus [F(6, 29) = 741.1 (p < 0.001)] of reserpine administered rats.

3.5. Effect of curcumin on biochemical indices

3.5.1. Effect of curcumin on reserpine-induced changes in lipid peroxidation

Lipid peroxide levels were increased significantly in the cerebral cortex and hippocampus of reserpine administered rats as compared to control group (Table 2). Treatment with curcumin (100, 200 and 300 mg/kg) produced a significant reduction in lipid peroxide levels in the cerebral cortex [F(6, 29) = 67.23

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of curcumin (C) on neurotransmitter levels norepinephrine (NE), dopamine (DA) and serotonin (5-HT). Data are expressed as mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>NE (pg/mg tissue)</td>
</tr>
<tr>
<td>CtrlR</td>
<td>Cerebral cortex</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
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<tr>
<td></td>
<td>Cerebral cortex</td>
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<tr>
<td></td>
<td>Hippocampus</td>
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<tr>
<td>R + C1</td>
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<td></td>
<td>Hippocampus</td>
</tr>
<tr>
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<td>Cerebral cortex</td>
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<td></td>
<td>Hippocampus</td>
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<tr>
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</tr>
<tr>
<td>C3</td>
<td>Cerebral cortex</td>
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\[\text{Ctrl, control; R, reserpine (1 mg/kg); C1, curcumin (100 mg/kg); C2, curcumin (200 mg/kg); C3, curcumin (300 mg/kg).} \]

\[\text{\* Different from control group (p < 0.05).} \]

\[\text{\* Different from reserpine-administered group (p < 0.05).} \]

\[\text{\* Different from one another (p < 0.05).} \]
(p < 0.01)) and hippocampus [F(6,29) = 143.6 (p < 0.01)] of reserpine administered rats.

3.5.2. Effect of curcumin on reserpine-induced changes in the anti-oxidant profile
The non-protein thiols and enzymatic activity of superoxide dismutase and catalase significantly decreased in the cerebral cortex and hippocampus of reserpine administered rats as compared to control group (Table 2). This reduction was significantly and dose dependently restored with different doses of curcumin in the cerebral cortex and hippocampus of reserpine administered rats.

3.5.3. Effect of curcumin on reserpine-induced nitrosative stress
Total nitric oxide was significantly elevated in cerebral cortex (2-fold) and hippocampus (2-fold) of reserpine administered animals (Table 2). Curcumin (100, 200 and 300 mg/kg) treatment significantly inhibited this increase in nitrite levels in the cerebral cortex [F(6,29) = 81.89 (p < 0.01)] and hippocampus [F(6,29) = 123.3 (p < 0.01)] of reserpine administered rats.

3.6. Effect of treatment on TNF-α and IL-1β levels
3.6.1. Effect of curcumin on brain TNF-α level
There was 2 fold and 3 fold increase in TNF-α level in the cerebral cortex and hippocampus (Table 3) of reserpine administered rats respectively. Treatment with curcumin (100, 200 and 300 mg/kg) produced a significant reduction in TNF-α levels in a dose-dependent manner in cerebral cortex [F(6,29) = 36.71 (p < 0.001)] and hippocampus [F(6,29) = 36.17 (p < 0.001)] of reserpine administered rats.

Table 2  Effect of curcumin (C) on lipid peroxide (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase and nitrite levels. Data are expressed as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LPO (nmol/mg pr)</th>
<th>GSH (nmol/mg pr)</th>
<th>SOD (U/mg pr)</th>
<th>Catalase (U/mg pr)</th>
<th>Nitrite (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>Cerebral cortex</td>
<td>1.65 ± 0.20</td>
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<td></td>
<td>Hippocampus</td>
<td>4.14 ± 0.21</td>
<td>0.12 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.16 ± 0.00</td>
</tr>
<tr>
<td>R + C1</td>
<td>Cerebral cortex</td>
<td>7.23 ± 0.25</td>
<td>0.26 ± 0.03</td>
<td>0.48 ± 0.03</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>2.17 ± 0.09</td>
<td>0.18 ± 0.01</td>
<td>0.49 ± 0.04</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>R + C2</td>
<td>Cerebral cortex</td>
<td>4.87 ± 0.13</td>
<td>0.36 ± 0.01</td>
<td>0.68 ± 0.05</td>
<td>0.58 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>1.58 ± 0.08</td>
<td>0.22 ± 0.02</td>
<td>0.73 ± 0.01</td>
<td>0.58 ± 0.01</td>
</tr>
<tr>
<td>R + C3</td>
<td>Cerebral cortex</td>
<td>3.60 ± 0.17</td>
<td>0.41 ± 0.03</td>
<td>0.89 ± 0.03</td>
<td>0.81 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>0.89 ± 0.08</td>
<td>0.28 ± 0.02</td>
<td>1.02 ± 0.04</td>
<td>0.83 ± 0.01</td>
</tr>
<tr>
<td>C3</td>
<td>Cerebral cortex</td>
<td>1.75 ± 0.18</td>
<td>0.55 ± 0.02</td>
<td>1.42 ± 0.05</td>
<td>0.89 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>0.87 ± 0.05</td>
<td>0.39 ± 0.03</td>
<td>1.35 ± 0.11</td>
<td>0.88 ± 0.03</td>
</tr>
</tbody>
</table>

