



Trade Science Inc.

# Natural Products

*An Indian Journal*

## Full Paper

NPAIJ, 3(1), 2007 [18-22]

### Protective Effect Of Curcumin Against Acute Immobilization Stress Induced Changes In Behavior And Biochemical Alterations In Mice



Corresponding Author

**Anil Kumar**

Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, (INDIA)

Ph.: +91-172-2534106; Telefax: +91-172-2541142

Email: kumaruips@yahoo.com

Received: 13<sup>th</sup> January, 2007

Accepted: 28<sup>th</sup> January, 2007

Web Publication Date : 25<sup>th</sup> February, 2007



Co-Author

**Richa Goyal**

Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, (INDIA)

#### ABSTRACT

The present study was designed to investigate protective effects of curcumin, in acute immobilization-induced certain behavioral and biochemical alteration in mice. Mice were immobilized for a period of 6 hr. curcumin (10 and 20mg/kg, i.p.) was administered 30 mins before subjecting the animals to acute stress. Behavioral tests (mirror chamber, actophotometer, tail flick test) and biochemical analysis (malondialdehyde level, glutathione, catalase, nitrite and protein) were done. Acute immobilization stress for a period of 6hr caused severe anxiety, analgesia and decreased locomotor activity in mice. Biochemical analyses revealed an increase in malondialdehyde, nitrite level and deplete glutathione and catalase activity in immobilized stressed brain. Pre-treatment with curcumin (10 and 20mg/kg, i.p.) significantly reversed immobilized stress-induced anxiety, analgesia and reduced locomotor activity. Biochemically curcumin treatment decreased malondialdehyde, nitrite activity and restored reduced glutathione level and catalase activity. Results suggest that curcumin has a neuro-protective effect and can be used in the treatment and management of stress and related disorders. © 2007 Trade Science Inc. - INDIA

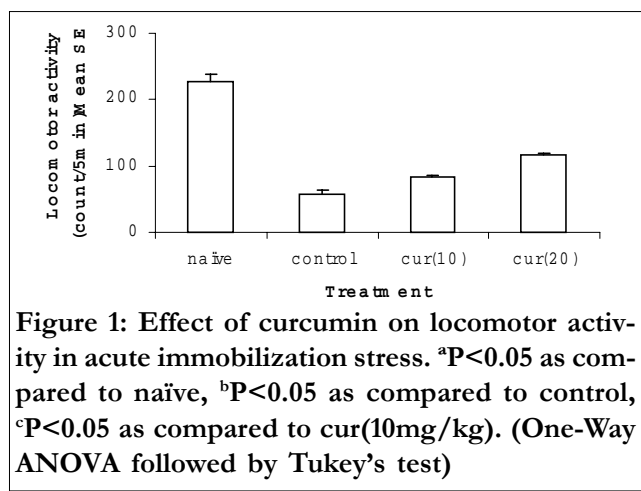
#### KEYWORDS

Anxiety;  
Analgesia;  
Curcumin;  
Immobilization stress;  
Lipid peroxidation;  
Locomotor activity.

## INTRODUCTION

Stress has been shown to affect several brain activities and promote long-term changes in multiple neural systems. Dietary bioactive compounds from different functional foods, herbs and nutraceuticals (ginseng, ginkgo, nuts, grains, tomato, soy phytoestrogens, curcumin, melatonin, polyphenols, antioxidant vitamins, carnitine, carnosine, ubiquinone, etc.) can ameliorate or even prevent diseases. Protection from chronic diseases of aging involves antioxidant activities, mitochondrial stabilizing functions, metal chelating activities, inhibition of apoptosis of vital cells, and induction of cancer cell apoptosis<sup>[1]</sup>. Curcumin (the primary active principle in turmeric, *Curcuma longa* Linn.) has been claimed to represent a potential antioxidant and anti-inflammatory agent with phytonutrient and bioprotective properties<sup>[2]</sup>. A variety of environmental and stressful stimuli have been shown to produce analgesia, a phenomenon often referred to as stress-induced analgesia<sup>[3]</sup>. There are various neuropsychiatric problems such as anxiety, memory dysfunction, depression is generally associated with stress<sup>[4,5]</sup>. Many of these effects have been hypothesized to involve stress-induced neurochemical and hormonal abnormalities that are often associated with oxidative stress<sup>[6,7]</sup>. Previous studies have demonstrated that various stress conditions induces hyperalgesia to thermal, chemical and mechanical stimuli<sup>[8]</sup>.

