



Gastroprotective and antidepressant effects of a new zinc(II)–curcumin complex in rodent models of gastric ulcer and depression induced by stresses

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ABSTRACT

Curcumin, a yellow pigment found in the rhizome of *Curcuma longa*, has been used to treat a variety of digestive and neuropsychiatric disorders since ancient times in China. Curcumin can chelate various metal ions to form metallocomplexes of curcumin which show greater effects than curcumin alone. This study investigated the antiulcerogenic and antidepressant effects of a Zn(II)–curcumin complex on cold-restraint stress (CRS)-induced gastric ulcers in rats, and on the forced swimming test (FST), tail suspension test (TST) and 5-hydroxy-L-tryptophan (5-HTP)-induced head twitch test in mice. CRS disrupted the rat mucosal barrier and induced gastric ulcers by decreasing the activities of the antioxidant enzymes, and increasing H^+K^+ -ATPase activity and malondialdehyde (MDA) level. Pretreatment with Zn(II)–curcumin (12, 24, and 48 mg/kg) dose-dependently reversed these trends, reduced gastric lesions and H^+K^+ -ATPase activity, and increased antioxidant activities compared with control groups. Zn(II)–curcumin significantly increased HSP70 mRNA, and attenuated increased iNOS mRNA in the mucosa. Zn(II)–curcumin (17, 34, and 68 mg/kg) also significantly decreased immobility time in the FST and TST, and enhanced 5-HTP-induced head twitches in mice. These results demonstrate that the Zn(II)–curcumin complex showed significant gastroprotective and antidepressant effects compared with curcumin alone via a synergistic effect between curcumin and zinc.

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1. Introduction

Stress is a major factor in the etiology of gastric ulcer formation and depression (Bale, 2005). Stress ulcers and stress-induced depression frequently occur in psychiatric diseases in 9–18% of the Western world (Xu et al., 2005). Depression with psychotic and somatic symptoms has been observed in patients with gastric ulcers (Guldahl, 1977). Clinical studies have shown that antidepressant drugs, such as dothiepin, tianeptine, trazodone, and venlafaxine, benefit patients with ulcers (Suleyman et al., 2009). Current antidepressant drugs exert undesirable side effects including weight gain, fatigue, and diarrhea. The development of safe antidepressant drugs from traditional herbs may alleviate some of the side effects that accompany typical antidepressant drugs.

Curcumin ($C_{21}H_{20}O_6$), a yellow pigment found in the rhizome of *Curcuma longa*, also known as turmeric, has been used since ancient times in China to treat a variety of digestive and neuropsychiatric disorders (Ravindran et al., 2007). The presence of both phenolic OH and CH_2 groups in the β -diketone moiety of this natural compound contribute significantly to its potent antioxidant properties (Mahattanadul et al., 2009).

Curcumin acts as a potent antiulcer and antioxidant compound, and shows a remarkable curative property against cold restraint stress (CRS)-induced chronic gastric ulcers, by preventing the generation of reactive oxygen species (ROS) (Mahattanadul et al., 2009; Chattopadhyay et al., 2006). Curcumin has been shown to exert antidepressant-like effects in a behavioral despair paradigm in mice through the central monoaminergic system, mainly by enhancing serotonergic and dopaminergic synaptic availability (Kulkarni et al., 2008).

Curcumin can chelate various metal ions to form metallocomplexes of curcumin, which show greater effects than curcumin alone. The Cu(II)–curcumin complex induces superoxide dismutase (SOD) activity and promotes the neutralization of free radicals, this scavenging of ROS has been reported to block $A\beta$ aggregation potential, and reacts with 2,2-Vdiphenyl-1-picryl hydrazyl radicals with a rate constant 10 times less than that of parent curcumin (Barik et al., 2005; Barik et al., 2007). A vanadyl curcumin complex ($VO(cur)_2$) was shown to be several-fold more effective than curcumin as an inhibitor of synovioocyte proliferation, a measure of antiarthritic potential (Thompson et al., 2004). Comparisons of antioxidant potentials among the complexes indicated that the predominant determinant of antioxidant capacity was the ligand, with $VO(cur)_2$ roughly twice as effective as curcumin alone (Mohammadi et al., 2005). $Mn(cur)(OAc)$ showed significant protective effects in a transient ischemia/reperfusion mouse model of neuronal damage (Vajragupta et al., 2003). A curcumin–gold complex ($Au(cur)_2Cl$) has been reported to have antiarthritic properties in an adjuvant-induced

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rat polyarthritis model, in which paw swelling was reduced after 3 weeks of Au(cur)₂Cl injections (Sharma et al., 1987).

Zinc, a transition metal with antioxidant properties, is essential for the proper healing of wounds. Zinc homeostasis is also important for the integrity of gastric mucosal cells. Zinc deficiency leads to stress and activation of macrophages and monocytes, resulting in the increased generation of inflammatory cytokines (Cannali et al., 2000). Recent studies performed in rodents suggest a causative role for zinc deficiency in the induction of depressive-like symptoms, such as reduced physical activity, anhedonia, anxiety, and anorexia (Whittle et al., 2009). Zinc induces the expression of heat shock proteins (HSPs) in thermotolerant HeLa cells (Hatayama et al., 1993), as well as in gastric mucosal and hepatic cells *in vivo* (Odashima et al., 2006). Zinc has also shown antidepressant-like activity in several models of depression in rodents such as in olfactory bulbectomy and chronic unpredictable stress (Cieslik et al., 2007). Several preclinical and clinical studies have indicated low zinc blood concentrations in depressed patients (Nowak et al., 2003), and high plasma zinc levels in recovering depressed patients (Lobato et al., 2008). Zinc supplementation may enhance antidepressant therapy in patients with unipolar depression, and enhance the antidepressant actions of classical antidepressants (imipramine and citalopram) in the forced swim test (FST) (Szewczyk et al., 2009).

In our previous work, we demonstrated that the Zn(II)–curcumin complex prevented pylorus-ligation-induced lesions in rats by inhibiting NF-κB activation and the subsequent production of proinflammatory cytokines (Mei et al., 2009). In this study, the gastroprotective and antidepressant effects of the Zn(II)–curcumin complex were investigated in a rat CRS-induced gastric ulcer model, and in mouse models of depression, including the forced swim test (FST), the tail suspension test (TST) and 5-hydroxy-L-tryptophan (5-HTP)-induced head twitch test.

