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## Effect of Kihito Extract Granules on cognitive function in patients with Alzheimer-type dementia

**Short title:** Usefulness of Kihito for cognitive function

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## **Abstract**

It has been recently suggested that Japanese herbal (kampo) medicines, such as Kami-Untan-To, may improve cognitive function in elderly subjects with Alzheimer's disease. Polygalae Radix is thought to be a useful component of Kami-Untan-To because it enhances the activity of choline acetyltransferase in cultured neuronal cells. The purpose of the present study was to investigate the safety and usefulness of Kihito Extract Granules, a commercially available Japanese herbal medicine that contains Polygalae Radix, for elderly patients with senile dementia. Seventy-five elderly subjects ( $84.4 \pm 6.4$  years) with senile dementia of Alzheimer type according to DSM-IV criteria were randomly assigned to the Nontreatment, Goshajinkigan (control kampo medicine), or Kihito groups. Each medicine was given three times a day for 3 months. There was no severe adverse event in all groups. We examined the Mini Mental State Examination (MMSE), the activities of daily living (ADL) scale, and cerebrovascular single photon emission computed tomography (SPECT) before and after treatment. MMSE scores were significantly improved only in the Kihito group ( $+1.65 \pm 0.53$ ) but not in the Nontreatment ( $-0.3 \pm 0.67$ ) and Goshajinkigan ( $-0.58 \pm 0.49$ ) groups. ADL scores remained unchanged in all groups. Treatment with Kihito was not associated with an increase in CBF. These results propose that Kihito may be useful and has a potential to be tested as a medicine for Alzheimer-type senile dementia, although further examination is required to clarify the mechanism of the improving effect of Kihito on cognitive function.

**Key words:** cognitive function, dementia, herbal medicine, Goshajinkigan, Kihito

## Introduction

Alzheimer-type dementia is a common form of dementia in the elderly, and the morbidity of Alzheimer's disease has been increasing in Japan. Previous reports have shown a loss of acetylcholine (Ach) in the brains of patients with Alzheimer's disease, a decrease in the activity of choline acetyltransferase (ChAT), the enzyme that biometabolizes Ach, and loss of presynaptic cholinergic neurons in the basal nucleus<sup>1-4</sup>. Because it is likely that the loss of central cholinergic activity is associated with cognitive worsening in patients with Alzheimer-type dementia, it has been hypothesized that cholinergic augmentation could improve the cognitive ability of these patients. Recently, clinical trials with donepezil<sup>5-7</sup>, revastigmine<sup>8</sup>, and galanthamine<sup>9</sup> have demonstrated that cholinesterase inhibitors, which inhibit breakdown of acetylcholine and increase its availability to synapses, show overall beneficial effects in cognitive function in patients with Alzheimer's disease.

There are many reports that herbal medicines may be useful for dementia in animal models and humans<sup>10-12</sup>. Some of these reports demonstrate the effect of herbal medicines on metabolism of Ach. It was reported, for example, that a single preparation of *Polygalae Radix*, an herbal medicine, considerably enhanced ChAT activity in cultured rat septal neurons<sup>13</sup>. Furthermore, it was reported that Japanese herbal (kampo) medicines such as *Kami-Untan-To* (KUT), a cocktail of herbal medicines containing *Polygalae Radix*, increased Ach synthesis through enhancement of ChAT activity<sup>14-16</sup> and that KUT prevented the progression of dementia in Alzheimer's disease<sup>7</sup>. Of importance, the omission of *Polygalae Radix* from the basal cocktail of KUT dramatically reduced ChAT activity<sup>13</sup>. However, KUT is not commercially available in Japan. Kihito is a kampo medicine containing *Polygalae Radix* that is commercially available in Japan for persons of frail constitution with anemia, anxiety, palpitation, and

isomnia<sup>17</sup>. The purpose of the present study was to evaluate the efficacy and safety of Kihito in the clinical context for patients with mild to moderate Alzheimer-type dementia.

