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Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease

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ABSTRACT

Curcuminoids (a mixture of curcumin, bisdemethoxycurcumin and demethoxycurcumin) share vital pharmacological properties possessed by turmeric, a well known curry spice, considered useful in Alzheimer's disease (AD). The aim of this study was to evaluate if curcuminoids possess acetylcholinesterase (AChE) inhibitory and memory enhancing activities. The in-vitro and ex-vivo models of AChE inhibitory activity were used along with Morris water maze test to study the effect on memory in rats. Curcuminoids inhibited AChE in the in-vitro assay with IC_{50} value of 19.67, bisdemethoxycurcumin 16.84, demethoxycurcumin 33.14 and curcumin 67.69 μM. In the ex-vivo AChE assay, curcuminoids and its individual components except curcumin showed dose-dependent (3–10 mg/kg) inhibition in frontal cortex and hippocampus. When studied for their effect on memory at a fixed dose (10 mg/kg), all compounds showed significant ($p<0.001$) and comparable effect in scopolamine-induced amnesia. These data indicate that curcuminoids and all individual components except curcumin possess pronounced AChE inhibitory activity. Curcumin was relatively weak in the in-vitro assay and without effect in the ex-vivo AChE model, while equally effective in memory enhancing effect, suggestive of additional mechanism(s) involved. Thus curcuminoids mixture might possess better therapeutic profile than curcumin for its medicinal use in AD.

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1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease with progressive loss in memory (Blennow et al., 2006). AD is characterized by the deposition of the senile plaques mainly composed of β-amyloid (Aβ) fragment and neurofibrillary tangles (Blennow et al., 2006; Selkoe, 2001). Despite intensive advancement in research, available therapeutic options are limited, thus, increasing demand for new drugs. In the recent past, medicinal plants attracted attention due to their potential role in dementia (Akhondzadeh et al., 2003; Le Bars et al., 1997; Ng et al., 2006; Wettstein, 2000).

Turmeric (rhizome of *Curcuma longa*) is a medicinal herb of high repute all over the world particularly in South Asia, where it is also used as curry spice in foods (Gilani et al., 2005; Goel et al., 2008). Turmeric has been traditionally used for its medicinal value in wound healing, inflammation, asthma, epilepsy, gall bladder stones, abdominal cramps, high cholesterol, congestion and AD (Duke, 2002; Kapoor, 1990; Nadkarni, 1986), but underlying pharmacological mechanism in most disorders particularly in AD is not yet clear. There is some evidence that curry consumption in old age is associated with better cognitive function (Ng et al., 2006), but there is lack of scientific evidence supporting the use of turmeric in AD.

Literature search revealed the presence of different active principles isolated and characterized in search for compound(s) with potential pharmacological properties, which include curcumin, bisdemethoxycurcumin, demethoxycurcumin, eugenol, dihydrocurcumin, azulene, D-camphene, caprylic acid, cineol, turmerone and zingiberine (Duke, 1992). It is also reported to contain β-caryophyllene, β-bisabolene and β-sesquiphellandrenendrene (Qin et al., 2007).

Compounds derived from the turmeric have shown an array of pharmacological properties. Curcumin, which is considered the main active constituent responsible for majority of the medicinal properties of turmeric, has been studied most extensively with particular focus on anticancer activity (Aggarwal et al., 2003; Duvoix et al., 2005; Kamat et al., 2007; Kunnumakkara et al., 2008). Curcumin is present in the largest quantity (75–80%) in curcuminoids and it is one of the well studied biologically active molecule of the turmeric exhibiting antioxidant and anti-inflammatory activities (Eybl et al., 2006; Mukhopadhyay et al., 1982; Reddy and Lokesh, 1994; Zhao et al., 1989), along with having potential in transgenic mouse model of AD (Lim et al., 2001). It has also been shown that curcumin through metal binding (Baum and Ng, 2004; Daniel et al., 2004), inhibiting Aβ fibril formation and destabilizing preformed fibril may provide protection in AD (Ono et al., 2004b). On the other hand, curcuminoids is a mixture of

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curcumin 75–80%, demethoxycurcumin 15–20% and bisdemethoxycurcumin 3–5% (Aggarwal et al., 2007) and possess the ability to inhibit lipid peroxidation in rat brain homogenates better than α-tocopherol (Sreejayan and Rao, 1994). Curcuminoids enhance Aβ clearance by promoting uptake through macrophages (Zhang et al., 2006). In addition to this, the potential of curcuminoids to protect cells from Aβ (1–42) insults (Kim et al., 2001) and to inhibit in-vitro Aβ fibril formation (Kim et al., 2005) indicate that curcuminoids may have potential to get place in therapeutics for the treatment of AD.

