Interactions of *Magnolia* and *Ziziphus* extracts with selected central nervous system receptors

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Aim of the study: To identify pharmacological targets, extracts of *Magnolia officinalis* (ME), *Ziziphus spinosa* (ZE), and a proprietary fixed combination (MZE) were tested for affinity with central nervous system receptors associated with relaxation and sleep.

Methods: In vitro radioligand binding and cellular functional assays were conducted on: adenosine A1, dopamine (transporter, D1, D2S, D3, D4a and D5), serotonin (transporter, 5-HT1A, 5-HT1B, 5-HT4, 5-HT6 and 5-HT7) and the GABA benzodiazepine receptor.

Results: Interactions were demonstrated with the adenosine A1 receptor, dopamine transporter and dopamine D1 receptor (agonist activity), serotonin receptors (5-HT1B and 5-HT6 antagonist activity) and the GABA benzodiazepine receptor at a concentration of 100 μg/ml or lower. ME had an affinity with adenosine A1 (Kᵢ of 9.2 ± 1.1 μg/ml) and potentiated the GABA activated chloride current at the benzodiazepine subunits of the GABA receptor (maximum effect at 50 μg/ml). ME had a modest antagonistic action with 5-HT6 and ZE with the 5-HT1B receptor.

Conclusion: The interactions in the receptor binding models are consistent with the traditional anxiolytic and sleep-inducing activities of *Magnolia officinalis* bark and *Ziziphus spinosa* seed.

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1. Introduction

*Magnolia officinalis* bark and *Ziziphus spinosa* seed have a history of use in traditional Asian medicine for mild anxiety, nervousness and sleep-related problems. Both are listed in the Pharmacopoeia of the People’s Republic of China (English Edition, 2005). Additionally, *Magnolia officinalis* bark is listed in the Japanese Pharmacopoeia XIV (English Edition, 2001). Extracts of both botanicals have demonstrated activity in rodent studies. *Magnolia* extracts, in traditional combinations with other herbs, have demonstrated anti-depressant effects in the tail suspension test, the forced swimming test as well as anxiolytic activity in the elevated plus-maze assay in mice (Kuribara et al., 2000; Luo et al., 2000). The activity in the plus-maze assay was traced to a constituent in *Magnolia* bark; namely honokiol (Kuribara et al., 2000). An extract of *Ziziphus spinosa* demonstrated anxiolytic activity in the elevated plus-maze assay and the black and white test in mice. Furthermore, the extract demonstrated a sedative effect in prolonging hexobarbital-induced sleeping time in mice (Peng et al., 2000). Activity guided fractionation identified spinosin as an active constituent of *Ziziphus spinosa* (Li and Bi, 2006). Spinosin augmented pentobarbital-induced sleep, increasing sleep time and reducing sleep latency in mice (Wang et al., 2008).

Extracts of *Magnolia officinalis* bark and *Ziziphus spinosa* seed were combined in a proprietary product called Seditol® (MZE) that is produced by Next Pharmaceuticals. MZE is characterized as containing a minimum of 2.7% honokiol and 0.1% spinosin. MZE is marketed as a dietary supplement in the USA for the improvement of sleep difficulties associated with restlessness, stress or anxiety. Seditol was subjectively assessed for tolerability and benefit in 295 volunteers with mild to moderate sleep difficulties in an uncontrolled, open-label study. The participants took the product for a minimum of 2 weeks and over 80% subjectively reported that Seditol helped them to relax, assisted in a restful sleep and was effective in reducing fatigue due to lack of sleep (unpublished).