## Methods

### *Study subjects*

A total of 75 elderly subjects with senile dementia of Alzheimer type were recruited from patients who were admitted and had undergone medical investigation at Hanwa Daini Senboku Hospital, Japan. Senile dementia of Alzheimer type was diagnosed based on the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV, American Psychiatric Association, Washington D.C., 1994)<sup>18</sup>. All subjects showed a Hachinski ischemic score of 4 points or less. Patients had Mini Mental State Examination (MMSE) scores ranging between 10 and 26 at the time of initial imaging. We also excluded patients with following diseases in uncontrolled and severe status; hypertension (systolic blood pressure  $\geq 160$ mmHg, diastolic blood pressure  $\geq 100$ mmHg), diabetes mellitus (HbA1c  $\geq 8.0\%$ ), dyslipidemia (total cholesterol  $\geq 260$ mg/dl), cardiovascular diseases (NYHA III or IV, peripheral artery disease with class III or IV of Fontaine classification), renal failure (serum creatinine  $\geq 2.0$ mg/dl), and a mental disorder such as depression. We also excluded patients with prominent cerebral infarction diagnosed by magnetic resonance imaging. None of the assigned patients received cholinesterase inhibitors.

### *Study drugs*

We used Goshajinkigan Extract Granules for Ethical Use (TUMURA & Co. Tokyo, Japan, 7.5g/day) and Kihito Extract Granules for Ethical Use (TUMURA & Co. Tokyo, Japan, 7.5g/day). For 7.5 g of Goshajinkigan (GJG), there are 4.5 g of extract of

following dried medical herbs; Moutan Cortex (3.0 g), Corni Fructus (3.0 g), Dioscoreae Rhizoma (3.0 g), Rehmanniae Radix (5.0 g), Cinnammoni Cortex (1.0 g), Aconiti Tuber (1.0 g), Achyranthis rRadix (3.0 g), Alismatis Rhizoma (3.0 g), Plantaginis Semen (3.0 g), and Poria (3.0 g). For 7.5 g of Kihito Extract Granules, there are 4.5 g of extract of following dried medical herbs; Angelicae Radix (2.0 g), Longan Arillus (3.0 g), Polygalae Radix (2.0 g), Saussureae Radix (1.0 g), Zizyphi Spinosi Semen (3.0 g), Astragali Radix (3.0 g), Atractylodis Rhizoma (3.0 g), Ginseng Radix (3.0 g), Glycyrrhizae Radix (1.0 g), Zingiberis Rhizoma (1.0 g), Zizyphi Fructus (2.0 g), and Poria (3.0 g). We chose GJG as a control for Kihito because GJG contains extracts of herbs that are completely different from Kihito, except Poria. Throughout the study, all subjects were hospitalized to check compliance. During the trial, no other major new medication was allowed.

### ***Study protocol***

Subjects were randomly assigned to one of three groups: the Nontreatment group, the Goshajinkigan group (GJG group), or the Kihito group. Patients assigned to the GJG and Kihito groups received GJG (7.5g/day) and Kihito (7.5g/day), respectively, after meals three times a day for 3 months.

MMSE scores and activities of daily living (ADL) scores were assessed at the beginning of the trial and after 3 months of medication. The evaluation of MMSE and ADL were performed with single blind method. Single photon emission computed tomography (SPECT) was performed at the beginning of the trial and after 3 months of medication in 10 patients to evaluate changes in cerebral blood flow (CBF). SPECT was performed using the model Toshiba E-Cam in 6 patients from the GJG group and 4 patients from the Kihito group with <sup>99m</sup>Technesium-ethyl cysteinate dimer (<sup>99m</sup>Tc-ECD).

Mean CBF (mCBF) was analyzed by Patlak plot analysis with the reference in the aortic arch; then, regional CBF (rCBF) was estimated by applying Lassen's correction algorithm<sup>19</sup> and three-dimensional stereotaxic ROI template (3D-SRT)<sup>20</sup> to objectively measure rCBF. The rCBF was calculated in the anterior cingulate, posterior cingulate, frontal, parietal, temporal, occipital, and hippocampus segments. Increased cases of CBF were determined by an increase in at least one rCBF measurement of more than 15% compared to the basal level. We set cutoff point at 15% based on the following information; (1) The data of reproducibility provided by the manufacturer indicated that inter-measurement variability of rCBF is between 5-10% using Patlak plot analysis with 3D-SRT, (2) Original article<sup>20</sup> of 3D-SRT showed that CBF values after treatment with placebo distributed within 15% changes of CBF values before treatment in 95% of 352-pair examinations.