Until now, acetylcholinesterase (AChE) inhibitors are the major class of drugs approved for AD, providing symptomatic relief and resulting in improvement in cognitive function (Blennow et al., 2006). Curcuminoids as a mixture can serve as a better treatment option for AD or curcumin, the main component of the curcuminoids, is an open question; furthermore, it would be interesting to know if the curcuminoids along with individual compounds possess AChE inhibitory activity. In this report, curcuminoids as a mixture of three compounds is compared with the individual components for their AChE inhibitory along with memory enhancing activities.

2. Materials and methods

2.1. Drugs and chemicals

Physostigmine, electric eel acetylcholinesterase, acetyl thiocholine iodide and 5, 5-dithiobis (2-nitrobenzioc) acid were obtained from the Sigma Chemical Company, St. Louis, MO, U.S.A. Curcuminoids >95% purity (having bisdemethoxycurcumin 4.15%, demethoxycurcumin 16.53% and curcumin 79.52%) and its individual components, bisdemethoxycurcumin (78% purity), demethoxycurcumin (98% purity) and curcumin (98.35% purity) were generous gifts from the Sabinsa Group of Companies, 70 Ethel Road West, Unit 6, Piscataway, NJ 08854, USA. Purity of the curcuminoids and individual components was established by the Sami Labs Ltd, Banglore, India (part of the Sabinsa Group of Companies) through HPLC. Drug solutions were made fresh on the day of experiment.

2.2. Animals

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996) and the protocol was approved from the Ethical Committee for Research on Animals (ECRA), Aga Khan University. Male, Sprague–Dawley rats (180–250 g) were bred and housed in the animal house of the Aga Khan University under a controlled environment (23–25 °C). Animals were given tap water *ad libitum* and a standard diet consisting of (g/kg): flour 380, fiber 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium meta bisulphate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

2.3. In-vitro acetylcholinesterase assay

The modified method of Ellman et al. (1961) was used. Electric eel acetylcholinesterase was used, while acetyl thiocholine iodide (ATCI) was used as a substrate of the reaction. 5, 5-dithiobis (2-nitrobenzioc) acid (DTNB) was used for the measurement of AChE activity. In this procedure, 150 µl of 0.1 M sodium phosphate buffer (pH 8.0), 10 µl test compound solution (in ethanol), and 20 µl of enzyme solution (0.09 units/ml) were mixed and incubated for 15 min at 25 °C. 10 µl of DTNB (10 mM) was then added and reaction was initiated by the addition of substrate (10 µl of ATCI, 14 mM solution). The hydrolysis of the ATCI can be measured by the formation of the colored product 5-thio-2-nitrobenzoate anion formed by the reaction of DTNB and thiocholine, which is released by the hydrolysis of enzyme. The formation of the coloured product was measured at 410 nm wave length after 10 min. Physostigmine, a standard AChE inhibitor, was used as positive control, which was dissolved in ethanol. Percent acetylcholinesterase inhibition was calculated using following formula.

$$\text{Percentage inhibition} = 100 - \left[\frac{\text{Absorbance of the test compound}}{\text{Absorbance of the solvent}} \times 100\right]$$

2.4. Ex-vivo acetylcholinesterase assay

AChE activity in rat brain was measured as described earlier (Isoma et al., 2002). Different doses of the test compounds were administered intra-peritoneally. Animals were sacrificed by decapitation under ether anesthesia 1 h after the drug administration. The frontal cortex and hippocampus were dissected out in ice cold 0.1 M phosphate buffer saline (pH 8.0). These tissues were homogenized in ice cold 0.1 M phosphate buffer saline (pH 8.0) using homogenizer. The homogenates were centrifuged at 1000 ×*g* for 10 min at 4 °C, and supernatant was used as a source of enzyme in AChE assay adopting same assay procedure as explained above. Protein concentration in the supernatant was measured using Bradford (1976) method.

2.5. Morris water maze test

The testing procedure was same as that described previously by Morris (1984). The experimental apparatus consisted of a circular water tank (120 cm in diameter, 45 cm high). An invisible platform (15 cm in diameter, 35 cm high) was placed 1.5 cm below the surface of water. Water temperature was kept at 21–23 °C. The pool was located in a test room and many clues external to the maze were visible from the pool (e.g., pictures, lamps, etc.), which could be used by the rats for spatial orientation. The position of the cues was kept constant throughout the task.