2. Materials and methods

2.1. Animals

Male adult Sprague–Dawley (SD) rats (6–7 weeks, 200–250 g) and male NIH mice (6–7 weeks, 18–22 g) were housed in a facility at the Laboratory of Traditional Chinese Medicine and Marine Drugs, School of Life Sciences, Sun Yat-Sen University approved by the Guangdong Experimental Animals Association (Guangzhou, China). Animals were housed in a storage room under conditions of constant temperature (23 ± 1 °C), relative humidity of 50 ± 5% and under a 12:12 h light: dark cycle (lights on at 07:00 h) until initiation of the experiment. Seventy SD rats were included in the CRS-induced ulcer experiment, and 60 NIH mice were included in the FST, TST and 5-HTP-induced head twitch test. The animals were maintained on a standard pellet diet and water *ad libitum*. All procedures regarding animal care and use were carried out based on the guidelines of the animal ethics committee of Sun Yat-Sen University (Guangzhou, China). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] was manufactured by Guangdong Zhongda Greenfield Biotech. Co. (Guangzhou, China). Polyvinylpyrrolidone K30 (PVP) was purchased from BASF Chemical Ltd. (New Jersey, USA). Lansoprazole tablets were obtained from Shengfan Pharmaceutical Company Limited (Henan, China). Fluoxetine Hydrochloride capsules were obtained from Eli Lilly and Company (Suzhou, China). 5-hydroxy-L-tryptophan (5-HTP) was obtained from Sigma Chemical Company (St. Louis, MO, USA).

2.3. Synthesis of Zn(II)–curcumin and solid dispersions (SDs)

The Zn(II)–curcumin complex was synthesized by mixing equimolar amounts of zinc acetate and curcumin in dry ethanol and refluxing the mixture for 3 h under a nitrogen atmosphere. The Zn(II)–curcumin complex was precipitated, and the solid was separated by filtration and washed several times with water and ethanol to remove any unreacted curcumin and zinc acetate. The molecular formula of the Zn(II)–curcumin complex is shown in Fig. 1. Zn(II)–curcumin and PVP in a ratio of 1:6 (w/w) were added to dry ethanol to reach 5% final concentration of Zn(II)–curcumin, produce a suspension by cryo-grinding under a nitrogen atmosphere. SDs of Zn(II)–curcumin/PVP were produced with a spray dryer. The operating parameters were: inlet temperature, 70 °C; outlet temperature, 50 °C; feed rate, 2–3 mL/min; atomization air pressure, 2 kg/cm²; and inspiration, –280 mmWC. Curcumin SDs (1:6, w/w) were also produced using the same procedure.

2.4. Experimental design

Rats were randomly divided into seven experimental groups. Each group consisted of 10 animals. The normal and control groups received PVP vehicle (300 mg/kg) throughout the course of the experiments. The treatment groups received different doses of Zn(II)–curcumin solid dispersions (SDs) (equivalent to Zn(II)–curcumin 12, 24 and 48 mg/kg, p.o.). The curcumin group received curcumin SDs (equivalent to curcumin 24 mg/kg, p.o.), and lansoprazole (7.8 mg/kg, p.o.) was used as the positive control for a period of 7 d.

NIH mice were randomly divided into six experimental groups. Each group consisted of 10 animals. The control group received PVP vehicle (420 mg/kg, p.o.) throughout the course of the experiments. The treatment groups received different doses of Zn(II)–curcumin SDs (equivalent to Zn(II)–curcumin 17, 34, 68 mg/kg, p.o.). The curcumin group received curcumin SDs (equivalent to curcumin 34 mg/kg, p.o.), and fluoxetine (3 mg/kg, p.o.) was used as the positive control for a period of 7 d.

2.5. CRS-induced gastric ulcer test

Gastric ulcers induced by CRS in rats are known to resemble human peptic ulcers, both grossly and histologically, and are widely used for studying the effects of drugs on healing rats (Konturek et al., 2003). The CRS-induced gastric ulcer model was used in this study. After fasting for 24 h prior to the experiment, the rats were positioned on a board with their heads up and were immersed in water at 8–10 °C, up to the level of the xiphoid. Normal control rats did not receive this treatment. Four hours after the stress treatment, the rats were killed by cervical dislocation.

After the CRS treatment, the area of mucosal injury was measured. The rat stomach was removed, the mucosa was exposed by cutting along the greater curvature, and was then rinsed with normal saline to remove any gastric contents and blood clots. The severity of the mucosal lesions was assessed using a magnifier and rated for gross

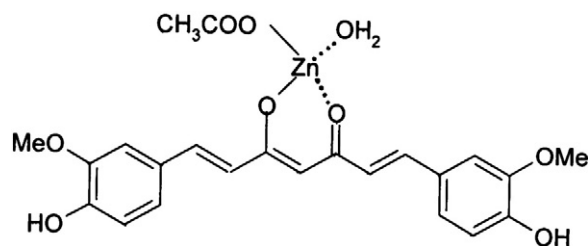


Fig. 1. Molecular structure of the Zn(II)–curcumin complex.

pathology according to the ulcer score described by Dekanski et al. (1975) as follows (Dekanski et al., 1975):

- 0 = no damage.
- 1 = blood at the lumen.
- 2 = pin-point erosions.
- 3 = one to five small erosions <2 mm.
- 4 = more than five small erosions <2 mm.
- 5 = one to three large erosions >2 mm.
- 6 = more than three large erosions >2 mm.

The inhibition percentage was calculated using the following formula (Demirbilek et al., 2004): $[(UI_{\text{non-treated}} - UI_{\text{treated}}) / UI_{\text{non-treated}}] \times 100$.

2.6. Measurement of antioxidant enzymes, $H^+ - K^+ - ATPase$ activity and MDA level

Following the macroscopic analyses, SOD, catalase (CAT) and $H^+ - K^+ - ATPase$ enzyme activities, and malondialdehyde (MDA) level in rat stomach tissues were determined. To prepare the tissue homogenates, stomach tissues were homogenized with liquid nitrogen using a mortar and pestle. The homogenized tissues (0.5 g each) were then mixed with 4.5 mL of homogenization Tris-buffer (10 mM, pH 7.4). The mixtures were homogenized on ice using an Ultra-Turraks homogenizer for 15 min. Homogenates were filtered and centrifuged at $1000 \times g$ at $4^\circ C$ for 20 min using a refrigerated centrifuge. The supernatants were then used to determine enzymatic activities.

SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium (NBT) to form a formazan dye (Sun et al., 1988). SOD activity was then measured at 560 nm by the degree of inhibition of this reaction. Enzyme activity leading to 50% inhibition was considered as 1 unit/mg protein. Decomposition of H_2O_2 in the presence of CAT was measured at 240 nm (Aebi, 1984). CAT activity was defined as the amount of enzyme required to decompose $1 \mu\text{mol}$ of H_2O_2 per second per milligram protein, at $37^\circ C$. Results were expressed as units per mg protein.

The remaining gastric tissues were homogenized using Tris-Cl buffer and used to measure $H^+ - K^+ - ATPase$ activity. The enzyme activity was assayed following the description by Reyes-Chilpa et al. (2006) (Reyes-Chilpa et al., 2006) in the presence and absence of different doses of Zn(II)-curcumin or lansoprazole. The reaction mixture (1 mL) contained enzyme in 20 mM Tris-HCl, pH 7.4, 2 mM $MgCl_2$ and 2 mM KCl. The reaction was started by the addition of 2 mM ATP and incubated for 30 min at $37^\circ C$. The reaction was terminated by the addition of ammonium molybdate and trichloroacetic acid mixture followed by centrifugation at $2000 \times g$. The amount of inorganic phosphate released from ATP was determined spectrophotometrically at 400 nm. The $H^+ - K^+ - ATPase$ activity was expressed as micromoles of P_i released per hour per mg of protein.

Tissue MDA levels were determined by the method described by Ohkawa et al. (1979) (Ohkawa et al., 1979). The corpus mucosa was homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added to a solution containing 0.2 mL of 80 g/L sodium lauryl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL of distilled water. This mixture was heated at $98^\circ C$ for 1 h, and 5 mL of n-butanol:pyridine (15:1) was added when the mixture had cooled. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. Absorbance of the supernatant was measured at 532 nm. A standard curve was obtained by using 1, 1, 3, 3-tetramethoxypropane. The recovery was over 90%. The results were expressed as nmol per mg of protein.

2.7. Detection of HSP70 and iNOS mRNA by reverse transcription polymerase chain reaction (RT-PCR)

Full-thickness specimens from the stomach were obtained to detect HSP70 mRNA and iNOS mRNA using RT-PCR. Some of the rat tissue samples were immersed in RNA Stabilization Reagent and stored at $-70^\circ C$. Total RNA was extracted using this reagent, according to the protocol provided by the manufacturer, and quantified by measuring the absorbance at 260 nm. Complementary DNA was synthesized using $1 \mu\text{g}$ of total RNA from each sample in 20 μL of reaction buffer along with SuperScript II reverse transcriptase. cDNAs for HSP70 and iNOS were amplified by PCR using the primers listed in Table 1. PCR products were separated on 2% agarose gels and visualized by ethidium bromide staining.

2.8. Forced swim test (FST)

This test was conducted according to the method of Porsolt et al. (1978), except that the water level was deeper. Drugs were administered for 7 days before the pretest and 1 h before the test session. Each NIH mouse was forced to swim in a transparent cylindrical polypropylene tank (45 cm height \times 20 cm diameter) containing 38 cm of water at $25 \pm 3^\circ C$, without the possibility of escaping. The resulting anxiety produces vigorous swimming activity and attempts at escaping by diving or climbing the walls of the cylinder. After an initial 2 min period of vigorous activity, animals ceased all movements, except those necessary for survival (keeping the head above the water). A mouse was judged immobile when it floated in an upright position, and made only small movements to keep its head above water. The duration of immobility was measured as recommended by a blind observer (Carbajal et al., 2009). The changes in the duration of immobility of separate groups of mice were recorded. Following each swimming stress session, the mice were towel-dried, then returned to their home cages and were able to access food and water for the remainder of the day.

2.9. Tail suspension test (TST)

The TST was performed according the method described by Belozertseva et al. (2007). Mice were individually suspended on a paper adhesive tape, 65 cm above the tabletop. The tape was placed approximately 1 cm from the tip of the tail. Mice were allowed to hang for 6 min and the duration of immobility was recorded. Mice were considered immobile only when hanging passively and completely motionless. A blind observer measured the immobility period in seconds.

2.10. 5-hydroxy-L-tryptophan (5-HTP)-induced head twitch test

The 5-HTP-induced head twitch test was performed according the method described by Nakagawasai et al. (2003). The mice were allowed to adapt for 1 h in an observation cage (25 \times 18 \times 13 cm) before injection of 5-HTP (100 mg/kg i.p.). The number of head twitches (rapid movements of the head with little or no involvement

Table 1
PCR primer and conditions.

cDNA	Primers	Product size
HSP70 U	5' TCATCAGCGGGCTGTATCAG 3'	354 bp
HSP70 D	3' TACCACAAAGGAAGGACGCT5'	
iNOS U	5' CACATACTTTACGCCACTAACA 3'	461 bp
iNOS D	3' TCCAGAGCCTGAGGTTAGA 5'	
β -actin U	5' TCACCAACTGGGACGATA 3'	207 bp
β -actin D	3' ACAACAGGGACATACGGA5'	

of the trunk) was counted for 15 min after the administration of 5-HTP by a blind observer.

2.11. Statistical analysis

The values were expressed as the mean \pm S.D. for 10 animals in each group. The data were analyzed by SPSS/13 software. Hypothesis testing methods included one-way analysis of variance (ANOVA), followed by Dunnett's T3 multiple comparisons test. The significance levels were analyzed at $P < 0.001$, $P < 0.01$, and $P < 0.05$.

3. Results

3.1. Anti-gastric effects of Zn(II)-curcumin on CRS-induced gastric ulcers

Histopathologic examination of the gastric mucosa showed stress-induced damage on the surface epithelial cells. As shown in Fig. 2A, no macroscopic or microscopic lesions were observed in the normal control group. Rats in the control group exposed to CRS at 8–10 °C for 4 h showed severe hemorrhagic ulcers, with elongated-band erosions in the glandular portion of the stomach, which occurred in 100% of animals in the CRS groups (Fig. 2B). A water temperature lower than 8 °C often leads to death within 1 h. As reported in Table 2 and Fig. 2C–E, Zn(II)-curcumin showed a dose-effect relationship, causing effective preventive and therapeutic effects against stress ulcers in rats. These ulcers only showed up as pinpoint erosions (petechia). Oral administration of Zn(II)-curcumin at 12–48 mg/kg decreased ulcer index by 34.9 ± 8.7 – 16.4 ± 5.9 (59.7–81.1% protection) in comparison to control 86.6 ± 11.1 ($P < 0.001$). As shown in Table 2, Fig. 2D–G, the antiulcerogenic effects of Zn(II)-curcumin at a dose of 24 mg/kg in reducing ulcer index (25.9 ± 6.8) was greater than curcumin alone at the same dose (36.8 ± 6.3). This difference was statistically significant at $P < 0.01$. Lansoprazole (7.8 mg/kg) was more potently reduced gastric index (11.6 ± 5.3), which was slightly better than that of Zn(II)-curcumin at a dose of 48 mg/kg (16.4 ± 5.9) ($P > 0.05$).