The study protocol was approved by the ethical committee of Hanwa Daini Senboku Hospital, and all subjects and/or their families gave written informed consent to participate in the study. All procedures followed were in accordance with the provisions of the Declaration of Helsinki in 1995.

### ***Statistical analysis***

Statistical analysis was performed using StatView (Abacus Concepts Inc., Berkeley, CA). Effects of treatment on each parameter were analyzed by analysis of variance (ANOVA), followed by Fisher's protected least significant difference test. Effects of treatment for three months on changes in MMSE scores were assessed by paired *t*-tests. Interaction between the effects of treatment and changes in MMSE scores were analyzed ANOVA with repeated measurement. Prevalence of hypertension and diabetes mellitus among the three groups was analyzed by chi-square test. Comparisons

of changes in MMSE and semi-quantitatively measured CBF with SPECT between the GJG and Kihito groups were performed with chi-square tests. A value of  $P < 0.05$  was considered statistically significant.

## **Results**

### ***Study safety***

We performed statistical analysis in 64 patients except withdrawal cases. The subjects numbers used in the analysis were 20 for the Nontreatment group, 24 for the GJG group, and 20 for the Kihito group. Characteristics of these patients are presented in Table 1.

Eleven subjects withdrew before the end of study because of withdrawal of consent within a month (GJG 3 subjects, Kihito 3 subjects), transfer to other hospital for non-medical reasons (GJG 1 subject, Kihito 1 subject), worsening of ADL by accidental fracture (Kihito 1 subject), or possible adverse effects (GJG 1 subject (diarrhea), Kihito 1 subject (hypertension)). The major reason of withdrawal of consent was due to bitter taste of the medicine. In patients possibly suffered adverse events, the complaints disappeared after discontinuation of the assigned drug. All laboratory findings, including liver function, were within normal range during the oral administration of GJG and Kihito. There was no severe adverse event in all groups. There was no significant difference between the GJG and Kihito groups in terms of the overall safety rating (Table2).

### ***Effect of treatment on MMSE, ADL, and CBF***

There was no significant difference in baseline characteristics among the three treatment groups (Table 1).



MMSE scores showed significant improvement at 3 months after treatment in the Kihito group, but not in the Nontreatment or GJG groups (Fig. 1). MMSE scores at 3 months after treatment compared to pretreatment scores also showed an increase by treatment only in the Kihito group (Fig.2). Improvement of mental state in the Kihito group was most manifested as changes in orientation and attention compared with those in memory and language (Fig.3).

Basal levels of ADL did not differ among the three groups (Table 1), and ADL was unchanged at three months after treatment in all groups.

Treatment with Kihito was not associated with an increase in CBF. Regarding the increased area of CBF, it was increased mainly in frontal and cingulate in both GJG and Kihito groups. However, we could not perform comparison of increased area between the GJG and Kihito groups because the number of subjects with increased rCBF was less for statistical analysis.

## **Discussion**

The present study demonstrated that Kihito treatment significantly improved cognitive function as estimated by MMSE, especially for scores in orientation and attention, compared with nontreatment and GJG treatment in patients with Alzheimer-type dementia. Differences in MMSE scores between before and after treatment in our Kihito group were about 2 points, which was similar to improvements reported previously in studies assessing the effect of herbal medicine on cognitive function<sup>7,21</sup>. Previous reports using donepezil demonstrated a similar or lesser extent of improvement in MMSE compared with our study using Kihito<sup>22-24</sup>. An increase of MMSE scores in the Kihito group in contrast to a decrease or no change in other treatment groups suggests the possibility of usefulness of Kihito for patients with

Alzheimer-type dementia.

We chose Kihito because it contains *Polygalae Radix*, is commercially available in Japan, and has been widely used for the elderly. Kihito is generally prescribed in Japan for persons of frail constitution with anemia, anxiety, palpitation, and insomnia<sup>17</sup>. We chose GJG as the control medicine in addition to a nontreatment group because: (1) GJG does not contain *Polygalae Radix* and other components of GJG have not been reported to affect cognitive function; (2) in comparison with non-herbal medicines, GJG and Kihito are similar in taste, color, and shape; and (3) both Kihito and GJG are frequently prescribed for elderly people. GJG has been prescribed in Japan for elderly people suffering from lumbago, back pain, and oliguria. Kihito and GJG have only one ingredient, *Poria*, in common. However, *Poria* demonstrates differential pharmacologic action in each combination because of interaction with the different components in each medicine.