2.5.1. Training trial

The training trials were carried out before administration of scopolamine, or other drugs. Each rat received 2 trials per day for 7 consecutive days. At the start of a trial, rats were placed randomly at one of four fixed starting points facing the wall (designated North, South, East and West) and were allowed to swim for 90 s, or until they escape the task by finding the platform. The platform was located in a constant position throughout the test period in the middle of one quadrant, equidistant from the center and edge of the pool. In each training session, the latency to escape to the hidden platform was recorded. If the rat found the platform, it was allowed to remain there for 20 s and then returned to its home cage. The rats that could not reach the platform in 20 s on the 7th day were excluded.

2.5.2. Test trial

Immediately after the fourteenth training trial on the 7th day, the trained rats were injected scopolamine (2 mg/kg, i.p.); 30 min later, test compounds (doses in mg/kg, i.p.) were injected. One hour after the administration of test compounds, rats were allowed to swim and the time spent to reach the platform was recorded.

2.6. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM; n = number of experiments). Median inhibitory concentrations (IC_{50} values) are represented with 95% confidence intervals (CI). Results were analyzed statistically using "Graphpad Prism" software and taken as significant only if the p value was less than 0.05.

3. Results

3.1. Effect on in-vitro acetylcholinesterase activity

When studied for its possible inhibitory effect in the in-vitro assay, curcuminoids mixture showed AChE inhibitory activity in a dose-

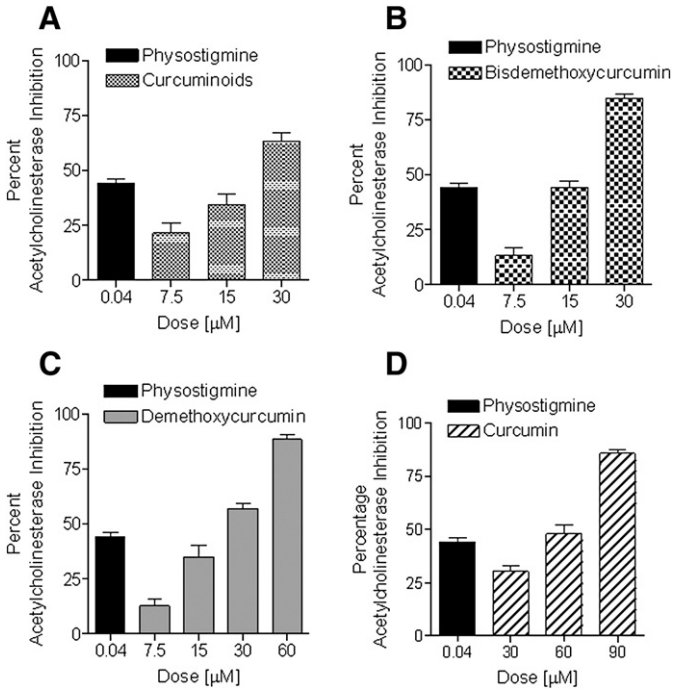

Fig. 1. Bar diagrams showing effect of curcuminoids mixture and its individual components in the in-vitro AChE assay: All experiments on 4 different test materials were conducted at the same time using physostigmine as a single positive control. Error bars represent mean±SEM; $n=5$. Note: Dose ranges on the *X*-axis are selected to show AChE inhibitory effect with in the range of 15–80% response, thus allowing to calculate IC_{50}values through regression analysis.

dependent manner with an IC_{50} value of 19.67 μM (14.31–25.02; 95% CI) as shown in Fig. 1. When individual components of curcuminoids were studied, all of the test compounds were able to inhibit AChE activity dose-dependently with varying potency. Bisdemethoxycurcumin was the most potent with IC_{50} values of 16.84 μM (15.07–18.60) while, demethoxycurcumin was found intermediate in potency with IC_{50} value of 33.14 μM (29.08–37.19) and curcumin was the least potent with IC_{50} value of 67.69 μM (63.71–71.66).

In another set of experiments, the effect of curcuminoids mixture was compared with that of individual components using a fixed dose (30 μM). Bisdemethoxycurcumin was found more effective than the parent curcuminoids ($p<0.01$), while, demethoxycurcumin shared comparable inhibitory effect ($p>0.05$) and curcumin was found less active ($p<0.001$) when compared to the parent curcuminoids mixture

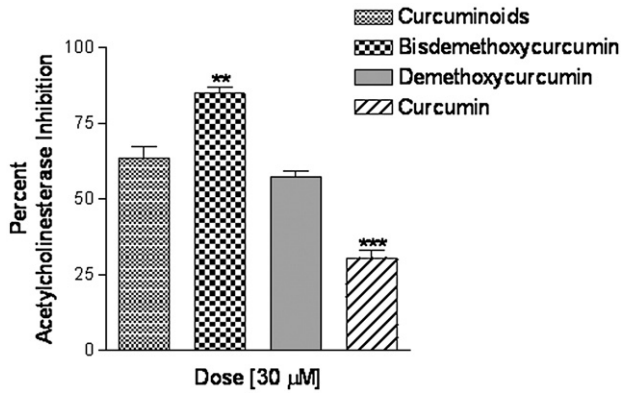

Fig. 2. Comparison of curcuminoids mixture with its individual components for AChE inhibitory activity using single dose (30 μM): Error bars represent mean±SEM; $n=5$. $^{**}p<0.01$ and $^{***}p<0.001$ compared with curcuminoids.