3.2. Influence of Zn(II)-curcumin on antioxidant enzymes, H^+ - K^+ -ATPase activity and MDA level

As shown in Table 2, following CRS, disorders of the nervous system and endocrine system led to increased gastric acid secretion and decreased gastric mucus secretion, thereby causing lesions in the gastric mucosa. Compared with the normal group, MDA levels and the activity of H^+ - K^+ -ATPase in gastric mucosa of the control group were elevated, while the activities of CAT and SOD in the gastric mucosa of the CRS model group decreased ($P < 0.001$). These data showed that Zn(II)-curcumin at doses of 12, 24, and 48 mg/kg significantly reduced MDA levels and H^+ - K^+ -ATPase activity in the gastric mucosa, and increased the activity of CAT and SOD in a dose-dependent manner ($P < 0.05$, $P < 0.001$, $P < 0.001$, respectively), compared with control animals. The inhibitory effects of lansoprazole (7.8 mg/kg) on MDA level and H^+ - K^+ -ATPase activity were weaker than Zn(II)-curcumin at a dose of 48 mg/kg, which was similar to the enhancement of SOD and CAT activities with lansoprazole and Zn(II)-curcumin. Compared with the control group, curcumin at a dose of 24 mg/kg significantly increased the activity of CAT and SOD in rat gastric mucosa ($P < 0.05$) and decreased MDA levels ($P < 0.01$) and H^+ - K^+ -ATPase activity ($P < 0.001$). Moreover, curcumin at a dose of 24 mg/kg produced weaker effects on the decrease in MDA level, and increase in SOD and CAT when compared with Zn(II)-curcumin at the same dose ($P < 0.05$). Compared to normal control value, Zn(II)-curcumin at a dose of 48 mg/kg recover approximately 80% of activity of CAT and SOD in rat gastric mucosa and MDA levels ($P < 0.01$).

3.3. Zn(II)-curcumin increased HSP70 mRNA and attenuated iNOS mRNA expression in gastric mucosa

Gene expression of HSP70 was detected in the gastric mucosa (Fig. 3). Compared with normal controls, a significant increase in HSP70 mRNA expression in the ulcerated mucosa was observed in CRS-induced rats ($P < 0.001$). Treatment of ulcerated rats with Zn(II)-curcumin at doses of 24 and 48 mg/kg led to a gradual dose-related increase in HSP70 expression, and statistically significant differences were observed at $P < 0.01$ and $P < 0.001$. In rats treated with curcumin at a dose of 24 mg/kg, no significant increase in HSP70 expression was evident when compared with controls. The mRNA expression of HSP70 in the groups treated with lansoprazole at a dose of 7.8 mg/kg was slightly lower than that recorded in the Zn(II)-curcumin (48 mg/kg) treated group, but showed similar values to the Zn(II)-curcumin (24 mg/kg) treated group. Compared with curcumin at a dose of 24 mg/kg, Zn(II)-curcumin at the same dose had significantly greater protective effects as a results of increased HSP70 expression ($P < 0.05$).

There are two types of NOS: constitutive (cNOS) and inducible (iNOS). iNOS is found mainly in macrophages and smooth muscle cells, it is cytotoxic and participates in the immune response, causing cell and tissue damage. As seen in Fig. 4, iNOS expression in the control group increased compared with that of the normal group ($P < 0.001$). iNOS expression following treatment with Zn(II)-curcumin at doses of 12, 24 and 48 mg/kg decreased with increasing dosage. Compared with the model group, statistical significance was seen at $P < 0.05$ and $P < 0.01$. The inhibitory effect of Zn(II)-curcumin at a dose of 48 mg/kg on iNOS expression was stronger than lansoprazole (7.8 mg/kg).

3.4. Effect of Zn(II)-curcumin on the FST

Fig. 5 illustrates the effect of Zn(II)-curcumin on the duration of immobility in the FST model. The results revealed a significant main effect of Zn(II)-curcumin on the duration of immobility in mice, where a dose-dependent decrease in the immobility period was observed when compared with the control group. Treatment with Zn(II)-curcumin at doses of 17, 34 and 68 mg/kg decreased the immobility time of rats to 108.5 ± 19.8 , 98.6 ± 15.4 and 90.3 ± 14.3 s in a dose-related manner, respectively, when compared with the control group (139.3 ± 13.8 s; $P < 0.001$). Curcumin alone is represented by bar E (Fig. 6), and the data show that curcumin alone at a dose of 34 mg/kg had a weaker effect on the immobility period than Zn(II)-curcumin at the same dose ($P < 0.05$). Immobility time in mice was decreased to 118.1 ± 19.5 s by curcumin at a dose of 34 mg/kg, whereas an immobility period of 108.5 ± 19.8 s was produced by Zn(II)-curcumin (34 mg/kg). When compared with the control group (139.3 ± 13.8 s), fluoxetine at a dose of 3 mg/kg significantly decreased the immobility time to 95.4 ± 18.9 s ($P < 0.001$). The effect of Zn(II)-curcumin was comparable to that of fluoxetine, which was used as a positive control in the FST. Zn(II)-curcumin significantly decreased the duration of immobility indicating an antidepressant effect.