The mechanism by which Kihito improves cognitive function may be related to the elevation of Ach levels by activation of ChAT, primarily attributable to *Polygalae radix*, as previously shown in cultured rat septal neurons<sup>13</sup>. This hypothesis is supported by the preventive effect of KUT, a cocktail of herbal medicines containing *Polygalae Radix*, on the progression of dementia in Alzheimer's disease<sup>7</sup> and by reduction of ChAT activity when *Polygalae Radix* is omitted from KUT<sup>13</sup>. The mechanism of activation of ChAT activity is reported to be caused by transcriptional activation of ChAT mRNA<sup>25,26</sup>. On the other hand, donepezil increases Ach levels in the brain via inhibition of Ach degradation by acetylcholine esterase<sup>27-29</sup>. Furthermore, KUT increases nerve growth factor mRNA in cerebral cortex cells<sup>25</sup>. Therefore, *Polygalae Radix* in Kihito may affect cognitive function via multiple pathways.

Another possible mechanism by which Kihito improves cognitive function is

an effect on CBF. In previous studies, both Nakano et al<sup>30</sup> and Nobili et al<sup>31</sup> reported that rCBF in critical areas was maintained but not improved after long-term treatment (12 months) and the MMSE score declined significantly both in untreated and in treated group with donepezil. Those reports are consistent with the report of Rogers et al<sup>32</sup> that cognitive improvements gained early with Ch-E inhibitors gradually fade away after several months of treatment. On the other hands, Carvolo et al<sup>33</sup> and Mega et al<sup>34</sup> reported that MMSE score was significantly improved and improvement in the rCBF pattern correlated with cognitive improvement with Ch-E inhibitors after short-term treatment (3-4 months). The present study, short-term treatment, demonstrated that improvement of CBF by Kihito was not superior to that elicited by GJG. This result may be interpreted by several possible causes. The first possibility is that our evaluation system was less sensitive to detect quantitatively changes in rCBF compared with the previously reported methods such as the statistical parametric mapping programs and three-dimensional stereotaxic surface projections<sup>33,35</sup>. The second possibility is that role of an increase of CBF in the improvement in MMSE by Kihito is small compared with activation of ChAT or other unknown pathways. The third possibility is that GJG treated group was inappropriate as control because GJG *per se* is known to increase peripheral vascular flow<sup>36</sup>. Further studies are required to clarify the precise mechanism.

The present study demonstrated improvement of mental state, especially in orientation and attention, in the Kihito group. Ushijima et al.<sup>37</sup> reported an association between rCBF and MMSE scores in patients with Alzheimer's disease. They demonstrated a relationship between a decline in rCBF in the parietal cortex and hippocampus and disorientation. In addition, there was a decrease in rCBF in the anterior temporal cortex associated with registration; in the frontal cortex associated with attention and calculation; in the medial temporal cortex associated with recall; and

in the posterior temporal cortex associated with language. As described above, donepezil increases rCBF, including in the prefrontal cortex. These reports are consistent with the previous report of a multicenter, randomized, controlled trial that donepezil-treated patients showed improvements in tests of attention and psychomotor speed<sup>38</sup>. We need further study to clarify whether Kihito might improve attention and orientation by any specific regional increases in CBF.

There are several study limitations in our study to conclude the effect of Kihito on MMSE including its precise mechanism. We evaluated MMSE and ADL with single blind method. Although MMSE consists of simple questions and inter-observer reliability is high<sup>39</sup>, double blind evaluation would be more reliable. Another study limitation is that the number of subjects who were performed SPECT was too small to conclude the relation between changes in CBF and changes in MMSE. Further studies are required to clarify the role of Kihito on regional changes in CBF and the relation between changes in MMSE and rCBF by Kihito. Taking these limitations into consideration, we conclude that Kihito would be a safe and probably effective herbal medicine for symptomatic treatment in Alzheimer-type dementia and has opened the window to investigations of its usefulness in a larger-scale trial.