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In order to better understand the pharmacological targets for ME and ZE extracts and their combination MZE, the preparations were tested for binding affinity with a number of central nervous system receptors associated with relaxation and sleep. Adenosine is a neurotransmitter known to play an important role in the initiation of sleep (Basheer et al., 2004). Dopamine plays an important role in mood and internal reward systems (Runyon and Carroll, 2006). Serotonin (5-hydroxytryptamine, 5-HT) is involved in a number of physiological functions including sleep, appetite, pain perception, and sexual activity (Tatarczynska et al., 2004). Gamma-aminobutyric acid (GABA) modulates arousal and attention, anxiety and sleep (Khom et al., 2006). The selected receptor/receptor subtypes for testing were adenosine A1, dopamine (transporter, D1, D2S, D3, D4, and D5), serotonin (transporter, 5-HT1A, 5-HT1B, 5-HT2, and 5-HT7) and the GABA benzodiazepine receptor. This work expands on previous receptor binding studies on extracts of Magnolia officinalis (Squires et al., 1999; Ai et al., 2001; Li and Bi, 2006) and Ziziphus spinosa (Liao et al., 1995).

2. Materials and methods

2.1. Test material

Seditol (MZE) is a proprietary blend of a patented extract of the bark of Magnolia officinalis Rehder and Wilson [Magnoliaceae (ME)] and an extract of the seeds of Ziziphus spinosa (Bunge) Hu ex. Chen. (syn. Ziziphus jujube var. spinosa (Bunge) Hu ex HF Chow) [Fam. Rhamnaceae] (ZE). ME is produced with carbon dioxide, and is the subject of two US patents (Nos. 6,582,735 and 6,814,987) describing composition and methods of preventing, treating in managing sleeplessness, restlessness and weight gain due to stress or lack of sleep. ZE is an ethanolic/water extract with a ratio of 4–6:1. MZE is characterized as containing a minimum of 2.7% honokiol, an active constituent of Magnolia officinalis bark and 0.1% spinosin, a chemical marker of quality for Ziziphus spinosa seeds. The raw plant materials were identified and supplied by the manufacturer’s contacts in the People’s Republic of China. Voucher specimens of the crude botanicals are deposited at the extract manufacturers’ facilities. Samples of the individual extracts (ME and ZE) and their blend (MZE) are retained by the manufacturer and by the authors.

2.2. Radioligand binding assays

2.2.1. Adenosine binding assays

The binding assays were conducted as described elsewhere (Müller et al., 2002). In short, the extracts were investigated in radioligand binding assays at A1 adenosine receptors of rat brain cortical membranes using the A1-selective radioligand [3H]2-chloro-N6-cyclopentyladenosine ([3H]CCPA). The extracts were dissolved in dimethyl sulfoxide (DMSO) and a final concentration of 2.5% DMSO was used in the assays. Membranes were preincubated with 0.2 lU/mg protein of adenosine deaminase in order to remove endogenous adenosine. Radioligand binding to rat brain cortical membranes was carried out in Tris–HCl buffer 50 mM, pH 7.4. Assays were performed by incubating the mixtures at 23 °C for 90 min. Nonspecific binding was defined using 10 μM of 2-chloroadenosine (CADO). [3H]CCPA was used in a final concentration of 1 nM. Protein (ca. 50 μg/well containing a final volume of 0.2 ml) was added to start the reaction. Incubations were terminated by rapid filtration using a Brandel 96-channel cell harvester (Brandel, Gaithersburg, MD, USA) through Packard 96-well GF/B-glass fibre filter plates. Filters were rinsed three times with 0.2 ml of Microscint-20 scintillation cocktail (Packard Bioscience). All experiments were performed in triplicate.

Data were analyzed using GraphPad PRISM® Version 4.0 (San Diego, CA, USA). For nonlinear regression analysis, the Cheng–Prusoff equation and K0-values of 0.2 nM for [3H]CCPA, 8 nM for [3H]MSX-2, 0.41 nM for [3H]PSB-603 and 4.9 nM for [3H]PSB-11 were used to calculate K0-values from IC50-values.

