

*Full Length Research Paper*

# Analysis of brain cell activation by nanosized particles of *Ginkgo biloba* extract

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It is quite difficult to treat Alzheimer's dementia, where one of the problems is inadequate absorption of drugs. Revolution in manufacturing drugs is expected by the development of nanotechnology. Animal test was conducted to see whether the *Ginkgo biloba* extract processed with nanotechnology changed the function of cerebral cortical synapses and hippocampal nerve action. Rats underwent euthanasia 1 month after nanosized *G. biloba* extract administered. Synthesis and release activity of acetylcholine was measured, also the population spike and excitatory postsynaptic potential measured using hippocampal slice. Additionally, measurement with quantum resonance spectrometer was performed for human blood, artery and cardiac muscle. The rats with nanosized *G. biloba* extract administered showed a trend of acetylcholine release from cerebral cortical synapses more promoted than the control rats. Also, these rats showed greater amplitude of population spike at hippocampal CA1 pyramidal cell layer than the control rats. Remarkable effect on human blood, artery and cardiac muscle was also indicated with quantum resonance spectrometer. Test result showed that the nanosized particles of *G. biloba* extract resulted in easier absorption. However, acetylcholine synthesis is not explained completely, and this is to be one of the research themes in nano-medicine to be solved.

**Key words:** *Ginkgo biloba* extract, brain cell activation, nanotechnology, synapse, acetylcholine, hippocampus.

## INTRODUCTION

*Ginkgo biloba* extract has been widely marketed with an expectation of brain cell activation (Tadano and Kisara, 1997). However, the existing powder of *G. biloba* extract cannot achieve a remarkable effect for brain cell activation because the granule size is big and the plant cell wall is not destroyed, which results in insufficient absorption of the active ingredient in the body.

The purpose of this research is to compare the novel sample of *G. biloba* extract processed with

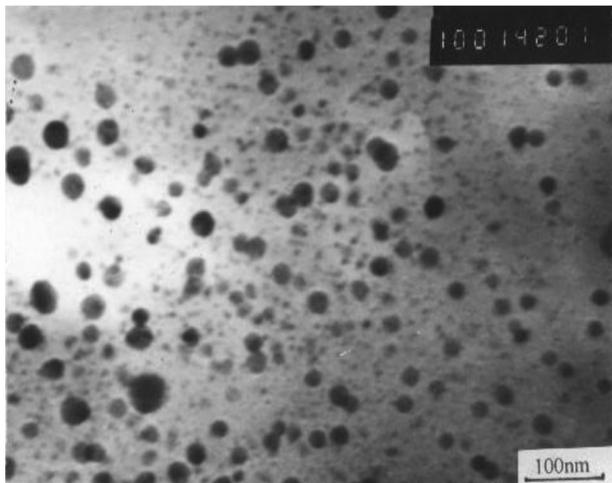
nanotechnology to the existing products for activity by measuring the functional change of cerebral cortical synapses and the change of hippocampal nerve action for the rats who take the novel sample of *G. biloba* extract orally to demonstrate the effect of the sample on brain function.

## MATERIALS AND METHODS

### Nano material and process of manufacture

*G. biloba* is extracted with 40% of ethyl alcohol. The extract is concentrated by adsorption of porous resin and powered after drying with high temperature to obtain the coarse powder of

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**Photo 1.** Particles of approx. 30 nm were observed with a transmission electron microscope.

*G. biloba* extract (Watanabe et al., 2005). Then, the coarse powder of *G. biloba* extract is ground with the break-down method, which is the combination method of dry and wet processes, to produce the nanosized particles (Suzuki et al., 2007). The high-speed rotating impact grinder, jet mill, ball mill, compression-shearing mill (Ongmill) or roller mill are used for dry ultrafine grinding. The wet process makes finer grinding possible than the dry process, since the wet process prevents the formation of pulverulent body, which is caused by attachment and aggregation for dry grinding, and reduces the cushion effect that obstructs fine processing. Eventually, the novel sample of granule size less than 100 nm is produced by the combination method of dry process (gas-phase grinding technique) and wet process (liquid-phase grinding technique) (Photo 1).