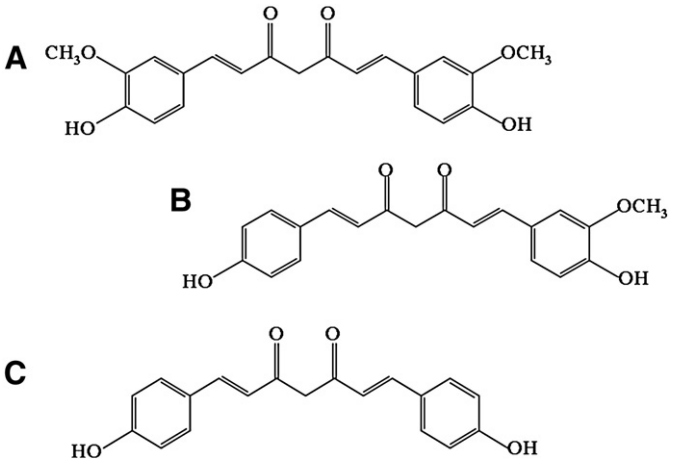

Fig. 3. Chemical structure of curcumin (A), demethoxycurcumin (B) and bisdemethoxycurcumin (C).

(Fig. 2). It appears that the adding methoxy group (-OCH_3) in the chemical structure reduces AChE inhibitory activity (Fig. 3).

3.2. Ex-vivo acetylcholinesterase assay in frontal cortex and hippocampus

Knowing the fact that there is cholinergic hypofunction in AD (Whitehouse et al., 1982), our next question was to see if curcuminoids also inhibit AChE in hippocampus and frontal cortex. One hour after

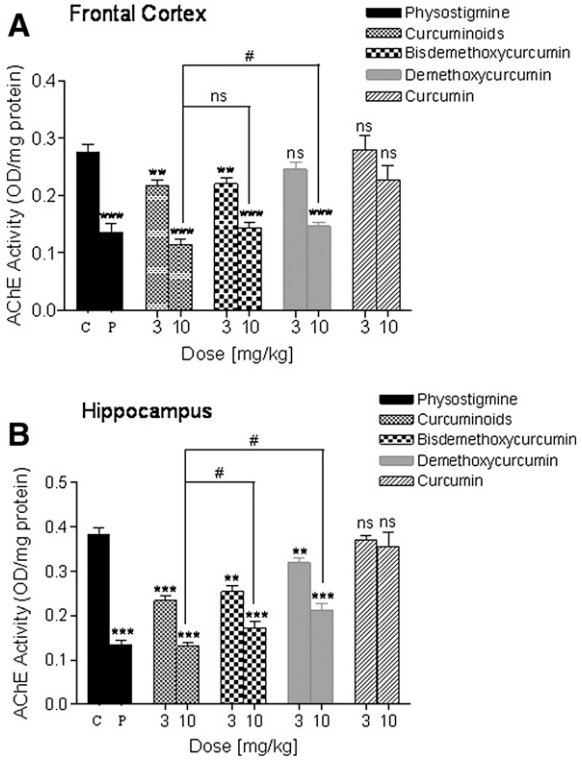

Fig. 4. Bar diagrams showing comparison of curcuminoids mixture with its individual components for their AChE inhibitory activity in the ex-vivo assay in rats: One hour after the i.p. administration of test compounds (3 and 10 mg/kg), AChE assay was carried out in frontal cortex (A) and hippocampus (B). Physostigmine was used as a standard AChE inhibitor. Error bars represent mean±SEM; $n=5$. $^{**}p<0.01$ and $^{***}p<0.001$ compared with control group marked as C (saline treated group). P=Physostigmine (0.06 mg/kg). ns=Non significant ($p>0.05$). #$p<0.05$ curcuminoids compared with bisdemethoxycurcumin and demethoxycurcumin at the 10 mg/kg dose.