In view of this, the present study was designed to investigate protective effects of curcumin, a bioflavonoid in acute immobilization-induced certain behavioral and biochemical alteration in mice.



**Figure 1: Effect of curcumin on locomotor activity in acute immobilization stress. <sup>a</sup>P<0.05 as compared to naïve, <sup>b</sup>P<0.05 as compared to control, <sup>c</sup>P<0.05 as compared to cur(10mg/kg). (One-Way ANOVA followed by Tukey's test)**

## MATERIALS AND METHODS

## Animals

Albino mice (Laca strain) weighing between 22-30g bred in Central Animal House facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each group consists of minimum 5 animals. All the experiments were carried out between 900 and 1500h. The experimental protocols were approved by Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

## Drugs and treatment schedule

Curcumin (10 and 20mg/kg, ip) was dissolved in 0.25% CMC and administered intraperitoneally 30 min before the animals were subjected to immobilized stress.

## Immobilization stress

Animals were immobilized for 6h by taping all the four limbs to board after placing them on their backs using zinc oxide hospital tape. Release was affected by unraveling the tape after moistening with acetone. In unstressed group, the mice were handled without any stress<sup>[9]</sup>.

## Behavioral assessment

Various behavioral parameters were assessed in mice after administration of acute stress.

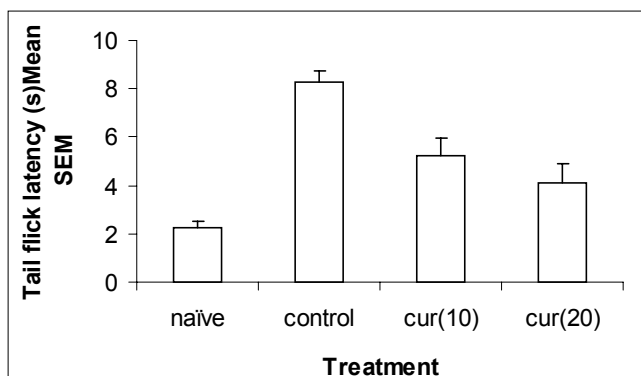
## Measurement of locomotor activity

The locomotor activity was recorded using actophotometer for a period of 5 min. Ambulatory activity was recorded and expressed in terms of total photo beam counts for 5 min per animal<sup>[10]</sup>.

## Measurement of anxiety: mirror chamber test

The mirror chamber consisted of a wooden chamber having a mirror chamber enclosed within it. During the 5 min test session, following parameters were noted-a) latency to enter the mirror chamber, b) total time spent in mirror chamber, c) number of en-

## Full Paper



**Figure 2: Effect of Curcumin on pain perception in acute immobilization stress. <sup>a</sup>P<0.05 as compared to naïve, <sup>b</sup>P<0.05 as compared to control, <sup>c</sup>P<0.05 as compared to Cur (10 mg/kg). (One-Way ANOVA followed by Tukey's test)**

tries in mirror chamber. Animals were placed individually at the distal corner of the mirror chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber<sup>[11]</sup>.

### Measurement of antinociception

The nociceptive threshold was determined as the tail flick latencies elicited in response to radiant heat<sup>[12]</sup>. Baseline latencies to tail flick withdrawal from the radiant heat source(3-5s) were established. A cut-off time of 10s was observed to prevent any injury to the tail<sup>[13]</sup>.

### Biochemical parameters

All the animals were sacrificed by decapitation on the same day following behavioral assessment. The brains removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation of the homogenate at 12000×g for 20 min at 4°C.