## Test procedures

### Animal

Male 27-month Wistar rats (mean weight 409 g) were used for the test.

### Administration of the sample of *G. biloba* extract

Rats were sorted into the following 3 groups after the handling performed 1 week prior to the administration for the purpose of reducing the stress in administration: administration group of *G. biloba* extract sample of 30 nm particles (GK30); administration group of the same sample of 100 nm particles (GK100); and administration group of the same sample of 200  $\mu$ m particles (GK200) as a control group. Each sample was suspended in water (7.84 mg/ml), and 1.96 mg (calculated by the standard dose of 240 mg/50 kg) of *G. biloba* extract was administered to a rat once a day through a stomach sonde (feeding needle) ( $n = 3/\text{group}$ ). Whereas water was administered to the control rats ( $n=5$ ). The period for administration was 1 month. Solid feed and water was provided *ad libitum* during the period for administration.

### Preparation of synaptosome

Synaptosome was prepared using whole cerebral cortex of rats by

Ficoll's discontinuous density gradient centrifugation (Tanaka and Ando, 1990).

### Measurement of synthesis and release activity of acetylcholine (ACh)

Synaptosome was suspended in Krebs-Ringer solution containing 200  $\mu$ M of eserine (acetylcholinesterase inhibitor) and incubated at 37°C for 30 min. After the incubation, 0.1 N of perchloric acid was added to terminate the reaction. The known amount of ethylhomocholine (EHC) was added as internal standard material and centrifuged to obtain the supernatant.

Some of the synaptosome incubated at 37°C for 30 min was washed with Krebs-Ringer solution containing eserine and then suspended in the buffer solution. Krebs-Ringer solution containing high concentration of potassium was added to this suspension. The final concentration of potassium was adjusted into 10 and 40 mM and incubated at 37°C for 5 min. EHC was added and centrifuged to obtain the supernatant. These samples were measured for the amount of synthesis and release of ACh by the high-performance liquid chromatography (EICOM 300) with electrochemical detector (the amounts of ACh, both synthesized and released, were calculated per unit synaptosomal protein).

### Measurement of population spike and excitatory postsynaptic potential using hippocampal slice

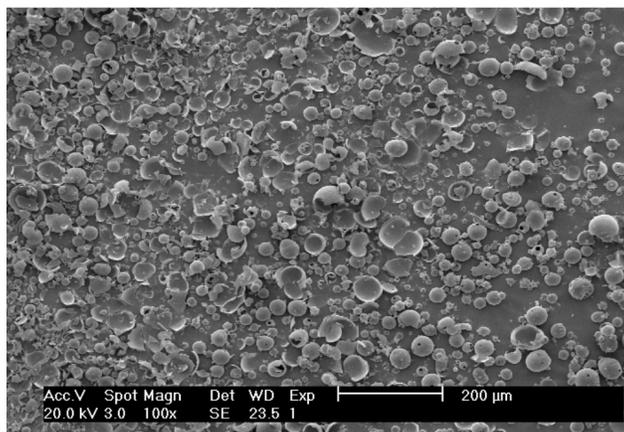
Hippocampus was removed to make 400  $\mu$ m thickness of hippocampal slice by a rotary slicer. This was used for the test after the incubation for 2 h in the artificial cerebrospinal fluid (ACSF) with mixed gas (95% O<sub>2</sub> + 5% CO<sub>2</sub>) sufficiently dissolved. The hippocampal slice was placed in the measurement chamber. The stimulation electrode was inserted into the Schaffer collateral, and the recording electrodes were inserted into the CA1 pyramidal cell layer and stratum radiatum. Electrical pulse stimulation was applied every 10 s, and the population spike and the excitatory postsynaptic potential were recorded. After ensuring that the stable baseline was obtained, electrical pulse stimulation was changed between 0.04 - 0.25 mA and the response to each stimulus intensity was measured.