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**Table 1. Patient characteristics**

	Nontreatment	GJG	Kihito	Statistics
Number	20	24	20	
Age, years	82.8±8.1	84.2±6.4	86.1±5.0	N.S
Male/Female	4/16	2/22	5/15	N.S
Education, years	9.0±0.5	8.6±0.5	8.8±0.5	N.S
Hypertension, %	55.0	37.5	35.0	N.S
Diabetes mellitus, %	5.0	12.5	25.0	N.S
Hachinski ischemic score	2.3±1.5	2.7±1.3	2.1±1.0	N.S
MMSE score	17.6±5.0	18.1±4.2	17.6±4.1	N.S
ADL score (Barthel index)	9.5±5.3	12.4±5.9	12.5±5.9	N.S
Serum albumin, g/dl	3.48±0.37	3.57±0.32	3.48±0.33	N.S
Serum creatinine, mg/dl	0.88±0.53	0.91±0.57	0.85±0.39	N.S
Hemoglobin, mg/dl	10.82±1.68	11.11±1.37	10.88±1.42	N.S

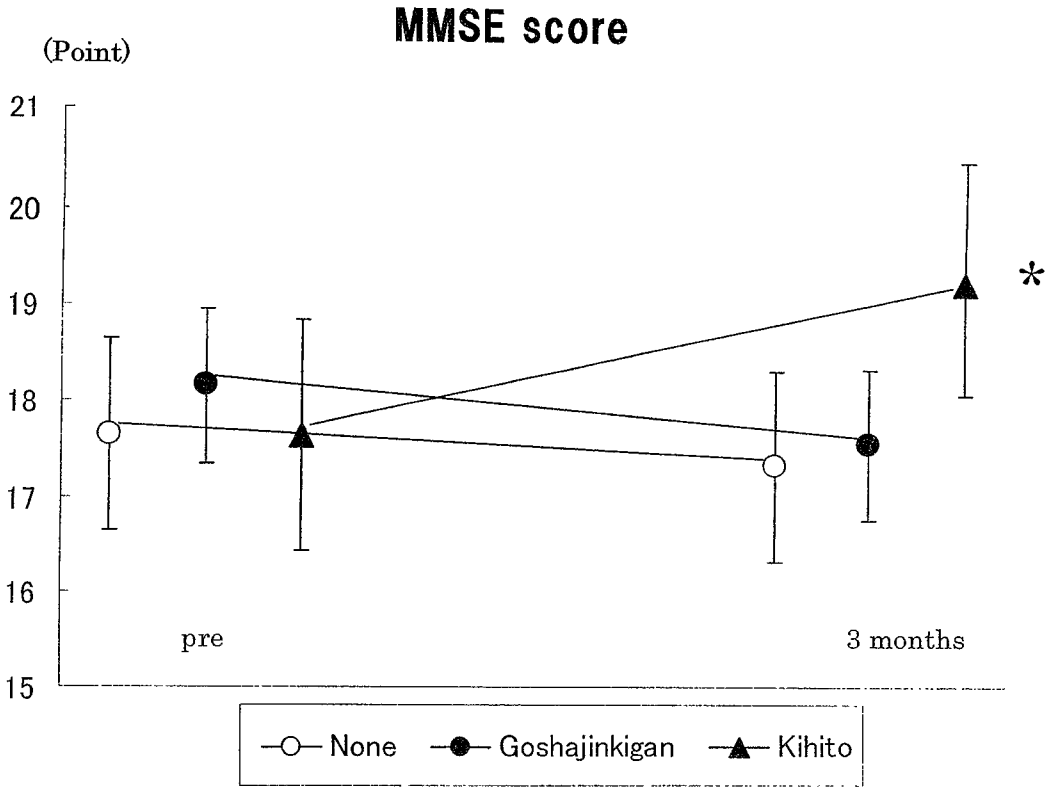
GJG, Goshajinkigan; MMSE, mini mental state examination; ADL, activity of daily life.

N.S; not significant. Values are mean±SD.