3.5. Effect of Zn(II)-curcumin on the TST

As seen in Fig. 6, pretreatment with Zn(II)-curcumin had a significant effect in the TST. Zn(II)-curcumin at doses of 17, 34 and 68 mg/kg significantly decreased the immobility time of mice to 56.4 ± 12.1 , 37.1 ± 13.8 and 29.9 ± 10.6 s, respectively. When compared with the control group (77.5 ± 12.2 s), significant differences were observed ($P < 0.001$). Moreover, Zn(II)-curcumin at a dose of 68 mg/kg was able to shorten the immobility time to the same extent as fluoxetine. Compared with the control group, the immobility time was significantly decreased to 35.6 ± 13.5 s at 3 mg/kg of fluoxetine ($P < 0.001$). The data in Fig. 7 show that curcumin at a dose of 34 mg/kg significantly decreased immobility time to 60.4 ± 14.4 s, whereas an immobility period of 37.1 ± 13.8 s was produced by Zn(II)-curcumin (34 mg/kg). Pretreatment

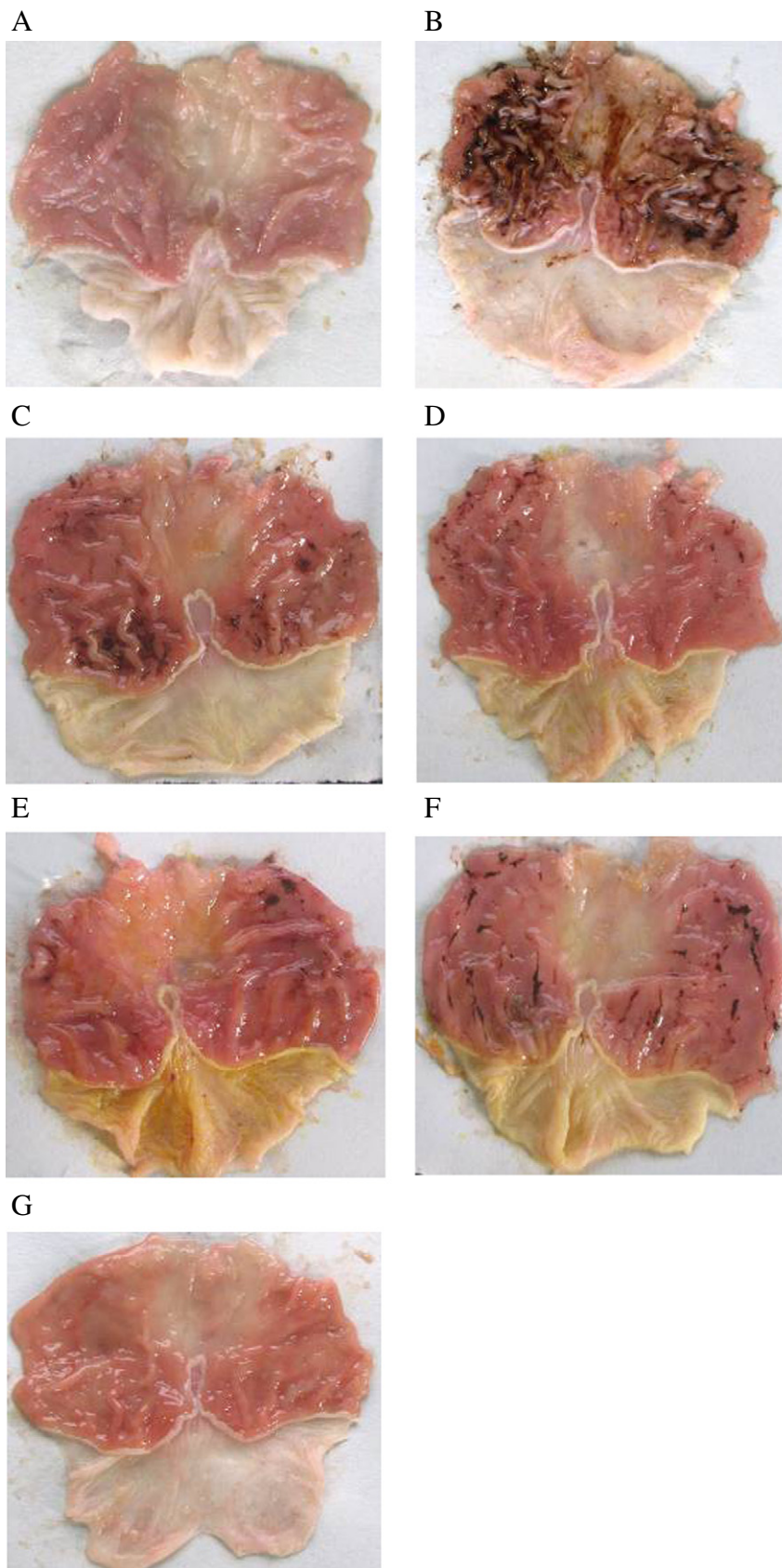


Fig. 2. Effect of Zn(II)-curcumin on gastric ulcers induced by cold-restraint stress (CRS) in rats. A: normal control group; B: control group damaged by CRS after pretreatment with PVP 300 mg/kg; C-E: Zn(II)-curcumin group treated with CRS after pretreatment with Zn(II)-curcumin 12, 24 or 48 mg/kg. F: Curcumin group treated with CRS after pretreatment with curcumin 24 mg/kg. G: Lansoprazole group treated with CRS after pretreatment with lansoprazole 7.8 mg/kg.

Table 2Effect of Zn(II)–curcumin on ulcer indexes, MDA, CAT SOD and H⁺–K⁺–ATPase from rat mucosa induced by CRS.

Treatment	Dose (mg/kg)	Ulcer indexes	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	H ⁺ –K ⁺ –ATPase (μmol Pi/mg protein/h)
Normal (PVP)	300	–	1.39 ± 0.40***	120.5 ± 18.1***	39.93 ± 9.90***	6.69 ± 1.74***
Control (PVP)	300	86.6 ± 11.1	3.13 ± 0.56	56.3 ± 10.6	18.67 ± 4.06	17.17 ± 4.40
Curcumin	24	36.8 ± 6.3***	2.67 ± 0.37**	75.8 ± 12.9*	22.96 ± 2.80*	8.15 ± 2.64***
Zn(II)–curcumin	12	34.9 ± 8.7***	2.60 ± 0.32**	79.7 ± 12.6*	24.45 ± 4.97*	12.52 ± 3.52*
	24	25.9 ± 6.8***##	2.29 ± 0.34***	88.2 ± 10.1***	27.01 ± 5.12***	10.51 ± 1.36***
	48	16.4 ± 5.9***	1.93 ± 0.29***	96.9 ± 16.2***	31.22 ± 7.35***	8.51 ± 2.47***
Lansoprazole	7.8	11.6 ± 5.3***	1.99 ± 0.58***	87.0 ± 21.2***	29.30 ± 8.15**	13.03 ± 2.55*

Each values represents the mean ± SD (n = 10 per group). **p* < 0.05, ***p* < 0.01, ****p* < 0.001, compared to control group (ANOVA followed by Dunnett's T3 multiple comparisons test). #*p* < 0.05, ##*p* < 0.01, compared to curcumin group (ANOVA followed by Dunnett's T3 multiple comparisons test).

with Zn(II)–curcumin at a dose of 34 mg/kg showed stronger antidepressant activity than curcumin at the same dose (*P* < 0.05).