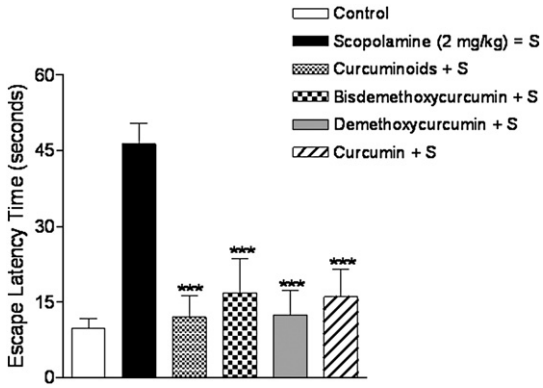

Fig. 5. Bar diagram showing comparison of curcuminoids mixture with its individual components for their effect on memory using Morris water maze test in rats: Saline was administered in a control group of animals and scopolamine (2 mg/kg i.p.) was administered in scopolamine group. Curcuminoids and individual compounds were studied for their effect on memory in scopolamine-induced amnesia at a single dose (10 mg/kg i.p.). Error bars represent mean±SEM; $n=8–10$. ***$p<0.001$ compared with scopolamine treated group.

the treatment with test compounds in two different doses (3 and 10 mg/kg; i.p.), animals were sacrificed; frontal cortex and hippocampal tissues were isolated. Supernatants were collected as explained in methods. Protein was estimated in supernatants using Bradford method and adjusted to 5 mg/ml. Results showed that curcuminoids at the doses of 3 and 10 mg/kg caused AChE inhibitory activity in the frontal cortex as well as in hippocampus (Fig. 4A and B).

When individual components of the curcuminoids were evaluated for their AChE inhibitory activity in frontal cortex and hippocampus, both bisdemethoxycurcumin and demethoxycurcumin inhibited AChE activity in a dose-dependent manner, while curcumin showed negligible effect ($p>0.05$) even at the highest dose used (10 mg/kg) as shown in Fig. 4A and B. Moreover, when compared with individual components at the dose of 10 mg/kg, curcuminoids mixture exhibited AChE inhibitory activity higher than that of demethoxycurcumin in frontal cortex and hippocampus; when compared with bisdemethoxycurcumin, it showed similar effect in frontal cortex but stronger effect in hippocampus (Fig. 4A and B).

3.3. Effect of curcuminoids on memory in rats

Curcuminoids mixture and its individual components were investigated for their effect on memory using Morris water maze task (Morris, 1984). After the intraperitoneal injection of scopolamine (2 mg/kg), rats showed impairment in the spatial memory compared to that of the control group in which there was no change in the latency to find hidden platform (Fig. 5). The test compounds were injected intraperitoneally after the administration of scopolamine (2 mg/kg) to see if they can reverse scopolamine-induced amnesia. After treating with curcuminoids mixture or the individual components (10 mg/kg), there was significant reversal ($p<0.001$) seen in the memory impairment, induced by the scopolamine (Fig. 5), with no clear difference in activity amongst all the test compounds ($p>0.05$), which indicates that all compounds possess comparable memory enhancing effect in this model. Curcumin, which showed negligible AChE inhibitory effect in the ex-vivo model, was able to show comparable reversal of scopolamine-induced amnesia, thus indicating some additional mechanism(s) in its effectiveness for AD.

4. Discussions

Curcuminoids mixture due to its potential therapeutic profile represent as an emerging candidate particularly for AD. Although, curcumin, the main constituent of the curcuminoids mixture, has been shown to possess some potential therapeutic activities for AD conditions (Yang et al., 2005), but to our knowledge other components were not explored, except a few preliminary reports (Park and Kim, 2002) particularly for their effect on the cholinergic system, which represents the core component of the AD pathology (Coyle et al., 1983; Whitehouse et al., 1982).

Activation of muscarinic acetylcholine receptors enhances GABAergic transmission in the cortical pyramidal neurons (Zhong et al., 2003). This GABAergic inhibition is known to control the flow of information in cortical circuits, thus, critical for the execution of certain forms of memory (Constantinidis et al., 2002). Therefore, cholinergic hypofunction represents as one of the major problems resulting in cognitive impairment, which is one of the conditions in AD. The results of ex-vivo AChE assay revealed that curcuminoids mixture possesses AChE inhibitory activity in hippocampus and frontal cortex, which strongly suggests the ability to cross blood brain barrier and inhibit AChE enzyme providing longer time for acetylcholine to stimulate post-synaptic muscarinic receptors. Enhancing cholinergic transmission is likely to offer beneficial effects which may improve debilitated AD patient's conditions.