Ctrl, control; R, reserpine (1 mg/kg), C1, curcumin (100 mg/kg); C2, curcumin (200 mg/kg); C3, curcumin (300 mg/kg).

Different from control group (p < 0.05).

Different from reserpine-administered group (p < 0.05).

Different from one another (p < 0.05).
Table 3  Effect of curcumin (C) on TNF-α, IL-1β, p-65 of NFκβ and caspase-3 levels. Data are expressed as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TNF-α (pg/ml)</th>
<th>IL-1β (pg/ml)</th>
<th>p-65 of NFκβ (ng/mg of protein)</th>
<th>Caspase-3 (%Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>89.20 ± 6.10</td>
<td>24.21 ± 2.25</td>
<td>18.95 ± 2.93</td>
<td>100.0 ± 1.08</td>
</tr>
<tr>
<td>Cerebral</td>
<td>29.56 ± 2.87</td>
<td>12.30 ± 1.25</td>
<td>10.93 ± 0.46</td>
<td>100.0 ± 0.81</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>51.93 ± 4.70</td>
<td>35.29 ± 2.42</td>
<td>39.58 ± 2.35*</td>
<td>326.91 ± 2.18*</td>
</tr>
<tr>
<td>R + C1</td>
<td>151.43 ± 7.08*</td>
<td>43.61 ± 5.30*</td>
<td>31.46 ± 0.92*</td>
<td>281.27 ± 6.20*</td>
</tr>
<tr>
<td>R + C2</td>
<td>88.42 ± 6.06*</td>
<td>21.88 ± 2.32*</td>
<td>25.25 ± 0.98*</td>
<td>314.44 ± 8.81*</td>
</tr>
<tr>
<td>R + C3</td>
<td>69.53 ± 3.21*</td>
<td>15.57 ± 1.31*</td>
<td>18.02 ± 1.20*</td>
<td>205.35 ± 2.04*</td>
</tr>
<tr>
<td>C3</td>
<td>127.34 ± 5.07*</td>
<td>30.59 ± 3.97*</td>
<td>17.42 ± 0.87*</td>
<td>193.37 ± 2.54*</td>
</tr>
<tr>
<td>Cerebral</td>
<td>58.30 ± 3.95*</td>
<td>15.71 ± 1.97*</td>
<td>12.15 ± 1.21*</td>
<td>109.09 ± 2.36*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>29.61 ± 3.02</td>
<td>12.96 ± 2.13</td>
<td>10.22 ± 0.73</td>
<td>99.47 ± 0.95</td>
</tr>
</tbody>
</table>

Ctrl, control; R, reserpine (1 mg/kg); C1, curcumin (100 mg/kg); C2, curcumin (200 mg/kg); C3, curcumin (300 mg/kg).
* Different from control group (p < 0.05).
# Different from reserpinized group (p < 0.05).
$ Different from one another (p < 0.05).

3.6.2. Effect of curcumin on brain IL-1β levels
There was significant increase in the IL-1β level in the cortex and hippocampus (Table 3) of reserpinized administered rats as compared to control group. Curcumin (200 and 300 mg/kg) treatment significantly decreased IL-1β levels in the cerebral cortex [F(6,29) = 8.104 (p < 0.001)] and hippocampus [F(6,29) = 24.87 (p < 0.001)] of reserpinized rats.

3.7. Effect of curcumin on nuclear factor kappa beta (NF-κβ)
NF-κβ p56 subunit was significantly elevated in cerebral cortex [2.09-fold] and hippocampus [3.6-fold] (Table 3) of reserpinized administered rats as compared to control group. Curcumin treatment significantly (p < 0.05) and dose-dependently prevented NF-κβ p56 subunit levels in the nuclear fraction of cortex [F(6,29) = 29.15 (p < 0.001)] and the hippocampus [F(6,29) = 77.22 (p < 0.01)] of reserpinized rats.