3.6. Effect of Zn(II)–curcumin on the 5-HTP-induced head twitch test

As seen in Fig. 7, Zn(II)–curcumin administration led to a statistically significant head-twitch response, due to its inhibitory effect on 5-HT reuptake, which enhances 5-HT levels. Administration of 17 and 34 mg/kg Zn(II)–curcumin significantly increased the head twitch time to 46 ± 14, and 57 ± 19, respectively, in a dose-related manner, as compared with the normal control (29 ± 12; *P* < 0.05, *P* < 0.001). The maximum number of head twitches was observed after administration of 68 mg/kg Zn(II)–curcumin, demonstrating synergism between curcumin and the zinc ion. Pretreatment with Zn(II)–curcumin at a dose of 34 mg/kg, significantly increased the incidence of head twitches as compared with curcumin at the same dose (*P* < 0.05). Therefore, Zn(II)–curcumin enhances serotonergic-mediated behavior indicating the involvement of the serotonergic pathway in antidepressant activity.

4. Discussion

Stress ulcers are single or multiple mucosal defects, complicated by upper gastrointestinal bleeding during the physiologic stress of serious illness. The balance of offensive and defensive factors plays an important role in gastric hemorrhage and ulcer formation in the stomach. Various physical and psychological stressors cause gastric ulceration in humans and experimental animals (Demirbilek et al., 2004). Offensive factors include acid back-diffusion, acetic acid and oxidative stress while defensive factors involve SOD and CAT in tissues. Oxidative stress from oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in gastric mucosa, and scavenging these free radicals is one of the mechanisms implicated in the healing of gastric ulcers (Salim, 1991; Hung and Wang, 2004). An increased level of lipid peroxidation (LPO) is due to increased generation of ROS during stress leading to oxidative damage. SOD converts the reactive superoxide radical to H₂O₂, which if not scavenged by CAT, can cause LPO by the generation of hydroxyl radicals. Hence, a decrease in CAT levels can lead to increased accumulation of these ROS and thus increased LPO and tissue damage (Sairam et al., 2002). In the rodent, chronic mild stress-induced depression is accompanied by increased LPO in the cerebellum and the striatum (Lucca et al., 2009a, 2009b). MDA represents an end-product of the peroxidation of polyunsaturated fatty acids and related esters within cell membranes, and is currently regarded as a reliable index of oxidative tissue damage. Chakraborti et al. (2007) found that CRS increased MDA and decreased GSH, SOD and CAT levels in male and female rats (Chakraborti et al., 2007). Increases in serum LPO and decreases in SOD and CAT levels were observed in CRS-induced gastric ulceration (Tandon et al., 2004; Esterbauer et al., 1991).

Curcumin can effectively block indomethacin-induced increased LPO, thiol depletion, peroxidase inactivation, and overproduction of ·OH to prevent ROS-mediated gastric ulcers (Chattopadhyay et al., 2006). Curcumin (20, 40, and 80 mg/kg) exerted its antiulcer activity not only by affecting oxidative stress and total antioxidant capacity, but also by inhibiting IL-6 secretion and preventing apoptosis in a pylorus-ligated model (Tuorkey and Karolin, 2009). Zinc has different roles in protecting biological structures from damage by free radicals. It maintains an adequate level of metallothioneins, and is an essential component of SOD. Zinc may halt the progression of gastrointestinal disease by its participation in free radical scavenging, and therefore halt the inflammatory process. Zinc prevents indomethacin-induced gastrointestinal disease by free radical scavenging, which is due to its ability to stabilize sulfhydryl groups (Varghese et al., 2009). Zinc can protect against CRS-induced gastric ulcers by stabilization of lysosomal membranes (Cho et al., 1980). Zinc acexamate (100 mg/kg) prevents the development of deep erosions, which appear with high doses of aspirin (Bravo et al., 1990). A zinc deficient diet for 5 weeks significantly decreases serum zinc concentrations in rat stomachs, increase the gastric secretory volume, and increase acid and pepsin levels in CRS-induced ulceration in rat stomachs (Cho et al., 1987).

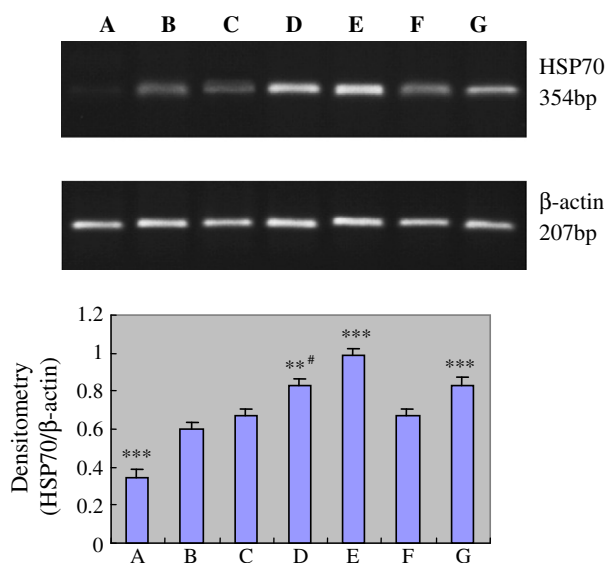


Fig. 3. Effect of Zn(II)–curcumin on HSP70 mRNA-expression in rat mucosa induced by CRS. After 4 h of CRS, the mRNA-expression of HSP70 was assayed by RT-PCR. The HSP70 mRNA-expression in the Zn(II)–curcumin treated groups increased in a dose-dependent manner (C, D and E). A: normal control group; B: control group damaged by CRS; C: Zn(II)–curcumin (12 mg/kg) group; D: Zn(II)–curcumin (24 mg/kg) group; E: Zn(II)–curcumin (48 mg/kg) group; F: curcumin (24 mg/kg) group; G: lansoprazole (7.8 mg/kg) group. Values are expressed as mean ± SD (n = 4/group). ***p* < 0.01, ****p* < 0.001, compared to control group (ANOVA followed by Dunnett's T3 multiple comparisons test). #*p* < 0.05, compared to curcumin group (ANOVA followed by Dunnett's T3 multiple comparisons test).