2.2.2. Dopamine, serotonin and GABA central binding

In vitro binding assays were performed using the general procedures shown in Tables 1 and 2. All receptors were human cloned, except for BZD and 5-HT1B, which were endogenous to the rat cerebral cortex and the Chinese hamster ovary (CHO) cells, respectively. The extracts were dissolved in DMSO at a concentration of 30 μg/ml and then diluted in water or saline for initial screening at a concentration of 100 μg/ml. Selected assays were repeated at a concentration of 10 μg/ml. Each determination was performed in...

### Table 1

Summary and general experimental conditions of dopamine and serotonin cellular functional assays.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Reference compound</th>
<th>Ligand</th>
<th>Conc. (nM)</th>
<th>Incubation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDZ</td>
<td>Diazepam (3 μM)</td>
<td>[3H]flunitrazepam</td>
<td>0.4</td>
<td>60 min, 4 °C</td>
<td>Speth et al. (1979)</td>
</tr>
<tr>
<td>DA transporter</td>
<td>BTCP (10 μM)</td>
<td>[3H]BTCP</td>
<td>4</td>
<td>120 min, 4 °C</td>
<td>Pristupa et al. (1994)</td>
</tr>
<tr>
<td>5-HT transporter</td>
<td>Imipramine (10 μM)</td>
<td>[3H]imipramine</td>
<td>2</td>
<td>60 min, 22 °C</td>
<td>Tatsumi et al. (1999)</td>
</tr>
</tbody>
</table>

### Table 2

Summary and general experimental conditions of dopamine and serotonin cellular functional assays.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Reference compound</th>
<th>Stimulus agonistic effect (control)</th>
<th>Reference compound</th>
<th>Stimulus agonistic effect (nM)</th>
<th>Incubation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Dopamine</td>
<td>None (10 μM dopamine)</td>
<td>SCH 23390</td>
<td>Dopamine (300)</td>
<td>30 min, 22 °C</td>
<td>Zhou et al. (1990)</td>
</tr>
<tr>
<td>D2S</td>
<td>Dopamine</td>
<td>None (100 nM dopamine)</td>
<td>(+)Butaclamol</td>
<td>Dopamine (30)</td>
<td>20 min, 37 °C</td>
<td>Missale et al. (1998)</td>
</tr>
<tr>
<td>D1</td>
<td>Dopamine</td>
<td>None (30 nM dopamine)</td>
<td>(+)Butaclamol</td>
<td>Dopamine (10)</td>
<td>10 min, 37 °C</td>
<td>Missale et al. (1998)</td>
</tr>
<tr>
<td>D4</td>
<td>Dopamine</td>
<td>None (300 nM dopamine)</td>
<td>Clozapine</td>
<td>Dopamine (100)</td>
<td>10 min, 37 °C</td>
<td>Missale et al. (1998)</td>
</tr>
<tr>
<td>D3</td>
<td>Dopamine</td>
<td>None (1 μM dopamine)</td>
<td>SCH 23390</td>
<td>Dopamine (50)</td>
<td>30 min, 22 °C</td>
<td>Sunahara et al. (1991)</td>
</tr>
<tr>
<td>5-HT1A</td>
<td>8-OH-DPAT</td>
<td>None (1 nM 8-OH-DPAT)</td>
<td>WAY 100635</td>
<td>8-OH-DPAT (10)</td>
<td>15 min, 22 °C</td>
<td>Newman-Tancredi et al. (2001)</td>
</tr>
<tr>
<td>5-HT1B</td>
<td>Serotonin</td>
<td>None (10 nM serotonin)</td>
<td>Methiothepin</td>
<td>Serotonin (100)</td>
<td>30 min, 33 °C</td>
<td>Giles et al. (1996)</td>
</tr>
<tr>
<td>5-HT2</td>
<td>Serotonin</td>
<td>None (1 μM serotonin)</td>
<td>GR 113808</td>
<td>Serotonin (30)</td>
<td>30 min, 22 °C</td>
<td>Mielat et al. (2000)</td>
</tr>
<tr>
<td>5-HT3</td>
<td>Serotonin</td>
<td>None (10 μM serotonin)</td>
<td>Methiothepin</td>
<td>Serotonin (100)</td>
<td>45 min, 37 °C</td>
<td>Kohen et al. (1996)</td>
</tr>
<tr>
<td>5-HT7</td>
<td>Serotonin</td>
<td>None (10 μM serotonin)</td>
<td>Mesulergine</td>
<td>Serotonin (100)</td>
<td>45 min, 37 °C</td>
<td>Ahmad et al. (1998)</td>
</tr>
</tbody>
</table>
Table 3
Effects of ME, ZE and their combination MZE on radioligand binding.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>ME</th>
<th>ZE</th>
<th>MZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA transporter</td>
<td>Interference (39)</td>
<td>–</td>
<td>64 (11)</td>
</tr>
<tr>
<td>D1</td>
<td>Antagonist: 14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D2S</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D5</td>
<td>Antagonist: 66 (21)</td>
<td>–</td>
<td>Antagonist: 21</td>
</tr>
<tr>
<td>5-HT transporter</td>
<td>Interference (21)</td>
<td>13</td>
<td>–</td>
</tr>
<tr>
<td>5-HT1A</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5-HT1B</td>
<td>Agonist: 102</td>
<td>Antagonist: 23</td>
<td>–</td>
</tr>
<tr>
<td>5-HT2A</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5-HT2B</td>
<td>Antagonist: 48 (15)</td>
<td>–</td>
<td>Antagonist: 20</td>
</tr>
<tr>
<td>5-HT7</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BZD</td>
<td>61</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The results are expressed as a percent inhibition of control specific binding with a concentration of 100 μg/ml (mean values; n = 2). Tests at a concentration of 10 μg/ml are included in parenthesis. (–) Indicates an inhibition of less than 10%. Blank squares indicate that the test was not run.

