AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Antidepressant Activity of Curcumin Loaded Solid Lipid Nanoparticles (C-SLNs) In Mice

Vandita Kakkar[1], Indu Pal Kaur[2]*

1. Doctoral Research Fellow, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India, 160014.

2. Associate Professor, Department of Pharmaceutics, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India, 160014.

ABSTRACT

Curcumin a hydrophobic poly-phenol is derived from turmeric, the rhizome of the herb *Curcuma longa* L. Curcumin has been shown to exert anti-depressant effects in rodent models. However, poor bioavailability of curcumin curbs its usage as a therapeutic agent. In view of the above curcumin loaded solid lipid nanoparticles (C-SLNs) were prepared and evaluated for the antidepressant effect of acute administration of C-SLNs (1, 2.5, 5 and 10 mg/kg, p.o.) in the forced swim model of depression in mice. C-SLNs exhibited 47.42%, 67.39%, 31.67% and 36.2% reduction in immobility time after administration of 1, 2.5, 5 or 10 mg/kg dose (p.o.) respectively. Free curcumin however did not result in a significant reduction, except at 2.5 mg/kg, which could produce a reduction of 21.7% but was still 2.83 times lower than the effect obtained with a similar dose of C-SLNs. The results obtained may be assigned to the therapeutic amounts of curcumin reaching the brain. Thus, C-SLNs with their improved bioavailability and permeability possess higher anti-depressant potential upon administration of a single and a much lower dose when compared to free curcumin.

Keywords: Curcumin, solid lipid nanoparticles, antidepressant, bioavailability, forced swim test

*Corresponding Author Email: indupalkaur@yahoo.com

Received 12 August 2011, Accepted 12 October 2011

Please cite this article in press as: Kaur I *et al.*, Antidepressant Activity of Curcumin Loaded Solid Lipid Nanoparticles (C-SLNs) In Mice . American Journal of PharmTech Research 2012.

INTRODUCTION

Depression is a common, debilitating, life-threatening illness with prevalence rate between 9% and 18% in the Western world [1]. According to World Health Organization it is the fourth leading cause of disability worldwide.Current antidepressant drugs, including various monoamine reuptake inhibitors and monoamine oxidase inhibitors, are erratically effective and are known to exert undesirable side effects. Thus it becomes imperative to look upon the development of safe and powerful antidepressant agents from traditional herbs.

Curcuma longa is commonly found in Chinese herbal medicines including Xiaoyao-san, *Xiaodingzhi-Wan*, *Banxia houpu-Tang*, *Xiaobuxin-Tang*, *Chaihu jia longgu muli- Tang*, *Baihe dihuang-Tang*, and *Gai mai dazao-Tang* used to treat the symptoms of mental stress, hypochondriac distensive pain and mania[2].

Although curcumin is a well known pleiotropic molecule, its therapeutic efficacy is limited, due to its low bioavailability (BA) and solubility. Envisaging the above, it is anticipated that enhancing the BA [3, 4] of curcumin would help to result in better therapeutic effects in depression. In a study by Bhuttani et al. the combination of piperine (2.5 mg/kg, i.p., 21 days), a bioavailability enhancer, with curcumin (20 and 40 mg/kg, i.p., 21 days) in a pretreatment study showed significant antidepressant effects as compared to free curcumin[5].

The current study was planned with an aim to assess the antidepressant effects of bio-enhanced C-SLNs administered per orally (p.o.; 1, 2.5, 5 and 10 mg/kg). The protocol was designed in view of the fact that the C-SLNs with their enhanced bioavailability (32-155 times as demonstrated by us in the earlier pharmacokinetic study[3], and pharmacodynamic study in Alzheimer's[6] would result in better therapeutic effects than free curcumin in alleviating depression.

MATERIALS AND METHODS

Animals

Male Lacca mice weighing 20–30 g (Central Animal House, Panjab University, Chandigarh) were used for the study. Animals were housed under standard (25 ± 2 °C, 60–70 % humidity) laboratory conditions on normal dark and light cycle. Standard rat chow pellets and water was allowed *ad libitum*. Animals were acclimatized to laboratory conditions before the test. All behavioural experiments were carried out between 10.00–17.00 hr. The experimental protocols were approved by the IAEC and conducted according to the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA).