### Measurement with quantum resonance spectrometer

Powders of *G. biloba* extract processed with nanotechnology and the powders not processed with nanotechnology were placed respectively on the measuring plate of CQRS-2 type quantum resonance spectrometer with living samples. Electromagnetic waves from living body and weak electromagnetic energy (electromagnetic waves) from *G. biloba* extract affected each other in about 1 min, which caused resonance phenomenon, and the quantificational values were obtained in quantum level.

## RESULTS AND DISCUSSION

The product of *G. biloba* extract prepared with existing powder process is considered to have low absorption efficiency of the active ingredient in the body because the granule size is big and the plant cell wall is destroyed insufficiently. The process of the powder of *G. biloba* extract using gas-phase grinding technique and liquid-phase grinding technique of this research can make nanosized particles where the plant cell wall is sufficiently



**Photo 2.** It was observed with a transmission electron microscope that the plant cell wall was destroyed.

**Table 1.** Result of the measurement with CQRS-2 type quantum resonance spectrometer.

Measurement item (Effect on human body)	<i>G. biloba</i> extract (before nanosized)	<i>G. biloba</i> extract (after nanosized)
Blood	5	24
Artery	17	29
Cardiac muscle	13	28

>20: Significantly beneficial effect, 17 – 20: Beneficial effect, 14 – 16: Good effect, <14: Ordinary effect.

destroyed (Photo 2).

### Result of the measurement with quantum resonance spectrometer

This process with nanotechnology can make the granule size of *G. biloba* extract smaller, and thus the absorption of active ingredient of *G. biloba* extract is expected to increase. The result of measurement with CQRS-2 type quantum resonance spectrometer shows that the granule size gets much smaller, which indicates the remarkable effect on human blood, artery and cardiac muscle (Table 1).

### Effect on synthesis and release of acetylcholine at cerebral cortical synapses

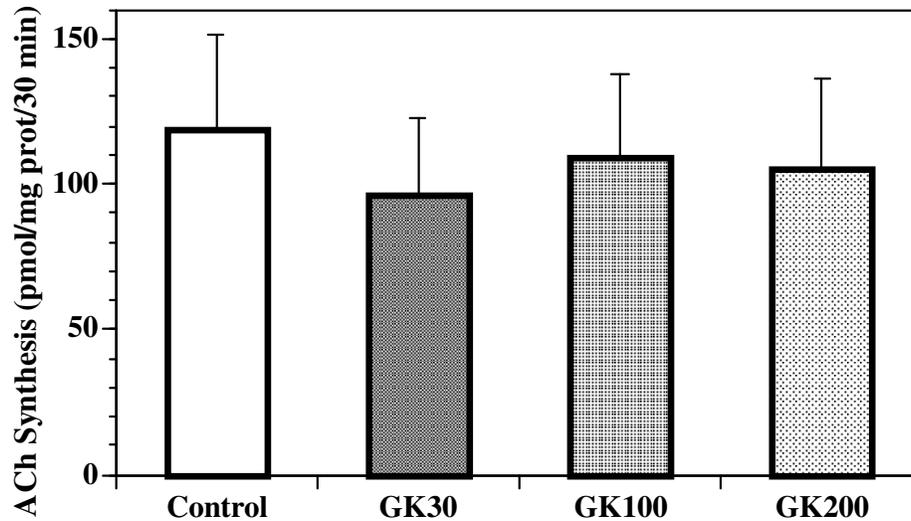
The administration effect of each *G. biloba* extract sample is shown in Figure 1A for ACh synthetic activity at cerebral cortical synapses. ACh synthetic activity for the rats of *G. biloba* extract administered is almost same with that of the control rats, which indicates that any *G. biloba* extract sample has no effect on ACh synthetic activity at cerebral cortical synapses.

Next, the effect on ACh releasing activity by depolarizing stimulation with high concentration of potassium was observed (Figure 1B). The rats of *G. biloba* extract administered show a trend of ACh release from cerebral cortical synapses more promoted than the control rats. Especially, the rats of novel sample of *G. biloba* extract (30 nm particles, GK30) administered show a significant promotion of ACh release compared with the control group. These results suggest that the *G. biloba* extract promotes ACh release efficiency by depolarizing stimulation without promoting ACh synthesis at the brain synapses of old-aged rats.