### Lipid peroxidation assay

**TABLE 2: Effect of curcumin on immobilized-induced biochemical alteration in whole brain of mice**

Treatment (mg/kg)	LPO (moles of MDA/mgpr)	Red.GSH (micromoles of GSH/mgpr)	Nitrite (µg/ml)	Catalase (µ Mole of H <sub>2</sub> O <sub>2</sub> /min/mgpr)
Naïve	0.168±0.03	0.065±0.0018	318±2.94	0.7050±0.03
Control	0.61±0.03 <sup>a</sup>	0.0147±0.002 <sup>a</sup>	649.75±4 <sup>a</sup>	0.128±0.03 <sup>a</sup>
Cur(10)	0.479±0.04 <sup>b</sup>	0.0412±0.002 <sup>b</sup>	462±4.16 <sup>b</sup>	0.298±0.01 <sup>b</sup>
Cur(20)	0.316±0.03 <sup>c</sup>	0.0501±0.0019 <sup>c</sup>	510±3.1 <sup>c</sup>	0.368±0.09 <sup>c</sup>

Values are expressed as Mean ± SEM. <sup>a</sup>P<0.05 as compared to naïve, <sup>b</sup>P<0.05 as compared to control, <sup>c</sup>P<0.05 as compared to Cur (10mg/kg). (One-Way ANOVA followed by Tukey's test).

The quantitative measurement of lipid peroxidation in the whole brain was measured according to the method of Wills<sup>[14]</sup>. The amount of malondialdehyde formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nanomole of malondialdehyde per milligram protein using the molar extinction coefficient of chromophore (1.56×10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup>).

### Estimation of reduced glutathione

Reduced glutathione in the brain was estimated according to the method of Ellman<sup>[15]</sup>. A 1.0 ml of homogenate was precipitated with 1.0 ml of 4% sulfosalicylic acid by keeping the mixture at 4°C for 1h and the samples were immediately centrifuged at 1200×g for 15 min at 4°C. The assay mixture contains 0.1ml of supernatant, 2.7ml of phosphate buffer of pH 8.0 and 0.2ml of 0.01M-dithiobisnitrobenzoic acid (DTNB). The yellow color developed was read immediately at 412nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nanomole GSH per milligram protein.

### Nitrite estimation

**TABLE 1: Anti-anxiety effect curcumin in mirror chamber test**

Drug Treatment (mg/kg)	Latency to enter mirror chamber (Mean± SEM) sec	No. of Entries in mirror chamber (Mean± SEM)	Time Spent in mirror chamber (Mean ± SEM) sec
Naïve	39±8.81	3±0.71	120±10.21
Control (Stressed)	82±5.09 <sup>a</sup>	0.5±0.29 <sup>a</sup>	1.25±0.75 <sup>a</sup>
Cur (10)	52.60±1.2 <sup>b</sup>	2.16±0.62 <sup>b</sup>	35.6±1.70 <sup>b</sup>
Cur (20)	59.16±2.06 <sup>c</sup>	2.99±0.72 <sup>c</sup>	41.86±2.98 <sup>b</sup>

Values are expressed as Mean ± SEM. <sup>a</sup>P<0.05 as compared to naïve, <sup>b</sup>P<0.05 as compared to control, <sup>c</sup>P<0.05 as compared to Cur (10 mg/kg). (One-Way ANOVA followed by Tukey's test)

Nitrite is the stable end product of nitric oxide (NO) in living system. Accumulation of nitrite was measured in cell free supernatants from brain homogenates by spectrophotometer assay based on Greiss reagent 15(1% sulphanilamide/0.1% naphthylethylenediamine dihydrochloride/2.5% phosphoric acid) and incubated at room temperature for 10 min to yield a chromophore. Absorbance was read at 543 nm spectrophotometrically. The nitrite concentration was calculated from a standard curve using sodium nitrite as standard and expressed as micro molar nitrite per milliliter homogenate<sup>[16]</sup>.

#### Protein estimation

The protein content was measured according to the method of Lowry using bovine serum albumin as standard<sup>[17]</sup>.

#### Catalase estimation

Catalase activity was assayed by the method of Luck<sup>[18]</sup>, wherein the breakdown of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H<sub>2</sub>O<sub>2</sub> phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and the change in absorbance was recorded at 240 nm. The results were expressed as micromole H<sub>2</sub>O<sub>2</sub> decomposed per milligram of protein/min.

#### Statistical analysis

All the values are expressed as mean±SEM. The data were analyzed by using one way analysis of variance followed by Tukey's test. P<0.05 was considered statistically significant.