Treatment schedule

The animals were divided into 10 groups (n = 6) and all the treatments were orally administered. Various treatment groups include; Group I (Control group): distilled water; Group II-blank SLNs; Group III- C-SLNs (1 mg/kg) body weight (bw); Group IV-SLN 2.5 : C- SLNs 2.5 mg/kg (bw); Group V -SLN 5 :C- SLNs 5 mg/kg bw; Group VI-SLN 10: C- SLNs 10 mg/kg bw; Group VII FC2.5: free curcumin (FC) 2.5 mg/kg bw; Group VIII-FC 5 mg/kg bw; Group IX- FC 10 mg/kg bw; Group X: FC 20 mg/kg bw. The experiments were conducted 60 min after the treatment.

Preparation of curcumin loaded solid lipid nanoparticles (C-SLNs)

Curcumin loaded solid lipid nanoparticles were prepared using micro-emulsification technique using the procedure as detailed. Polysorbate 80 (45.45%w/w), soy lecithin (0.58%w/w), and water were placed together in a beaker and heated to the lipid melt temperature. Lipid (7.27%w/w) was melted separately at 82-85ºC. Curcumin was added to the aqueous phase, following which the hot aqueous emulsifier mix, was dropped at once into the lipid melt, under magnetic stirring to obtain a clear microemulsion. The hot microemulsion thus formed, was transferred into an equivalent amount of cold water (~2°C) under continuous mechanical stirring (5000 rpm) for 1.5 h. In the aqueous medium, SLNs are formed by crystallization of the oil droplets present in the microemulsion [3, 7]. The prepared SLNs were stored in a refrigerator until further analysis.

Forced swim test (FST)

Following 60 min. post administration of free curcumin or C-SLNs at proposed doses, the forced swim test was performed. This study was carried out in mice (n =6/group) according to the method described by Porsolt et al[8] .

Mice were dropped individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water maintained at 23–25°C. Mice were left in the cylinder for 5 min. After the first 2 min, the total duration of immobility in mice was measured during a 3-min test. The mouse was judged to be immobile, and the time period recorded when it remained floating passively in the water.

Statistical Analysis

All data was analysed via one-way analysis of variance (ANOVA) using SigmaStat 2.0 software (Jandel Scientific); Data presented are mean ± SEM (n=6). A value of $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Antidepressant effects of curcumin in animal models are reportedly mediated by the modulation of central mono aminergic neurotransmitter systems. Previous studies have shown that curcumin inhibits the activity of monoamine oxidase (MAO) in C6 glial cells; MAO plays a central role in several psychiatric neurological disorders, including clinical depression and anxiety [9] there is evidence that MAO inhibitor-induced increase in mono aminergic neurotransmission can alleviate clinical depression[10]

In acute study, after single administration of free curcumin (FC) 2.5, 5, 10 and 20 mg/kg and C-SLNs (1, 2.5, 5 and 10 mg/kg), a marked decrease in the immobility time was observed in the forced swim test. (Figure 1;Table 1). Mice administered C-SLNs exhibited 47.42%, 67.39%, 31.67% and 36.2% reduction in immobility time after administration of 1, 2.5, 5 or 10 mg/kg dose (p.o.) respectively.

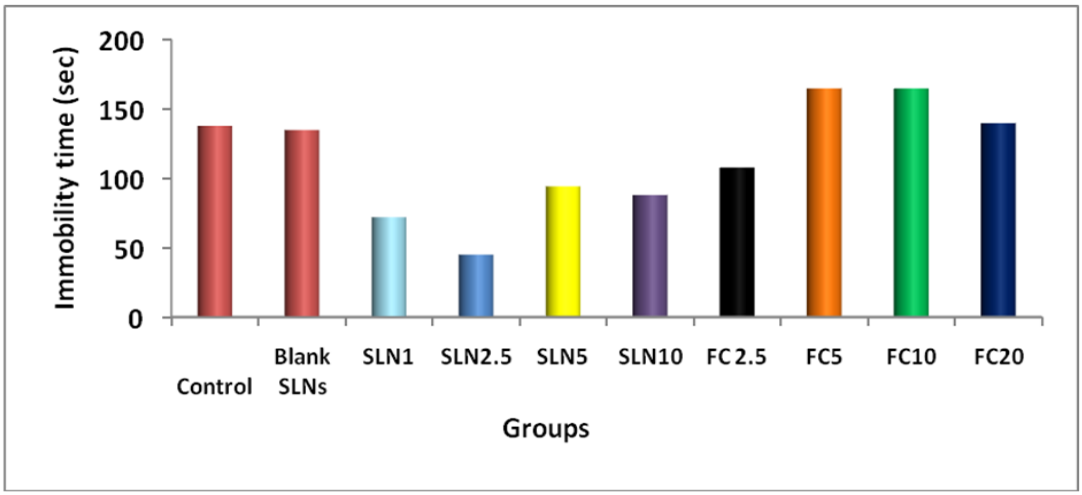

Figure 1. Effect of different doses of FC or C-SLNs on the immobility time in the mice forced swim test