duplicate. In each experiment, the respective reference compound was tested at a minimum of eight concentrations in duplicate to obtain a competition curve in order to validate the experiment.

For the receptor binding studies, the specific radioligand binding to the receptors was defined as the difference between total binding and nonspecific binding determined in the presence of an excess of unlabeled ligand. Results are expressed as a percent of control specific binding and as a percent inhibition of control specific binding obtained in the presence of the tested extracts.

For the cellular functional assays, the measured reaction product was the second messenger cyclic adenosine monophosphate (cAMP). The specific radioligand binding to the receptors was defined as the difference between total response and measured specific agonist response determined in the presence of an excess of ligand. Results are expressed as a percent of control specific response or as a percent inhibition of control specific agonist response obtained in the presence of the tested extracts. The conditions for specific assays are detailed in Table 2.

2.2.3. GABA<sub>A</sub> binding assays

The binding assays were conducted as described elsewhere (Khom et al., 2006). Briefly, the experiments were conducted with GABA<sub>A</sub> receptors expressed from Xenopus laevis. Stage V–VI oocytes were prepared and cRNA injected as previously described. Female Xenopus laevis (NASCO, USA) were anesthetized by exposing them for 15 min to a 0.2% MS-222 (methanesulfonate salt of 3-aminobenzoic acid ethyl ester; Sandoz, Germany) solution before surgically removing parts of the ovaries. Follicle membranes from isolated oocytes were enzymatically digested with 2 mg/ml collagenase (Type 1A, Sigma, Germany). Synthesis of capped run-off poly(A<sup>+</sup>) cRNA transcripts was obtained from linearized cDNA templates (pcMV vector). One day after enzymatic isolation, the oocytes were injected with 50 nL of DEPC-treated water (diethylpyrocarbonate, Sigma, Germany) containing the different rat cRNAs at a concentration of approximately 300–3000 pg/μl/subunit. The amount of cRNA was determined by means of a NanoDrop ND-1000 (Kisker-Biotech, Steinfurt, Germany). To ensure expression of the gamma2S subunit cRNAs encoding for α1, β2 and γ2S sub-units were mixed in a ratio of 1:1:10. Oocytes were stored at 18 °C in ND96 solution. Voltage clamp measurements were performed on the 1st and 2nd days after cRNA injection. Electrophysiological experiments were performed by the two-microelectrode voltage clamp method making use of a TURBO TEC 01C amplifier (npi electronic GmbH, Tamm, Germany) at a holding potential of ~70 mV. The bath solution contained 90 mM NaCl, 1 mM KCl, 1 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub> and 5 mM HEPES (pH 7.4).