In the in-vitro AChE assay, the individual components of the curcuminoids mixture showed AChE inhibitory activity in the decreasing order as bisdemethoxycurcumin, demethoxycurcumin and curcumin. When curcuminoids mixture was compared with the individual components for its in-vitro AChE inhibitory activity, it is evident that the parent mixture of curcuminoids was slightly less effective than bisdemethoxycurcumin, the most effective individual component (Fig. 2), however, in the ex-vivo assay, no individual compound showed better profile than the parent curcuminoids, rather curcuminoids mixture showed activity either comparable to that of bisdemethoxycurcumin or higher than other two individual compounds (Fig. 4A and B), while curcumin showed negligible effect ($p>0.05$). Different explanations can be made for the better profile of curcuminoids in the ex-vivo system. Curcuminoids as a mixture might be penetrating better through blood brain barrier or it could be because of some synergistic interaction between the individual components, when they are present in a formulation, close to the natural form. Interestingly, plant extracts and/or fractions which are known to contain multiple chemical entities have been shown to possess synergistic and/or side-effect neutralizing potential (Gilani and Rahman, 2005). Possibility also exists for different kinetics of the drug binding with enzyme at molecular levels, when in the form of a mixture.

Curcumin in the ex-vivo assay showed negligible inhibitory effect ($p>0.05$) while moderately effective in the in-vitro AChE assay, which needs explanation. It has been found that curcumin has poor absorption (Wahlstrom and Blennow, 1978) and limited penetration into the brain (Pan et al., 1999), which may explain its inability to show AChE inhibitory activity in the ex-vivo model. In the present study, bisdemethoxycurcumin was found the most active in the in-vitro AChE assay when compared with other components of the curcuminoids, but was found inactive in inhibiting lead-induced lipid peroxidation as opposed to other two components (Dairam et al., 2007), indicating that each single compound has varying degree of effectiveness in different models considered useful for AD; thus parent mixture of curcuminoids is likely to offer better therapeutic potential sharing wider range of activities. It appears as replacing the methoxy group on benzene ring with hydroxyl group increases AChE inhibitory activity, as bisdemethoxycurcumin being the most active amongst the curcuminoids. Interestingly, other studies have also similar observation where the number of hydroxyl groups in polyphenols and tannic acid (bisdemethoxycurcumin in this case "Fig. 3") have shown strong link with the anti-amyloidogenic and fibril-destabilizing activities (Ono et al., 2006, 2004a, 2003). A possibility exists that curcuminoids mixture might be more active

than curcumin for its anti-amyloidogenic and fibril-destabilizing activities.

Memory impairment in AD patients is a condition that makes them dependent upon their caregivers. Our data show that curcuminoids and the individual components exhibited memory enhancing effect in Morris water maze test. Furthermore, AChE inhibitory activity of the curcuminoids corresponded well with the memory enhancing effect while, curcumin failed to show this correlation at the similar doses (Figs. 4 and 5). The fact that, curcumin, which was relatively week in its AChE inhibitory effect and devoid of significant AChE inhibitory activity in the ex-vivo assay, but still shared comparable memory enhancing effect in Morris water maze test, suggests that curcumin enhances memory in this model possibly through mechanism(s) independent of the AChE enzyme inhibition. Interestingly, curcumin has also been reported to possess antioxidant (Dairam et al., 2008; Zhao et al., 1989), anti-inflammatory (Ammon et al., 1993) and calcium antagonist (Gilani et al., 2005) activities along with memory enhancing effect (Dairam et al., 2007). Memory enhancing effect shared by curcumin in the present study might be due to these known properties of curcumin, though additional mechanism(s) cannot be ruled out. Taken together, these properties of the curcumin and its ability to decrease plaque burden in AD (Garcia-Alloza et al., 2007; Yang et al., 2005), might be contributing for its effectiveness, thus attracted for clinical trail in AD (Baum et al., 2008).

We need to improve our understanding how curcuminoids as a mixture is pharmacologically different from purified curcumin. Curcuminoids mixture is known to inhibit lipid peroxidation in rat brain homogenates (Sreejayan and Rao, 1994), scavenge nitric oxide radicals (Sreejayan and Rao, 1997), protect cells from Aβ (1–42) insults (Kim et al., 2001), inhibit Aβ induced inflammatory cytokines expression (Giri et al., 2004), enhance memory (Frautschy et al., 2001) and inhibit in-vitro beta amyloid fibril formation (Kim et al., 2005) in addition to its strong AChE inhibitory activity observed in the present study. Curcumin though has little effect on AChE activity, it constitutes 75–80% of the curcuminoids mixture (Aggarwal et al., 2007); hence, curcumin is likely to play a major role towards the therapeutic success of curcuminoids, though the parent curcuminoids mixture has a merit of possessing additional AChE inhibitory activity primarily due to other two components. The doses at which curcuminoids show AChE inhibitory and memory enhancing effects are quite relevant from the clinical point of view, because clinical studies have shown safety of the curcuminoids up to the oral dose of as high as 12 g (Lao et al., 2006).