## RESULTS

#### Behaviour measurements (Locomotor, anxiety and analgesic activity)

6-hour acute immobilization stress significantly decreased the locomotor activity(as indicated by decreased ambulatory movements), anxiety (increased the latency to enter in mirror chamber, decreased number of entries and time spent in the mirror chamber) antinociceptive activity (increased tail flick latency). The effect was significant as compared to naïve mice(P<0.05). Pretreatment with curcumin(10 and 20mg/kg, ip) significantly improved ambulatory movements, anxiety(decreased time latency to enter in mir-

ror chamber, increased number of entries and duration in mirror chamber) and reduced tail flick latency. The effects were significant as compared to control(stressed mice) (P<0.05).

#### Measurement on biochemical estimations

6-hour acute immobilization stress significantly increased malondialdehyde, nitric oxide levels (as indicated by a rise in whole brain nitrite level) and depleted reduced glutathione and catalase activity as compared to naïve animals(P<0.05). Pretreatment with curcumin (10 and 20mg/kg, ip) significantly reversed the increase in malondialdehyde level, nitrite activity and restored depleted reduced glutathione and catalytic activity as compared to control (P<0.05).

## DISCUSSION

Stress has significant impact upon the immune, circulatory and nervous system<sup>[19-23]</sup> and cause immunological, cardiovascular, and neurodegenerative disorders<sup>[23,5]</sup>. Acute stress is a result of a traumatic event that makes a person fear and helplessness. Acute stress has been reported to influence behavioral activity such as motor activity, anxiety and produces antinociceptive effect<sup>[24-26]</sup>. Diets rich in vegetables and fruits are associated with reduced risk of several major diseases, including neurodegenerative disorders. Although some beneficial phytochemicals might function solely as antioxidants, it is becoming clear that many of the beneficial chemicals in vegetables and fruits evolved as toxins(to dissuade insects and other predators) that, at sub toxic doses, activate adaptive cellular stress-response pathways in a variety of cells including neurons<sup>[27]</sup>.

In the present study, immobilization stress significantly decreased motor activity after an acute stress of 6hr in mice. Its lucid that oxidative stress play role in the pathogenesis of motor activity<sup>[8]</sup>. It is also reported that immobilization stress decreased motor activity, modulate anxiety<sup>[26]</sup>, depression-related behaviors and alter the pain perception<sup>[24]</sup>. In the present study, curcumin decreased anxiety level and increased pain perception, suggesting its antistress effect.

Stress activates hypothalamus-pituitary-adrenal axis (HPA) axis and influence several neurological function at both central and peripheral level. Besides, neurotransmitters and neuropeptides also influence

## Full Paper

HPA axis activity by acting at the hypothalamic or suprahypothalamic level<sup>[28]</sup>. Oxidative stress has been implicated in the pathophysiology of many neurological disorders such as Alzheimer, Huntington disease etc<sup>[29,30]</sup>. Oxidative stress can cause cellular damage and neuro-degeneration by inducing the reactive oxygen species (ROS) that oxidize vital cellular components such as lipids, proteins and DNA<sup>[20]</sup>. In the present study, 6 hour immobilized stress significantly increased lipid peroxidation, nitrite activity and depleted reduced glutathione and catalase activity in stressed mice brains, suggesting oxidative stress due to immobilization in mice. Stress has also been known to increase the MDA levels and decreases the reduced glutathione activity<sup>[31,27]</sup>. Curcumin, a well known antioxidant, acts by scavenging of reactive oxygen/nitrogen species or their precursors, inhibition of ROS formation, binding of metal ions needed for the catalysis of ROS generation and up-regulation of endogenous antioxidant defenses<sup>[20,32,7]</sup>. In the present study, curcumin significantly reversed immobilization-induced oxidative stress indicators in mice, suggesting its beneficial role in stress related problem. However, the exact mechanism of its action is far from elucidation.

### CONCLUSION

The present study has shown that curcumin is effective at ameliorating immobilization stress-induced behavioral alterations and oxidative stress. Present findings further support the therapeutic potential of curcumin as neuroprotectant in the treatment of stress-related disorders.