Table 1. Mean values ± SEM of immobility time in FST for depression

Groups	Mean immobility time (sec)	% Reduction (w.r.t control)
Control	138±12	0
Blank SLNs	135±19	2.17
SLN1	72±9*	47.83
SLN2.5	45±5.58*	67.39
SLN5	94.3±7.72*	31.67
SLN10	88±13.2*	36.24
FC 2.5	108±12.4*	21.74
FC5	165±12.4	-19.57
FC10	165±16.25	-19.57
FC20	140±12.4	-1.45

* All the values are significantly different from the control group ($p \leq 0.05$).

On the other hand free curcumin resulted in a significantly lower effect W ($p \leq 0.05$) with 21.7% reduction in immobility at a dose of 2.5 mg/kg which was almost 2.83 times lesser than observed with C-SLNs at the similar dose. Further, it was observed that free curcumin at all the other doses (5, 10 and 20 mg/kg) did not produce any significant reduction ($p \leq 0.05$) with respect to control. Treatment with blank SLNs also did not show any reduction in immobility time as compared to the control group indicating the role played by curcumin in mitigation of depression when incorporated into SLNs.

Most of the studies discussing the antidepressant effects of curcumin have been conducted at doses higher than 20 mg/kg except a study [11]which explored the effectiveness of curcumin at doses as low as 1.25 mg/kg. Further, to this the effects were elicited only after chronic administration (both p.o. and i.p. route are reported in different studies) of curcumin varying from 14-21 days. Moreover, there exists no database on treatment with any novel curcumin formulation in alleviation of depression. However, combination of piperine (at a sub-optimal dose of 2.5 mg/kg, i.p. for 21 days) a bioavailability enhancer, with curcumin (20 mg/kg, i.p., 21 days) in a pretreatment study, showed a significant decrease in immobility time during the forced swim test, in comparison to curcumin used alone, and the effect matched the 40 mg/Kg dose of curcumin. However, the combination did not show any significant improvement in the MAO B activity[5].

In the present study, the antidepressant effect of curcumin and its developed solid lipid nanoparticles (C-SLNs) were evaluated after administration of a single dose, 60 min. prior to the 'forced swim task', a well established model of depression[8-12]. Administration of C-SLNs resulted in a significant reduction (47.83%) in the immobility time in the forced swim test at a dose as low as 1 mg/kg bw. The effect increased to 67.39% with 2.5 mg/kg bw dose and then decreased with higher doses. However, no dose-dependent reduction could be observed within C-SLN groups at $p < 0.05$. This could be attributed to the fact that the amount of curcumin absorbed (60–66% of the given dose) remains constant regardless of the dose indicating that administration of more curcumin does not result in higher absorption. Similar observation, that is, there is a dose-dependent limitation to bioavailability in rats has been reported earlier[13] and recently by us[3]. Furthermore, significant effects obtained with single doses of C-SLNs, in the present investigation, indicate an atypical antidepressant nature of curcumin; the same is reported earlier also[14].

The results obtained may be assigned to the therapeutic amounts of curcumin reaching the brain. C-SLNs possess high permeability to cross the blood brain barrier to reach the target sites.

Further, as a result of incorporation of curcumin in the lipidic core of the solid lipid nanoparticles, curcumin is protected from the physiological and enzymatic degradation. The above achieved protection spares greater amounts of active curcumin to produce therapeutic effects. As curcumin is known to alter the levels of serotonin and dopamine, it may be anticipated that with a more bioavailable form of curcumin, higher amounts reach the brain, thus producing greater alleviation in the antidepressant effects as compared to free curcumin. An effect obtained with such a low and a single dose (1 mg/kg) of C-SLNs in the present study indicates that even minute quantities of curcumin can elicit an effect i.e the rate limiting step is permeability across and delivery to the brain and not the concentration achieved in the brain. It seems that curcumin triggers a cascade of events which results in neuroprotection once it reaches the brain. This is expected out of neurohormetics and curcumin is proposed as a neurohormetic in in vitro experiments [15,16] Oobservations in volunteers and patients also suggest that curcumin might possess biological activity even at low oral doses[17].