To our knowledge this is the first report providing evidence for the AChE inhibitory and memory enhancing effect of the curcuminoids in scopolamine-induced amnesia in rats. Moreover, reported antioxidant, anti-inflammatory and inhibitory effects on Aβ fibril formation may highlight curcuminoids to be considered as a candidate for clinical trails. Our data provide substantial initial evidence for the therapeutic potential of the curcuminoids with raising several interesting questions and inviting further investigations. It will be interesting in the future to dissect out kinetics of the curcuminoids as well as individual components. Secondly, the effect of curcuminoids and individual components at molecular level may present interesting scenario, to see how they are changing cellular machinery of RNA and proteins to show their effects. To summarize, these data indicate that curcuminoids mixture possesses a wide range of pharmacological activities beneficial for AD. Hence, curcuminoids may find the place as potential therapeutic option.

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References

Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 2003;23:363–98.
Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv Exp Med Biol 2007;595:1–75.
Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. J Clin Pharm Ther 2003;28:53–9.
Ammon HP, Safayhi H, Mack T, Sabieraj J. Mechanism of antiinflammatory actions of curcumin and boswellic acids. J Ethnopharmacol 1993;38:113–9.
Baum L, Ng A. Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. J Alzheimer's Dis 2004;6:367–77 discussion 443–9.
Baum L, Lam CW, Cheung SK, Kwok T, Lui V, Tsoh J, et al. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. J Clin Psychopharmacol 2008;28:110–3.
Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet 2006;368:387–403.
Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
Constantinidis C, Williams GV, Goldman-Rakic PS. A role for inhibition in shaping the temporal flow of information in prefrontal cortex. Nat Neurosci 2002;5:175–80.
Coyle JT, Price DL, DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 1983;219:1184–90.
Dairam A, Limson JL, Watkins GM, Antunes E, Daya S. Curcuminoids, curcumin, and demethoxycurcumin reduce lead-induced memory deficits in male Wistar rats. J Agric Food Chem 2007;55:1039–44.
Dairam A, Fogel R, Daya S, Limson JL. Antioxidant and iron-binding properties of curcumin, capsaicin, and *S*-allylcysteine reduce oxidative stress in rat brain homogenate. J Agric Food Chem 2008;56:3350–6.
Daniel S, Limson JL, Dairam A, Watkins GM, Daya S. Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. J Inorg Biochem 2004;98:266–75.
Duke JA. Handbook of phytochemcial constituents of GRAS herbs and other economic plants. CRC Press; 1992.
Duke JA. Hand book of medicinal herbs. 2nd ed. CRC Press; 2002.
Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, et al. Chemopreventive and therapeutic effects of curcumin. Cancer Lett 2005;223:181–90.
Ellman GL, Courtney KD, Andres Jr V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.
Eybl V, Kotyzova D, Koutensky J. Comparative study of natural antioxidants—curcumin, resveratrol and melatonin—in cadmium-induced oxidative damage in mice. Toxicology 2006;225:150–6.
Frautschy SA, Hu W, Kim P, Miller SA, Chu T, Harris-White ME, et al. Phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. Neurobiol Aging 2001;22:993–1005.
Garcia-Alloza M, Borrelli LA, Rozkalne A, Hyman BT, Bacskai BJ. Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. J Neurochem 2007;102:1095–104.
Gilani AH, Rahman AU. Trends in ethnopharmacology. J Ethnopharmacol 2005;100:43–9.
Gilani AH, Shah AJ, Ghayur MN, Majeed K. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. Life Sci 2005;76:3089–105.
Giri RK, Rajagopal V, Kalra VK. Curcumin, the active constituent of turmeric, inhibits amyloid peptide-induced cytochemokine gene expression and CCR5-mediated chemotaxis of THP-1 monocytes by modulating early growth response-1 transcription factor. J Neurochem 2004;91:1199–210.
Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. Biochem Pharmacol 2008;75:787–809.
Isoma K, Ishikawa M, Ohta M, Ogawa Y, Hasegawa H, Kohda T, et al. Effects of T-82, a new quinoline derivative, on cholinesterase activity and extracellular acetylcholine concentration in rat brain. Jpn J Pharmacol 2002;88:206–12.
Kamat AM, Sethi G, Aggarwal BB. Curcumin potentiates the apoptotic effects of chemotherapeutic agents and cytokines through down-regulation of nuclear factor-kappaB and nuclear factor-kappaB-regulated gene products in IFN-alpha-sensitive and IFN-alpha-resistant human bladder cancer cells. Mol Cancer Ther 2007;6:1022–30.
Kapoor L. Handbook of ayurvedic medicinal plants. CRC Press; 1990.
Kim DS, Park SY, Kim JK. Curcuminoids from *Curcuma longa* L. (Zingiberaceae) that protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from betaA(1–42) insult. Neurosci Lett 2001;303:57–61.
Kim H, Park BS, Lee KG, Choi CY, Jang SS, Kim YH, et al. Effects of naturally occurring compounds on fibril formation and oxidative stress of beta-amyloid. J Agric Food Chem 2005;53:8537–41.
Kunnumakkara AB, Diagaradjane P, Guha S, Deorukhkar A, Shentu S, Aggarwal BB, et al. Curcumin sensitizes human colorectal cancer xenografts in nude mice to {gamma}-radiation by targeting nuclear factor-{kappa}B-regulated gene products. Clin Cancer Res 2008;14:2128–36.
Lao CD, Ruffin MTt, Normolle D, Heath DD, Murray SI, Bailey JM, et al. Dose escalation of a curcuminoid formulation. BMC Complement Altern Med 2006;6:10.