3.8. Effect of curcumin on caspase-3 activity
Caspase-3 levels were significantly elevated in cerebral cortex [3.26-fold] and hippocampus [4.36-fold] (Table 3) of reserpinized administered rats as compared to control group. Treatment with curcumin significantly (p < 0.05) inhibited caspase 3 activity in cortex [F(6,29) = 307.6 (p < 0.01)] and hippocampus [F(6,29) = 601.2 (p < 0.01)] of reserpinized rats in a dose-dependent manner.

4. Discussion
Clinical depression is a multifactorial and multisymptomatic disease, and apparently so is depression is associated with pain, that’s why we aimed to investigate pain perception in rats with depressive-like behaviour in comparison to non-depressed controls. In the present study, reserpinized rats exhibited increased pain sensitivity in tail flick latency (thermal hyperalgesia) and decreased paw-withdrawal threshold in Randall-sellito test (mechanical hyperalgesia) and von-Frey hair test (mechanical allodynia). These findings corroborate previous reports published from our lab, Kulkarni and Robert (1982) and from Nagakura et al. (2002) who found a time-dependent decrease in nociceptive threshold as observed in tail immersion test in reserpinized rats suggesting reserpine-induced hyperalgesia. Curcumin increased the pain threshold in reserpinized rats in all the behavioural paradigms of pain which is in line with evidence from previous studies done in our laboratory where curcumin attenuated the diabetic neuropathic pain (Sharma et al., 2006).

The enhanced pain sensitivity in reserpinized rats was also coupled with depression as indicated by increased immobility time in forced swim test which is in line with the results reported by Zeng et al. (2008) stating that the presence of depression-like behaviour in rats exacerbated mechanical allodynia under the condition of chronic neuropathic pain. Curcumin significantly and dose-dependently decreased the immobility time in forced swim test in reserpinized rats which is in conformity with the antidepressant activity of curcumin in various rodent models of depression (Xu et al., 2005).

Analyses of cerebrospinal fluid and serum from patients with chronic pain have suggested a decrease in biogenic amines, i.e., dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) (Russell et al., 1992). Basic neurobiological research as well as clinical studies has also revealed that the monoamines (5-HT, DA, NE) have a crucial role in the development of the depression syndrome (Elhuguei, 2004). Serotonin and norepinephrine are both important modulators in pain perception and depression in normal subjects, thus it is reasonable to suspect that disturbances in these functions may be the consequences of abnormalities in serotonin and norepinephrine metabolism and transmission (Kundermann et al., 2009). In the present study, curcumin restored 5-HT, norepinephrine and dopamine levels in different brain regions of reserpinized rats and the results are in accordance with the previous findings from our laboratory (Kulkarni et al., 2008). Xu et al. (2005) also found increased levels of serotonin, dopamine and norepinephrine in both the frontal cortex and hippocampus of mice treated with curcumin (10 mg/kg) and this effect was attributed to monoamine
oxidase inhibiting activity of curcumin. These findings suggest that the neuroprotective effects of curcumin may involve the modulation of central monoaminergic neurotransmitter systems.

Substance P is an active neuropeptide in the CNS and there are studies which show the role of substance P, as it lower pain thresholds (Malberg and Yaksh, 1992) and causes depression (Kramer et al., 1998). Substance P shows the strong negative correlation between serum concentrations of the primary serotonin metabolite, 5-hydroxyindoleacetic acid (Schwarz et al., 1999) and secondly NE may inhibit substance P, thus low NE could indirectly cause more nociception (Gureje et al., 1998). In the present study repeated administration of reserpine showed a significant increase in the substance P levels in both cortex and hippocampus regions of the rat brain and treatment with curcumin significantly relegated the increased levels of the Substance P. To the best of our knowledge, this is the first study which states the inhibitory effect of curcumin on substance P.

The second facet of our hypothesis involves nitroductive stress-induced neurogenic inflammation which may be responsible for the development and perpetuation of pain in depression. Reserpine is a monoamine depletor that exerts a blockade on the vesicular monoamine transporter for neuronal transmission or storage, promoting dopamine-autoxidation and oxidative catabolism by monoamine oxidase (Lohr et al., 2003). This accelerated mechanism leads to the formation of dopamine-quinones and hydrogen peroxide, related to the oxidative stress process (Bilska et al., 2007).