Further, free curcumin did not show any reduction in depression at doses of 5, 10, and 20 mg/kg. The dose of 2.5 mg/kg of curcumin although showed some effect, was 2-3 times lower than that obtained with C-SLNs at the same dose. A study report by Xu et al[11] in rats demonstrated 18.7%, 38.7%, 63.5% and 68.9% reduced immobility with curcumin administered chronically at 1, 2.5, 5 or 10 mg/kg dose (p.o.), respectively for 14 days, as compared with the control group. We could however, achieve better effects (2.54 times at 1mg/kg and 1.77 times at 2.5 mg/kg) after a single dose administration of C-SLNs at a 10 times lower dose, indicating the potential usefulness of incorporating curcumin into SLNs. Combination of piperine with 20 mg/Kg dose of i.p. administered curcumin resulted in about 59% reduction in immobility[5] All the other studies also report on antidepressant effects of curcumin after chronic administration only. Further, this is the first study report elaborating the antidepressant effect of curcumin after making it bioavailable using suitable formulation strategies. The present study gives a clear picture of role of pharmaceutical countering to result in a bioavailable molecule with enhanced therapeutic effect.

CONCLUSION

From the above study it can be claimed that C-SLNs with their improved bioavailability and permeability[3] possess much higher potential to act as an anti-depressant upon administration of a single and a much lower dose when compared to free curcumin.

ACKNOWLEDGEMENT:

The contingent grant provided by CSIR to Ms Vandita Kakkar is highly acknowledged.

REFERENCES:

1. Schloss P, Henm FA. New insights into the mechanisms of antidepressant therapy. Pharmacol Ther. 2004; 102:47- 60.
2. Kong LD, Cheng CHK, Tan RX. Monoamine oxidase inhibitors from rhizome of Coptis chinensis. Planta Med 2001; 67:74- 6.
3. Kakkar V, Singh S, Singla D, Kaur IP. Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin Mol Nut Food Res. 2011; 55:495-503.
4. Kakkar V, Singh S, Singla D, Sahwney S, Chauhan AS, Singh G, et al. Pharmacokinetic applicability of a validated liquid chromatography tandem mass spectroscopy method for orally administered curcumin loaded solid lipid nanoparticles to rats. J Chromatogr B. 2010; 878(32):3427-31.
5. Bhutani MK, Bishnoi M, Kulkarni SK. Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. Pharmacol Biochem Behav 2009;92(1):39-43.
6. Kakkar V, Kaur IP. Evaluating potential of curcumin loaded solid lipid nanoparticles in aluminium induced behavioral, biochemical and histopathological alterations in mice brain. Food Chem Toxicol 2011; 49:2906-13.
7. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol 2005; 27:127.
8. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1997; 229:327- 36.
9. Mazzio EA, Harris N, Soliman KF. Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells. Planta Med. 1998; 64:603- 6.
10. Dar A, Khatoon S. Behavioral and biochemical studies of dichloromethane fraction from the Areca catechu nut. Pharmacol Biochem Behav 2000; 65:1-6.
11. Xu Y, Ku B-S, Yao H-Y, Lin Y-H, Ma X, Zhang Y-H, et al. The effects of curcumin on depressive-like behaviors in mice. Eur J Pharmacol 2005; 518(1):40-6.
12. Kulkarni SK, Bhutani MK, Bishnoi M. Antidepressant activity of curcumin: involvement of serotonin and dopamine system. Psychopharmacology. 2008; 201(3):435-42.
13. Ravindranath V, Chandrasekhara N. Metabolism of curcumin--studies with [3H] curcumin. Toxicology. 1981; 22:337-44.

14. Janscar S, Leonard BE. The olfactory bulbectomized rat as a model of depression. In: Usdin E, Goldstein M, Friedhoff AJ, Georgotas A, editors. Frontiers in neuropsychiatric research. New York Macmillan Press; 1983:357-72.
15. Calabrese EJ, Baldwin LA. Defining hormesis. Hum Exp Toxicol 2002;21:91-7.
16. Kakkar V, Kaur IP. Evolving the concept of curcumin as a neurohormetic. Pharmacos 2011;37:25-8.
17. Sharma RA, Steward WP, Gescher AJ. Pharmacokinetics and Pharmacodynamics of Curcumin. Bharat B. Aggarwal Y-JSaSS, editor: Springer US; 2007.