### REFERENCES

- [1] C.K.Ferrari; *Biogerontology*, **5**, 275 (2004).
- [2] M.Balasubramanyam, A.A.Koteswari, R.S.Kumar, S.F.Monickaraj, J.U.Maheswari, V.Mohan; *J.Biosci.*, **28**, 715 (2003).
- [3] H.Imbe, Y.Iwai-Liao, E.Senba; *Front Biosci.*, **11**, 2179 (2006).
- [4] T.Esch, G.L.Fricchione, G.B.Stefano; *Med. Sci. Monit.*, **9**, RA 23 (2003).
- [5] B.S.McEWEN, N.Y. Ann; *Acad.Sci.*, **1032**, 1 (2004).
- [6] L.Jacobson, R.Sapolsky; *Endocr Rev.*, **12**, 118 (1991).
- [7] Y.G.Sherki, E.Melemed, D.Offen; *Neuropharmacology*, **40**, 959 (2001).
- [8] A.Dhir, S.V.Padi, P.S.Naidu, S.K.Kulkarni; *Eur.J. Pharmacol.*, **27**, 192 (2006).
- [9] T.K.Sur, D.Bhattacharya; *Indian J.Pharmacol.*, **29**, 318 (1997).
- [10] D.S.Reddy, S.K.Kulkarni; *Brain Res.*, **799**, 215 (1998).
- [11] S.K.Kulkarni, D.S.Reddy; *Method Find Exp.Clin Pharmacol.*, **18**, 219 (1996).
- [12] E.F.D'Amour, D.L.Smith; *J.Pharmacol.Exp.Ther.*, **72**, 74 (1941).
- [13] S.K.Kulkarni; 'Handbook of Experimental Pharmacology'. Vallabh Prakashan, 123 (1999).
- [14] E.D.Wills; *Biochem J.*, **99**, 667 (1966).
- [15] G.L.Ellman; *Arch.Biochem.Biophys.*, **82**, 48670 (1959).
- [16] L.C.Green, D.A.Wagner, J.Glagowski; *Anal. Biochem.*, **126**, 131 (1982).
- [17] O.H.Lowry, N.J.Rosenberg, A.L.Farr, R.J.Randall; *J.Biol.Chem.*, **193**, 265 (1951).
- [18] H.Luck; 'Catalase., Methods of Enzymatic Analysis', Bergmeyer HU, Ed. Academic Press, New York, 885 (1971).
- [19] R.L.Macdonald, R.W.Olsen; *Annu.Rev.Neurosci.*, **17**, 569 (1994).
- [20] F.Marzatico, L.Bertorelli, O.Pansarasa, P.Guallini, C.Torri, G.Biagini; *Intern J. of Stress Mgmt.*, **5**, 223 (1998).
- [21] T.Esch, G.B.Stefano, G.L.Fricchione, H.Benson; *Mod.Asp.Immunobiol.*, **2**, 187 (2002).
- [22] T.Esch, G.B.Stefano, G.L.Fricchione, H.Benson; *Med.Sci.Monit.*, **8**, 93 (2002).
- [23] T.Esch, G.B.Stefano, G.L.Fricchione, H.Benson; *Neuroendocrinol Lett.*, **23**, 199 (2002).
- [24] I.L.Torres, A.P.Vasconcellos, S.N.Silveira, C.Dalmaz; *Braz.J.Med.Biol.Res.*, **34**, 241 (2001).
- [25] G.A.Metz, N.M.Jadavji, L.K.Smith; *Eur.J. Neurosci.*, **22**, 1190 (2005).
- [26] S.Sevgi, M.Ozek, L.Eroglu; *Methods Find Exp.Clin. Pharmacol.*, **28**, 95 (2006).
- [27] M.P.Mattson, A.Cheng; *Trends Neurosci.*, **29**, 632 (2006).
- [28] A.G.Michalska, J.Bugajski; *Journal of Physiology and Pharmacology*, **54**, 449 (2003).
- [29] A.Kumar, P.S.Naidu, N.Seghal, S.S.V.Padi; *Pharmacology*, (in Press), (2006).
- [30] Puneet Kumar., S.S.V.Padi, P.S.Naidu, Anil Kumar; *Behavioural Pharmacology*, **17**, 485 (2006).
- [31] M.Nazar, M.Jessa, A.Plaznik; *J.Neural Transm.*, **104**, 733 (1997).
- [32] J.Liu, A.Mori; *International Journal of Stress management*, **1**, 249 (1999).