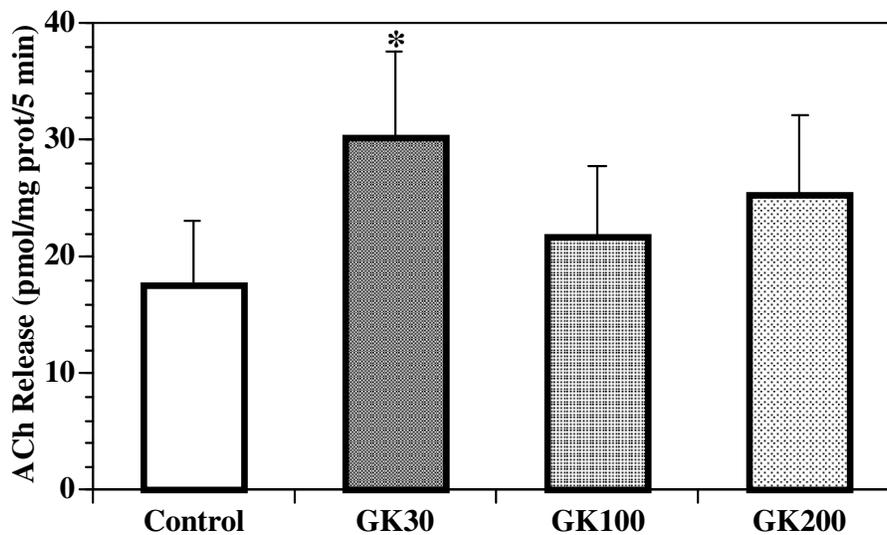
Calcium ion influx from voltage-dependent calcium channel can trigger the release of neurotransmitter substance at synapses. The change of calcium ion influx of *G. biloba* extract was measured using Fura2 of calcium ion sensitive dye. There was especially no result of calcium ion influx promoted by this sample.

### Effect on neuron activity at hippocampus

Figure 2A shows the amplitude of population spike at hippocampal CA1 pyramidal cell layer. The administration group of *G. biloba* extract shows greater amplitude of population spike than that of the control group. The



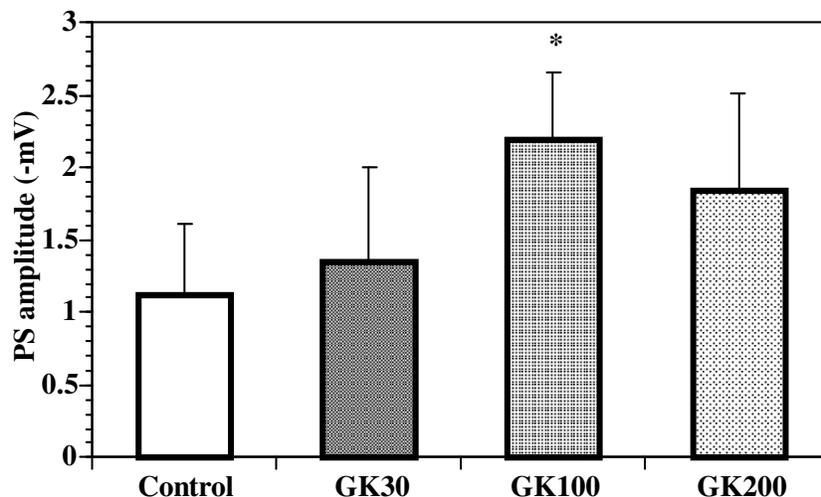
**Figure 1A.** The administration effect of each *G. biloba* extract sample is shown for ACh synthetic activity at cerebral cortical synapses ( $p < 0.05$ ).