GABA and ME were applied by means of an automated fast perfusion system (Baburin et al., 2006). To elicit I<sub>GABA</sub>, the chamber was perfused with 120 μl of GABA-containing solution at volume rates of 300 μl/s. ME was solved in DMSO at a concentration of 10 μg/ml and then diluted in bath solution to 10 μg/ml, 50 μg/ml and 100 μg/ml. Indicated concentrations of ME were co-applied with GABA EC<sub>3–10</sub> (effective concentration of GABA that induces 3–10% of maximal GABA-evoked current). Percent potentiation of I<sub>GABA</sub> by ME was calculated using formula: (R/C – 1) × 100%, where R is the amplitude of the chloride current evoked by co-application of control GABA EC<sub>3–10</sub> and the indicated concentration of ME, and C is the amplitude of the chloride current evoked by application of control GABA EC<sub>3–10</sub> alone. Bar graphs were built using Origin software (OriginLab Corporation, USA). Statistical significance was calculated using unpaired Student's t-test with a confidence interval of P < 0.05.

3. Results

The receptor binding results for selected receptors associated with the neurotransmitters dopamine, serotonin and GABA are summarized in Table 3. Results of interactions with the adenosine A<sub>1</sub> receptor are depicted in Fig. 1 and the results of interactions with the GABA benzodiazepine receptor are depicted in Fig. 2.

3.1. Adenosine

ME exhibited 48 ± 15% inhibition of radioligand binding at 100 μg/ml and a K<sub>i</sub> of 9.2 ± 1.1 μg/ml (Fig. 1). MZE had a small interaction with the adenosine A<sub>1</sub> receptor (14 ± 1% inhibition of radioligand binding at 100 μg/ml). This effect was due to ME as ZE was not active when tested individually at this concentration. Further testing investigated the nature of the interaction between ME and the adenosine receptor. ME at a concentration of 10 μg/ml completely inhibited forskolin-stimulated cAMP accumulation in CHO cells expressing the adenosine A<sub>1</sub> receptor. As this result was considered likely to be an artifact, the functional properties of ME were investigated in GTP-shift experiments using rat cortex membrane preparation containing adenosine A<sub>1</sub> receptors. ME did not cause a GTP-shift in this experiment.

3.2. Dopamine

The selected receptor/receptor subtypes associated with dopamine included the transporter, D<sub>1</sub>, D<sub>2S</sub>, D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub>. ME...
and MZE bound with the dopamine transporter and the D_{5} receptor (Table 3). ME inhibited binding to the DA transporter by 39% at 10 µg/ml with no results being determined at 100 µg/ml due to interference in the test system. ZE was not active at 100 µg/ml in this system. MZE inhibited binding to the DA transporter by 64% at 100 µg/ml and 11% at 10 µg/ml.

ME interacted with the D_{3} receptor causing a 66% inhibition at 100 µg/ml and 21% inhibition at 10 µg/ml. ZE was not active at 100 µg/ml in this system. MZE interacted modestly with the D_{3} receptor with 21% inhibition of control at 100 µg/ml.

3.3. Serotonin

The selected receptor/receptor subtypes associated with serotonin included the transporter, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{4e}, 5-HT_{6} and 5-HT_{7}. MZE bound modestly as an antagonist to the 5-HT_{1B} and 5-HT_{6} receptors (Table 3). ZE had an antagonist effect with the 5-HT_{1B} receptor with 23% inhibition of control binding at 100 µg/ml. MZE displayed 29% inhibition of control at that concentration. ME had no antagonist effect at 100 µg/ml and instead had an agonist effect, with 102% inhibition of control at 100 µg/ml. However ME appeared not to interfere with the activity of ZE when the two were combined in MZE.