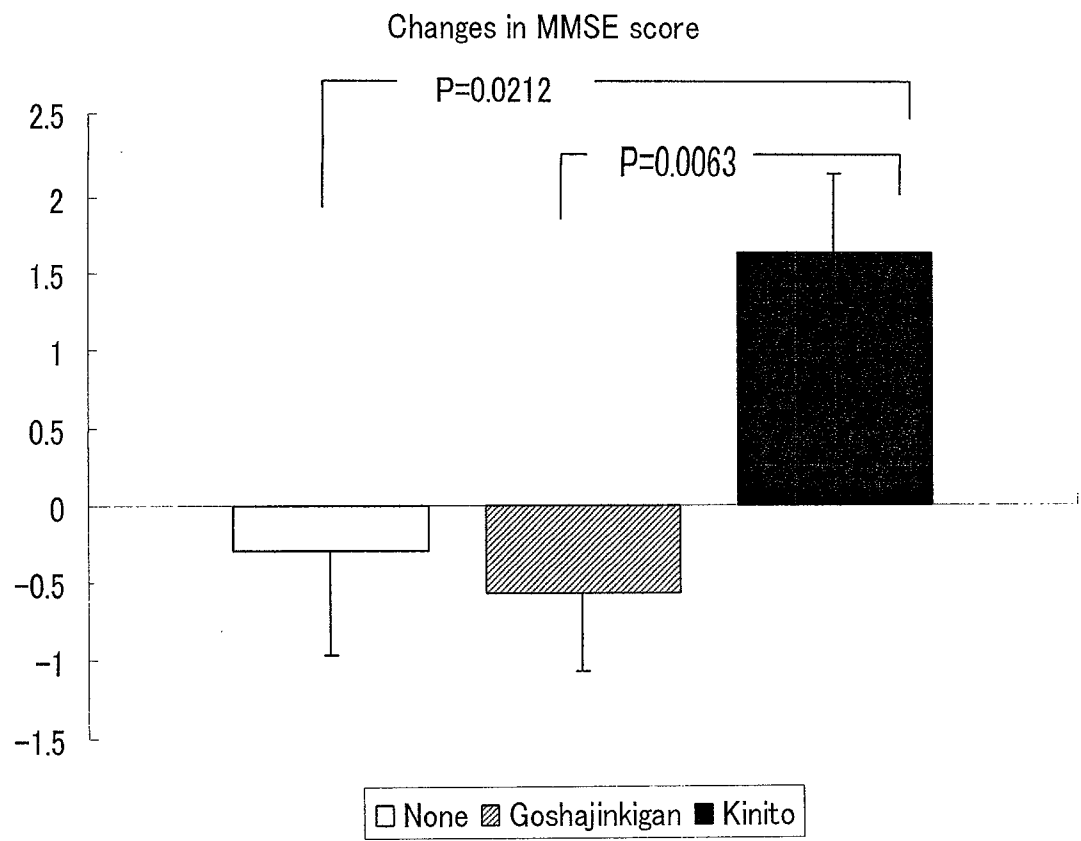
**Table 2. Comparison of blood biochemical analysis among the three groups.**

	Nontreatment		GJG		Kihito	
	Pre	3 months	Pre	3 months	Pre	3 months
Serum albumin, g/dl	3.48± 0.37	3.50± 0.37	3.57± 0.32	3.57± 0.34	3.48± 0.33	3.54± 0.33
Serum creatinine, mg/dl	0.88± 0.53	0.85± 0.49	0.91± 0.57	0.91± 0.63	0.85± 0.39	0.84± 0.32
Hemoglobin, mg/dl	10.82± 1.68	10.84± 1.96	11.11± 1.37	11.02± 1.38	10.88± 1.42	11.37± 1.34
Serum AST(asparatate aminotransferase), IU/l	21.80± 10.52	23.20± 14.02	24.16± 14.92	23.58± 13.09	19.20± 5.05	18.75± 5.81
Serum ALT(alanine aminotransferase), IU/l	14.85± 10.88	16.45± 14.94	18.75± 16.35	17.91± 12.78	12.40± 5.52	11.65± 7.30

GJG, Goshajinkigan; Values are mean±SD. There was no statistically significant difference among the three groups in blood biochemical analysis.