Le Bars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. A placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia. North American EGb Study Group. Jama 1997;278:1327–32.
Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 2001;21:8370–7.
Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11:47–60.
Mukhopadhyay A, Basu N, Ghatak N, Gujral PK. Anti-inflammatory and irritant activities of curcumin analogues in rats. Agents Actions 1982;12:508–15.
Nadkarni K. Indian materia medica. Popular Prakashan; 1986.
Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Curry consumption and cognitive function in the elderly. Am J Epidemiol 2006;164:898–906.
NRC. Guide for the care and use of laboratory animals. Washington, D.C.: National Academy Press; 1996.
Ono K, Yoshiike Y, Takashima A, Hasegawa K, Naiki H, Yamada M. Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. J Neurochem 2003;87:172–81.
Ono K, Hasegawa K, Naiki H, Yamada M. Anti-amyloidogenic activity of tannic acid and its activity to destabilize Alzheimer's beta-amyloid fibrils in vitro. Biochim Biophys Acta 2004a;1690:193–202.
Ono K, Hasegawa K, Naiki H, Yamada M. Curcumin has potent anti-amyloidogenic effects for Alzheimer's beta-amyloid fibrils in vitro. J Neurosci Res 2004b;75:742–50.
Ono K, Hamaguchi T, Naiki H, Yamada M. Anti-amyloidogenic effects of antioxidants: implications for the prevention and therapeutics of Alzheimer's disease. Biochim Biophys Acta 2006;1762:575–86.
Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. Drug Metab Dispos 1999;27:486–94.
Park SY, Kim DS. Discovery of natural products from *Curcuma longa* that protect cells from beta-amyloid insult: a drug discovery effort against Alzheimer's disease. J Nat Prod 2002;65:1227–31.
Qin NY, Yang FQ, Wang YT, Li SP. Quantitative determination of eight components in rhizome (Jianghuang) and tuberous root (Yujin) of *Curcuma longa* using pressurized liquid extraction and gas chromatography-mass spectrometry. J Pharm Biomed Anal 2007;43:486–92.
Reddy AC, Lokesh BR. Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. Mol Cell Biochem 1994;137:1–8.
Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001;81:741–66.
Sreejayan, Rao MN. Curcuminoids as potent inhibitors of lipid peroxidation. J Pharm Pharmacol 1994;46:1013–6.
Sreejayan, Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997;49:105–7.
Wahlstrom B, Blennow G. A study on the fate of curcumin in the rat. Acta Pharm Toxicol (Copenh) 1978;43:86–92.
Wettstein A. Cholinesterase inhibitors and *Gingko* extracts—are they comparable in the treatment of dementia? Comparison of published placebo-controlled efficacy studies of at least six months' duration. Phytomedicine 2000;6:393–401.
Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science 1982;215:1237–9.
Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, et al. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. J Biol Chem 2005;280:5892–901.
Zhang L, Fiala M, Cashman J, Sayre J, Espinosa A, Mahanian M, et al. Curcuminoids enhance amyloid-beta uptake by macrophages of Alzheimer's disease patients. J Alzheimer's Dis 2006;10:1–7.
Zhao BL, Li XJ, He RG, Cheng SJ, Xin WJ. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. Cell Biophys 1989;14:175–85.
Zhong P, Gu Z, Wang X, Jiang H, Feng J, Yan Z. Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease. J Biol Chem 2003;278:26888–96.