Bagis et al. (2005) demonstrated significantly higher serum levels of pentosidine and malondialdehyde, together with serum superoxide dismutase reduction in patients with chronic pain as compared with normal controls. This generation of advanced glycation end products that results from the increased nitroductive stress activates transcription factor NF-κB, leading to pro-inflammatory gene expression (Pall, 2007). It includes expression of cytokines and growth factors by macrophages and mesangial cells (IL-1β, IGF-1, TNF-α). In the present study, lipid peroxidation was significantly increased whereas the levels of nonprotein thiols, superoxide dismutase and catalase were significantly decreased in the cerebral cortex and hippocampus of reserpinised rats. Curcumin inhibited lipid peroxidation and restored endogenous antioxidant profile in a dose-dependent manner signifying its anti-oxidant potential and this is in addition to the powerful scavenger activity of curcumin for the superoxide anion, the hydroxyl radical and peroxynitrite (Unnikrishnan and Rao, 1995). In this study, nitrite levels were also significantly increased in cerebral cortex and hippocampus regions of reserpinised rats suggesting that NO is an important messenger molecule in signal transduction pathways that enhance nociceptive transmission in the central nervous system (Wu et al., 2001). Curcumin decreased nitrite levels in reserpinised rats.

Nitroductive stress is also linked to the generation of inflammatory cytokines and NF-κB. We also found increased levels of IL-1β and TNF-α in the reserpinised rats and our findings are in concurrence with the Huang et al. (2004), who found increased IL-1β levels in brains of reserpinised rats. Szelenyi et al. (2000) reported dramatically increased TNF-α levels in lipopolysaccharide treated mice on treatment with reserpine. Recently, Uceyler et al. (2007) had also reported that the patients with the complex regional pain have increased mRNA and protein levels for TNF-α. In our study, curcumin significantly reduced TNF-α and IL-1β levels in cortex and hippocampus of reserpinised rats which is attributed to the potent anti-inflammatory properties of curcumin (Jain et al., 2009). The current findings are further supported by results from Cho et al. (2007) who found a significant decrease in pro-inflammatory cytokines (TNF-α, IL-1β, IL-8) on treatment with curcumin (Cho et al., 2007).

We also observed a significant increase in levels of NF-κB and caspase-3 in the cerebral cortex and hippocampus of reserpine administered rats suggesting a possible role of apoptotic pathway in reserpine-induced pain depression dyad. Our findings are supported by observations from Ruster et al. (2005) who found activated NF-κB and higher N⁴-carboxymethyllysine levels in the serum of patients with chronic pain (Ruster et al., 2005). N⁴-carboxymethyllysine is the major advanced glycation end product in human tissues and a marker for cumulative oxidative stress. Increased formation of advanced glycation end products was observed in patients with chronic pain, and a relation to NF-κB activation was suspected (Hein and Franke, 2002). Kislinger et al. (1999) clearly demonstrated that N⁴-carboxymethyllysine adducts are ligands of the receptor for advanced glycated endproducts (RAGE). RAGE has been demonstrated to convert short-living redox-dependent signals to a sustained cellular response by perpetuated activation of NF-κB (Bierhaus et al., 2001). In the present study, treatment with curcumin significantly inhibited both NF-κB and caspase-3 in cerebral cortex and hippocampus of reserpin treated rats. These results are in accordance with the studies done by Bharti et al. (2003) who suggested curcumin as a potent inhibitor of nuclear transcription factor κB in several cell types (Bharti et al., 2003).

### 4.1. Possible mechanism of curcumin’s action

The results of the present study raised the possibility that curcumin showed multiple effects by virtue of its strong anti-inflammatory and antioxidant properties. Moreover, curcumin mediated increase in monoamine transmission may be a step in a potentially complex cascade of events that ultimately may result in antidepressant and anti-nociceptive activities (Bhutani et al., 2009).

Conclusively, the findings from the current study suggested that reserpine-induced neurochemical alterations and nitroductive-inflammatory cascade-induced apoptotic signalling may be responsible for inducing pain symptoms and associated depression in rats. Curcumin being a multi-targeted compound and thereby blocking various steps of this cascade has a potential to attenuate pain—depression syndrome in rats. However, further studies are needed to clarify the mechanism of curcumin action in the reserpinized animal and to establish the clinical effectiveness of curcumin in patients suffering from pain—depression dyad.

### Contributors

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors...
approved the final version to be published. Dr Kanwaljit had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design: Vipin Arora, Anurag Kuhad, Kanwaljit Chopra.

Acquisition of data: Vipin Arora, Vinod Tiwari.

Analysis and interpretation of data: Vipin Arora.

Role of the funding source

UGC provided research fellowship for meritorious students to Mr Vipin Arora and contingency to procure chemicals, kits and animals.

Conflict of interest

Authors have no conflict of interest.

References


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