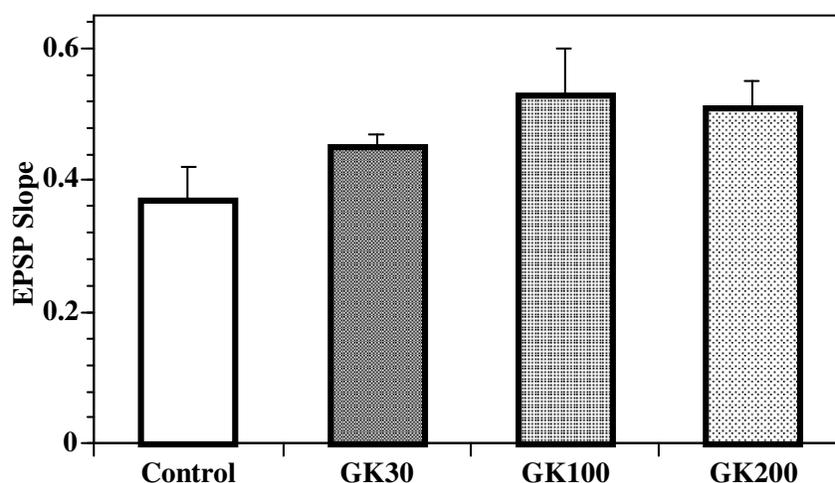
**Figure 1B.** The rats of *G. biloba* extract administered show a trend of ACh release from cerebral cortical synapses more promoted than the control rats ( $p < 0.05$ ).

significant amplification of population spike was observed especially for the rats of novel sample of *G. biloba* extract (100 nm particles, GK100) administered. In addition, the administration group of *G. biloba* extract shows the increasing trend of excitatory postsynaptic potential (EPSP), the indicator of neuronal excitability, compared with the control group (Figure 2B). In other words, this result suggests that the response of hippocampal pyramidal cell to stimulation is increased or the cell population that responds to stimulation is increased by the administration of *G. biloba* extract.

Williams et al. (2004) have concluded that the effect of the *G. biloba* extract (EGb 761) is postsynaptic, not presynaptic, because EGb 761 has no effect on paired pulse facilitation (PPF), though it promotes the hippocampal long-term potentiation of old-aged mice. However, the acetylcholine release from the presynaptic sample of synaptosome is significantly increased by the depolarizing stimulation for *G. biloba* extract, especially for the novel sample of *G. biloba* extract, which indicates that this nano sample has more beneficial effect than the existing sample for the release of neurotransmitter



**Figure 2A.** The amplitude of population spike at hippocampal CA1 pyramidal cell layer is shown ( $p < 0.05$ ).



**Figure 2B.** It is suggested that the response of hippocampal pyramidal cell to stimulation is increased or the cell population that responds to stimulation is increased by the administration of *Ginkgo biloba* extract ( $p < 0.05$ ).

substance from presynapse. Ramassamy et al. (1993) have reported that the decrease of membrane fluidity and the decrease of dopamine uptake occur by processing synaptosome with ascorbic acid/ $Fe^{2+}$  and by oxidation of synaptic membrane lipids, however, that these 2 parameters can recover with EGb 761 work. Also, Drieu et al. (2000) and Stoll et al. (1996) have reported that *G. biloba* extract can increase the brain cell membrane fluidity. The possibility can be also considered in this research that the novel sample of nanosized particles of

*G. biloba* extract increases the synaptic membrane fluidity, resulting in increasing the fusion efficacy of synaptic vesicle and synaptic plasma membrane.

GK30 (30 nm) affected ACh release activity at synapses of cerebral cortices, however, why did GK100 (100 nm), but not GK30, affect excitability at hippocampal pyramidal cell bodies? Exact reason is unclear at present. It may be raised from regional difference of the brain. Further study is necessary to elucidate the mechanisms of the effects of GK30 and GK100 on the

efficacy of ion channels that involved in both neurotransmitter release and generation of population spikes.

### Implications of current data

Despite the limitations cited above, the administration of the novel sample of *G. biloba* extract demonstrates the increase of acetylcholine releasing activity from cerebral cortical synapses and the improvement of stimulation response of hippocampal pyramidal cell.

Thus, the nanosized *G. biloba* extract is expected to activate the brain cell and work on the treatment of Alzheimer's dementia. For further clinical studies, it is expected to investigate how the nanosized *G. biloba* extract can work on different races, genders and ages.

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