ME displayed an antagonist effect with the 5-HT_{6} receptor with 48% inhibition of control at 100 µg/ml and 15% inhibition of control at 10 µg/ml. This effect was also seen with MZE, which had an antagonist effect with 20% inhibition of control at 100 µg/ml.

3.4. GABA

ME had a strong interaction with the GABA central benzodiazepine receptor at a concentration of 100 µg/ml, causing a 61% inhibition at that concentration. Neither ZE nor MZE had a significant interaction with the receptor at that concentration. The activity of ME was eliminated when the concentration was reduced 10-fold to 10 µg/ml (Table 3). This explains why there was no activity demonstrated with MZE. In a separate experiment, the Magnolia extract strongly potentiated the GABA activated chloride current at the benzodiazepine subunits of the GABA receptor in a bell-shaped dose–response curve with maximum effect at 50 µg/ml (Fig. 2).

4. Discussion

In the present series of assays, interactions with the adenosine A_{1} receptor, dopamine transporter and dopamine D_{5} receptor (antagonist activity), serotonin receptors (5-HT_{1B} and 5-HT_{6} antagonist activity) and the GABA benzodiazepine receptor were demonstrated. MZE demonstrated an interaction with the adenosine A_{1} receptor that appears to be due to the constituent ME. Adenosine A_{1} is known to play an important role in the initiation of sleep. During the day, adenosine accumulates in neurons and in the evening it is released into the nerve synapses where it prepares the body to enter into a relaxed state (Bashmeer et al., 2004). ME was tested for adenosine A_{1} agonist activity. The rationale for this is that it was hypothesized that the Magnolia extract might act in a similar means to an extract of Valerian root (Valeriana officinalis L.). Previously it has been reported that a Valerian extract counteracted functional central arousal caused by the oral administration of caffeine, a well known adenosine antagonist (Schellenberg et al., 2004). Results with the Valerian extract indicate that it is a partial agonist of adenosine A_{1} (Müller et al., 2002). The GTP-shift experiments with ME indicate that it may be either a partial agonist in analogy with the Valerian extract, or an antagonist.

Dopamine is a neurotransmitter that plays an important role in mood. Dopamine is increased as an internal reward system leading to feelings associated with pleasure and contentment. Dopamine transporter inhibitors (in this case MZE) increase the level of dopamine in neural synapses increasing and prolonging the action of dopamine. The dopamine transporter is a target for the development of pharmacotherapies for a number of central disorders including attention deficit hyperactivity disorder (ADHD), obesity, depression, and stimulant abuse (Runyon and Carroll, 2006). D_{1} and D_{2} receptor antagonism appear to play a role in the development of fear or anxiety (Inoue et al., 2005). MZE and ME displayed D_{2} antagonistic activity. In addition, weak binding at the 100 µg/ml level indicated potential D_{1} antagonistic for ZE.

Serotonin (5-HT) is a neurotransmitter involved in a number of physiological functions including sleep, appetite, pain perception, and sexual activity. Several pathological states such as migraine, depression, and anxiety have been linked to the serotonergic system. To date, there are 14 known serotonin receptor subtypes through which serotonin exerts its actions. One receptor in particular, known as 5-HT_{1B}, plays a crucial role in regulating serotonin transmission in the brain. Recent studies have suggested a role for the 5-HT_{1B} receptor in depression, as well as in obsessive-compulsive disorder, drug addiction, anxiety, aggression and sleep. 5-HT_{1B} antagonists reduce the latency to onset of anxiolytic behavior and play a role in stress regulation with activity comparable to diazepam (Tatarczynska et al., 2004). MZE exhibited a mild 5-HT_{1B} antagonist activity that appears to be due to the presence of ZE. ME tested individually had the opposite effect. Functional assays and competitive binding curves would be the next step to better understand the activity of each extract and their combination on this particular receptor subtype. MZE also displayed a modest antagonist effect with the 5-HT_{6} receptor. This activity appears to be due to the presence of ME. Selective 5-HT_{6} antagonists may have potential as anxiolytics and anti-depressants (Wesolowska and Nikiforuk, 2007).