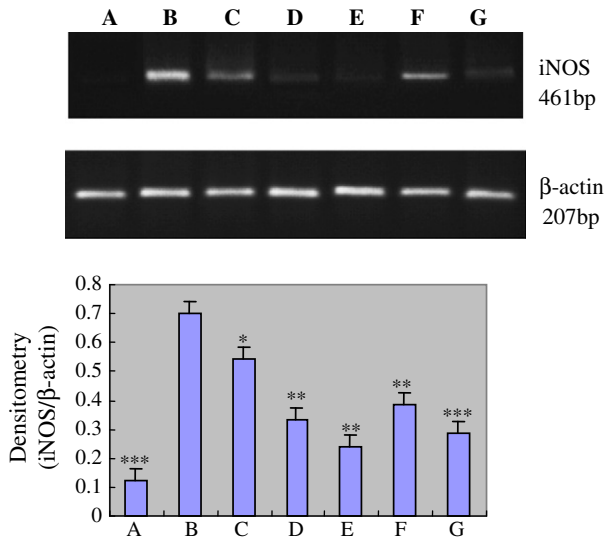


Fig. 4. Effect of Zn(II)-curcumin on iNOS mRNA-expression in rat mucosa induced by CRS. After 3.5 h of CRS, the mRNA-expression of iNOS was assayed by RT-PCR. A: normal control group; B: control group damaged by CRS; C: Zn(II)-curcumin (12 mg/kg) group; D: Zn(II)-curcumin (24 mg/kg) group; E: Zn(II)-curcumin (48 mg/kg) group; F: curcumin (24 mg/kg) group; G: lansoprazole (7.8 mg/kg) group. Values are expressed as mean \pm SD ($n=4$ /group). *** $p<0.01$, ** $p<0.001$, compared to control group (ANOVA followed by Dunnett's T3 multiple comparisons test). # $p<0.05$, compared to curcumin group (ANOVA followed by Dunnett's T3 multiple comparisons test).

In this study, MDA levels increased in the gastric tissue of stressed rats, indicating that CRS induces LPO. Zn(II)-curcumin at 48 mg/kg body weight (b.w.) protected against CRS-induced ulcer lesions by up to 81.1%, similar to that of lansoprazole (86.6%) at 8 mg/kg b.w., a known antiulcer drug. Furthermore, we extended our investigation by examining biochemical parameters such as alterations in H^+-K^+ -ATPase, MDA, and antioxidant enzyme levels including CAT and SOD, in the ulcerated stomach. The H^+-K^+ -ATPase of gastric parietal cells exchanges luminal K^+ for cytoplasmic H^+ , which results in gastric acidification. Hypersecretion of gastric acid, or a defect in its barrier function, is considered a principal casual factor in acid-related diseases such as gastric ulcers. Accordingly, the activity of gastric H^+-K^+ -ATPase was measured in the stomach homogenate, which showed a 3-fold upregulation of the enzyme in the ulcer condition which was normalized by treatment with Zn(II)-curcumin. These results were further substantiated by H^+-K^+ -ATPase inhibition by Zn(II)-curcumin at 12, 24 and 48 mg/kg, which was similar to lansoprazole (8 mg/kg), indicating the potential multi-targeted effect of Zn(II)-curcumin in preventing CRS-induced ulcers in experimental rats. Preventive antioxidant enzymes such as SOD and CAT are the first line of defense

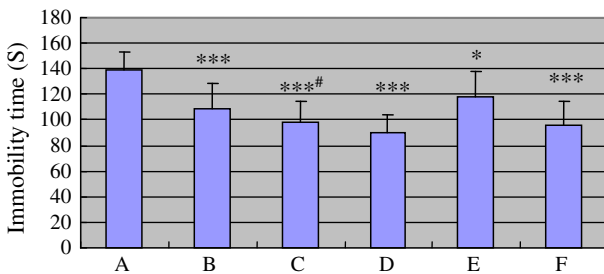


Fig. 5. Effects of Zn(II)-curcumin on the duration of immobility in the forced swim test in mice. A: control group; B: Zn(II)-curcumin (17 mg/kg) group; C: Zn(II)-curcumin (34 mg/kg) group; D: Zn(II)-curcumin (68 mg/kg) group; E: curcumin (34 mg/kg) group; F: fluoxetine (3 mg/kg) group. Values are expressed as mean \pm SD ($n=10$ /group). * $p<0.05$, *** $p<0.001$, compared to control group (ANOVA followed by Dunnett's T3 multiple comparisons test). # $p<0.05$, compared to curcumin group (ANOVA followed by Dunnett's T3 multiple comparisons test).

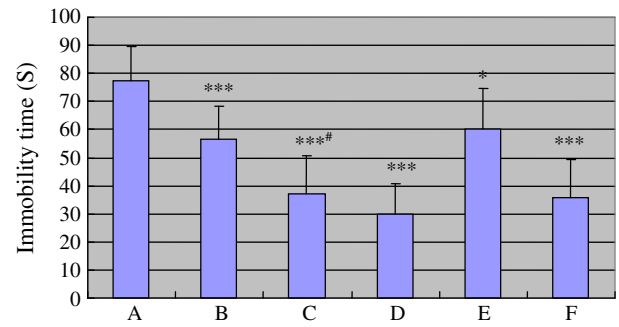


Fig. 6. Effects of Zn(II)-curcumin on the duration of immobility in the tail suspension test in mice. A: control group; B: Zn(II)-curcumin (17 mg/kg) group; C: Zn(II)-curcumin (34 mg/kg) group; D: Zn(II)-curcumin (68 mg/kg) group; E: curcumin (34 mg/kg) group; F: fluoxetine (3 mg/kg) group. Values are expressed as mean \pm SD ($n=10$ /group). ** $p<0.01$, *** $p<0.001$, compared to control group (ANOVA followed by Dunnett's T3 multiple comparisons test). # $p<0.05$, compared to curcumin group (ANOVA followed by Dunnett's T3 multiple comparisons test).