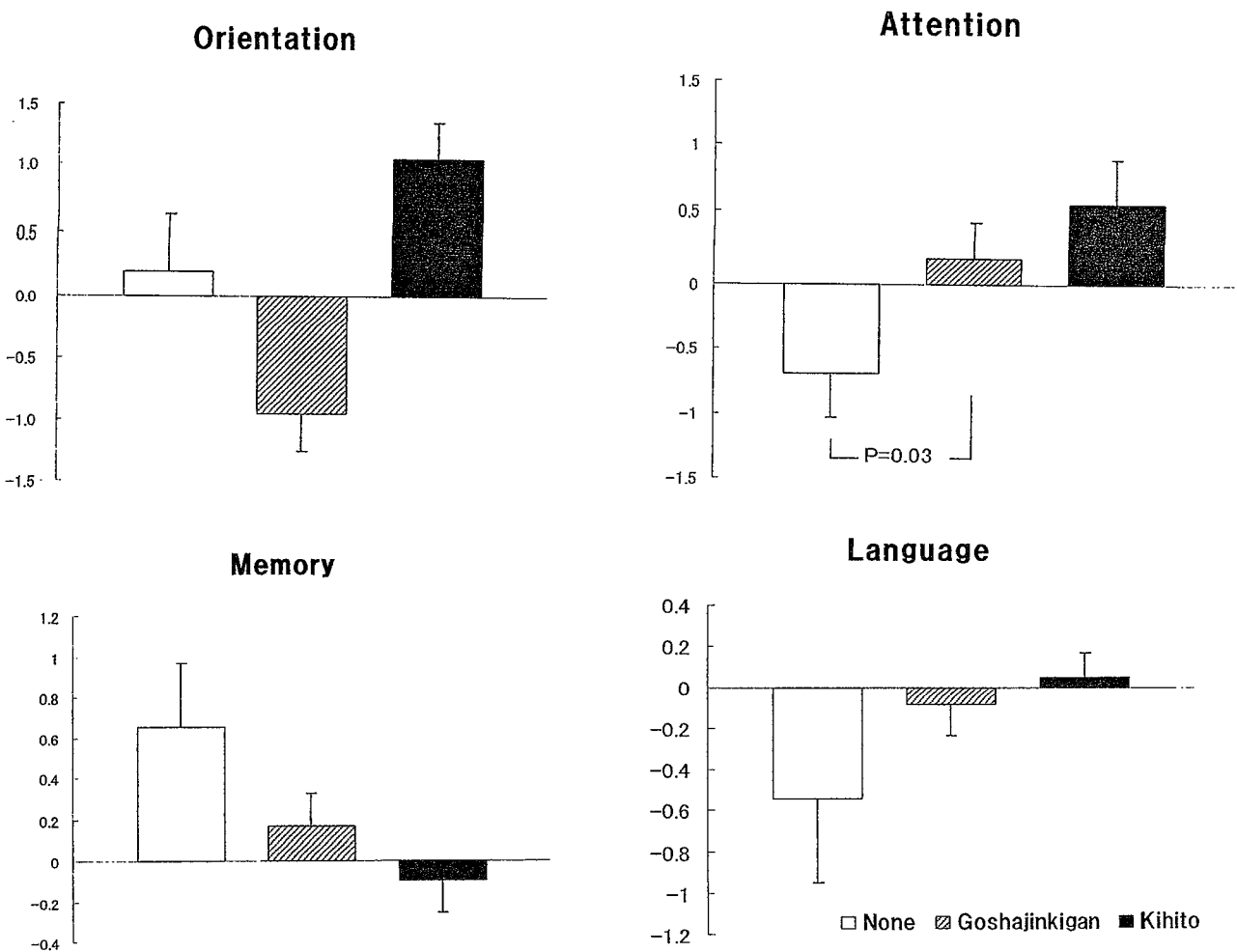


**Figure 1. Comparison of MMSE scores before and 3 months after treatment in three groups.** MMSE was performed at the beginning of the trial and after 3 months of medication. Data are given as mean  $\pm$  SEM. ANOVA with repeated measurement showed that F value of interaction between the effects of treatment and changes in MMSE scores were 4.55 ( $P=0.015$ ). Significant improvement of MMSE was observed only in the Kihito group (\*:  $P<0.01$  pretreatment vs. 3 months of treatment by paired t-test).



**Figure 2. Comparison of differences of MMSE scores between before and after treatment among three groups.** P values from post hoc analysis for multiple comparisons between two groups are shown in the figures. Data are given as mean  $\pm$  SEM.





**Figure 3. Comparison of differences of MMSE scores between before and after treatment among three groups: Subanalysis of four categories of mental states.** Questions of MMSE were categorized into four mental states;<sup>40</sup> orientation (10 points), memory (registration and recall, 6 points), attention (attention and calculation, 5 points), and language (9 points). Data are given as mean  $\pm$  SEM of average scores.

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