A previous study demonstrated an interaction between a Ziziphus seed extract (drug extract ratio of 7:3:1) and the serotonin receptor subtypes 5-HT_{1A} and 5-HT_{2} with a concentration of 10 mg/ml (Liao et al., 1995). This study did not duplicate those results. However ZE in this study was tested at a concentrations 100 times lower than the previous study, which is closer to that expected in the human body following traditional use.

GABA is a neurotransmitter that modulates arousal and attention, anxiety, sleep and muscle tone. Sedation and relief from

![Fig. 2. Interaction of ME with GABA. Potentiation of the chloride current through GABA receptors composed of α1, β2 and γ2S subunits by 10, 50 or 100 µg/ml of sample M. The Y-axis indicates the increase compared to the control current in %. Data are given as mean ± SEM from three different experiments. *Statistical significance from zero (P< 0.05).](image-url)
anxiety are the effects of agents acting on GABAergic neurotransmission. Benzodiazepines are a class of drugs that act in this manner, reducing anxiety and inducing sleep through interactions with the GABA_A receptor. ME was found to have a strong effect on the benzodiazepine binding site of the GABA_A receptor, working in the same direction as benzodiazepines. Previous studies have reported that honokiol, a chemical constituent of ME, interacts with the GABA_A receptor (Squires et al., 1999; Ai et al., 2001; Li and Bi, 2006). Magnolol, another constituent of ME, has been shown to be active in the oocyte assay used in this study (Kim et al., 2008). Honokiol and magnolol demonstrated activity in mice in the plus maze test with a dose of 0.2 mg/kg for 7 days. Honokiol exhibited activity at a dose similar to that of diazepam (a benzodiazepine), but did not exhibit the side effects known to be produced by this class of drugs (Kuribara et al., 1999a,b). Our studies did not duplicate a previous study that showed binding of a Ziziphus extract to the GABA_A site (Liao et al., 1995). The reason may be that the previous study used a concentration 100 times higher than that used in these experiments.

The affinity of the Magnolia extract with the A1 and GABA_A receptors is in line with earlier reports. The modest interaction of ME with the GABAA site (Liao et al., 1995). The reason may be that the pre-existence of interaction between the ZE and the 5-HT1B receptor.

The activities in the receptor binding models are in agreement with the traditional uses of the extracts of Magnolia and Ziziphus. The study results may also provide some clues as to the activity of their proprietary combination (Seditol). Further investigations using in vivo models incorporating bioavailability and biotransformation are important to verify these findings.

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References


Ai, J, Wang, X, Nielsen, M, 2001. Honokiol and magnolol selectively interact with the GABA receptor (Squires et al., 1999; Ai et al., 2001; Li and Bi, 2006). Magnolol, another constituent of ME, has been shown to be active in the oocyte assay used in this study (Kim et al., 2008). Honokiol and magnolol demonstrated activity in mice in the plus maze test with a dose of 0.2 mg/kg for 7 days. Honokiol exhibited activity at a dose similar to that of diazepam (a benzodiazepine), but did not exhibit the side effects known to be produced by this class of drugs (Kuribara et al., 1999a,b). Our studies did not duplicate a previous study that showed binding of a Ziziphus extract to the GABA_A site (Liao et al., 1995). The reason may be that the previous study used a concentration 100 times higher than that used in these experiments.

The affinity of the Magnolia extract with the A1 and GABA_A receptors is in line with earlier reports. The modest interaction of ME with the GABAA site (Liao et al., 1995). The reason may be that the pre-existence of interaction between the ZE and the 5-HT1B receptor.