against ROS. Administration of Zn(II)-curcumin at 12, 24 and 48 mg/kg resulted in a significant increase in SOD and CAT, and reduced MDA levels (Table 2), compared with those in the control groups ($P<0.05$, $P<0.001$), suggesting that Zn(II)-curcumin was effective in preventing free radical-induced damage during ulceration. This illustrated a synergistic effect between curcumin and zinc. HSP70 is the principal eukaryotic stress-inducible heat shock protein and promotes the ability of organisms to survive multiple environmental stresses. HSP70 is crucial for the maintenance of cell integrity during normal cellular growth, as well as during pathophysiological conditions. Induction of HSPs is an important physiological response, enabling organisms to adapt and respond to stress. HSPs help the cells to survive otherwise lethal conditions. HSP induction also inhibits signal transduction pathways including NF- κ B, mitogen activated protein kinase and stress-activated kinases in the stimulation of proinflammatory cytokines and other inflammatory agents. Induction of HSPs, especially HSP72, inhibits translocation of NF- κ B to the nucleus, thereby inhibiting iNOS induction. Restoration of HSP70 suppressed gastric mucosal iNOS expression. Previous experiments have shown that reduced expression of HSP72 after famotidine-treatment was dramatically increased when zinc derivatives were administered 6 h after famotidine administration (Wada et al., 2006). Geranylgeranylacetone is a nontoxic antiulcer drug, and has recently been shown to induce HSP70. This property makes it a useful intervention in stomach dysfunction and probably other digestive tract disorders (Kawai et al., 2000). Geranylgeranylacetone use has also proved to be beneficial in treating hepatocellular damage caused by either ethanol or oxidant-related pathology (Ikeyama et al., 2001), or

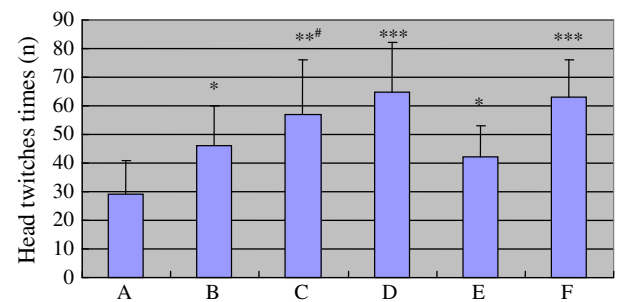


Fig. 7. Effects of Zn(II)-curcumin on the number of head twitches in the head twitch test induced by 5-hydroxy-L-tryptophan (5-HTP). A: control group; B: Zn(II)-curcumin (17 mg/kg) group; C: Zn(II)-curcumin (34 mg/kg) group; D: Zn(II)-curcumin (68 mg/kg) group; E: curcumin (34 mg/kg) group; F: fluoxetine (3 mg/kg) group. Values are expressed as mean \pm SD ($n=10$ /group). ** $p<0.01$, *** $p<0.001$, compared to control group (ANOVA followed by Dunnett's T3 multiple comparisons test). # $p<0.05$ compared to curcumin group (ANOVA followed by Dunnett's T3 multiple comparisons test).

damage due to hepatectomy (Oda et al., 2002). Statistical analysis showed a significant increase in HSP70 following oral zinc supplementation on the stress response, which indicated that dietary zinc was a chaperone inducer and a major determinant of the stress response in human lymphocytes (Putics et al., 2008). Several reports have shown that zinc treatment induces HSP70 expression *in vitro*, demonstrated in several mammalian cells and mucosal cells during transient stress-like conditions (Ambra et al., 2004). Zinc-carnosine was shown to prevent acetic acid-induced colitis in rats *via* induction of HSP72 and suppression of the transcription factor NF- κ B (Odashima et al., 2006). Zinc chloride exerted an antidepressant-like effect in the FST and showed an anti-immobility effect of zinc in the TST (Szweczyk et al., 2008). In mice, administration of WAY 1006335 (5-HT_{1A} antagonist) completely antagonized the antidepressant-like effect induced by zinc in the FST (Szweczyk et al., 2009). The synergistic antidepressant-like effect of zinc and the selective serotonin reuptake inhibitors (SSRI) fluoxetine, paroxetine and citalopram in mouse FST and TST indicates that zinc activity might be associated with modulation of serotonin neurotransmission (Cunha et al., 2008).

Pro-inflammatory cytokines and chemokines released after various stress stimuli (e.g. immobilization stress) initiate an inflammatory reaction characterized by the activation of inflammatory cells and the stimulated activity of enzymes, including iNOS, which produces high amounts of NO. Acute and chronic stress cause depression and anxiety-like behaviors in rats, and there are studies showing that the acute inhibition of NOS prevents these acute and chronic stress-induced behaviors, indicating that stress and NO may be involved in the generation of anxiety and depression (Sevgi et al., 2006). iNOS inhibitors or scavengers of NO are useful pharmacologic tools for stress-induced ulcers or depression.

Previous experiments have shown that zinc suppresses cytokine-induced iNOS expression or NO production, which in part, explains the anti-inflammatory property of zinc (Yamaoka et al., 2000). In a lung injury experiment produced by moderate zinc deficiency, iNOS activity was shown to increase in lungs and contribute to lung injury, which indicates that zinc ions suppress iNOS activity in pulmonary cells (Gomez et al., 2006). The antidepressant-like effect of zinc is mediated through a mechanism dependent on the blockade of NMDA receptors and on NOS inhibition (Rosa et al., 2003).

iNOS, which is mainly detected in gastric mucosa, was increased during the development of CRS-induced injury in rats. Administration of Zn(II)-curcumin at 12, 24, and 48 mg/kg b.w. inhibited this damage, and was accompanied by a decrease in the expression of iNOS. NF- κ B is a major transcription factor involved in iNOS gene expression. Our earlier studies suggested that Zn(II)-curcumin prevented pylorus-ligation-induced lesions in rats by inhibiting NF- κ B activation and the subsequent production of proinflammatory cytokines (Mei et al., 2009). Its antiulcer effect might be attributable to its capacity to reduce gastric acid secretion and enhance the mucosal defense mechanism through the suppression of NF- κ B-mediated inflammation.

In this study, we observed that Zn(II)-curcumin (12, 24 and 48 mg/kg) significantly decreased the immobility time in the FST and TST in mice, indicating an antidepressant effect. Moreover, Zn(II)-curcumin (12, 24 and 48 mg/kg) indirectly released 5-HT, which in turn activated 5HT receptors which then increased head twitch times.

In conclusion, these results show that the antilcerogenic and antidepressant activity of Zn(II)-curcumin indicates a synergistic effect between curcumin and zinc. The effect of Zn(II)-curcumin augments mucosal resistance by its participation in free radical scavenging and increasing HSP70 expression, thereby attenuating the increase in iNOS expression. The present experimental data show that Zn(II)-curcumin possibly produces its antidepressant effect by indirectly releasing 5-HT, which in turn activates 5-HT receptors as part of the mechanism of its antidepressant activity. Zn(II)-curcumin may be developed as a new drug for the treatment of ulcers and depression due to the synergism between curcumin and zinc.

Acknowledgments

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