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Liver mitochondrial activation and fat-lowering effects of rapid and synchronized dormancy-breaking ginger rhizome assumed to be administered orally

Toshihiro Ona [1,2,*], Teruyuki Seino [3], and Junko Johzuka [2,1]

[1] Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan;
[2] O'Atari Inc., Onojo, Fukuoka, Japan;
[3] Department of Material and Environmental Engineering, Hakodate National College of Technology, Hakodate, Hokkaido, Japan.

* Corresponding author: ona@agr.kyushu-u.ac.jp, ona@oatari-inc.com

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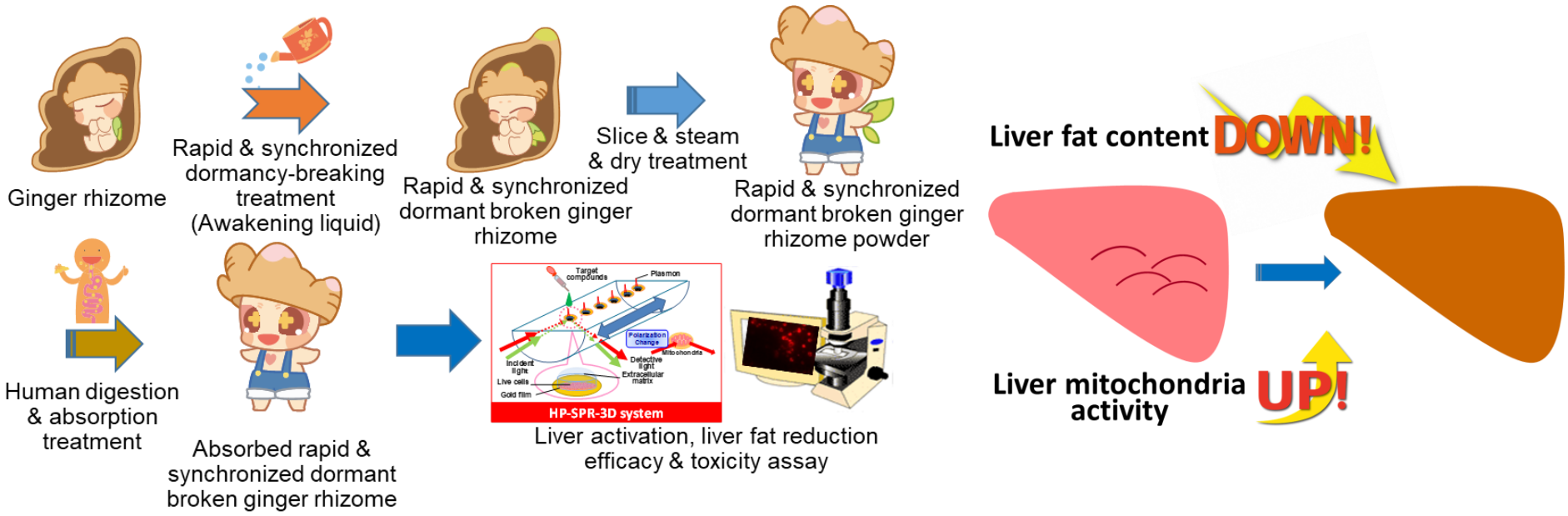
Graphical Abstract



Abstract: In ginger rhizome, the percentage of 6-gingerol (GING) is high before heating and the percentage of 6-shogaol (SHOG) increases after heating, and SHOG has been reported to activate liver metabolism. We have studied the method of rapid and synchronized dormancy-breaking treatment with Awakening liquid™ to promote phase transition of content components in seeds, resulting in improved functionality. In this study, we applied this method to ginger rhizome. After the treatment and heating, we evaluated liver activation and final fat-reduction before and after metabolism in the liver, assuming oral administration, including comparison between GING and SHOG. At 100nM, apoptotic toxicity was observed in GING both before and after metabolism, and promotion of metabolic activity was observed in SHOG, about 2 times greater after metabolism than before. In SHOG 1000nM, apoptotic toxicity was observed before metabolism, and promotion of metabolic activity was observed after metabolism by detoxification. The SHOG 100 nM decreased liver fat by about 20%. In dormancy-breaking ginger rhizome endosperm, SHOG was found to increase, containing about 8 times more than untreated and about 1.5 times more than non-dormancy-breaking one. There was also a concentration-dependent decrease in liver fat, which was about 27% at 2.0 mg/ml. However, apoptotic toxicity was observed at 3.0 mg/ml and above. Similarly, untreated sample showed little effect at 1.0 mg/ml, and apoptotic toxicity was observed at 2.0 mg/ml and above. In the model experiment assuming oral administration, rapid and synchronized dormancy-breaking ginger rhizome showed high effects on liver mitochondrial activation and fat lowering.

Keywords: liver; fat-reduction; dormancy-breaking; ginger rhizome; mitochondria

Introduction

Awakened Ginger Rhizome™ is rapid and synchronized dormant broken (awakened) rhizome using a proprietary Grandir recipe™. After the rhizome contents (ingredients) are converted by themselves, the rhizome is heated. This process increases both liver mitochondria activation and liver fat reduction efficacies and decreases toxicity of the ginger rhizome. The research was carried out at the Global Innovation Center, Kyushu University, Japan and at Department of Material and Environmental Engineering, Hakodate National College of Technology, Hakodate, Hokkaido, Japan.

Background

The liver breaks down sugar and synthesizes neutral fat as an energy source to accumulate. However, more than 30% of fat in the liver is called fatty liver. Fatty liver is caused by overeating (obesity), excessive drinking, stress, smoking, and diabetes [1]. As fatty liver progresses, it causes steatohepatitis, inflammation triggering fibrosis of the liver, cirrhosis, genetic mutations leading liver cancer, and death [1,2].

To resolve fatty liver, it is important to save drinking, eating and to do exercise. Activation of liver metabolism (mitochondria) and breakdown of fat (lipid), promotion of β-oxidation of lipids, are important in reducing liver fat.

Ginger has been cultivated in tropical Asia since 650 B.C., and has a long history of healthy eating habits as a crude drug, herbal medicine, and as a cooking ingredient such as condiments [3]. There were reports about extractives (extract) and ingredients of ginger rhizome on liver metabolism activation and they were submitted to clinical trials [4-6]. However, side effects of ginger are sometimes reported including heartburn, diarrhea, and irritation in the mouth [7].

The need for assured efficacy with minimized toxicity as food

Ginger rhizomes are in a dormant state in which growth activity is temporarily suspended until conditions are favorable for germination, with minimal metabolism and energy consumption [6]. However, once they wake up, they change the ingredients into a form which dissolves easily in water and break toxicity substances to inhibit germination [4]. In order for rhizomes to sprout and grow, a lot of nutrients as well as life itself reside. They include sugar, amino acids, minerals, hormones, and even substances which boost immunity to prevent diseases after sprouting. On the other hand, after blastogenesis or germination, rhizomes consume those ingredients. Additionally, extractives and extracts are acceptable, but real food is favorable to take without extraction or concentration considered from long human eating habits.

However, it is difficult to decide the endpoint of rhizome awakening by a conventional method. More importantly, the ingredients become uneven if rhizomes did not awake at the same time.

Therefore, a rapid method of awaking the ginger rhizome synchronizedly was developed as proprietary Grandir recipe™ with 2d of dormancy-breaking.

Here, it is very important to assess rapid and synchronized dormant broken ginger rhizome in order to assure bioavailability and efficacy with toxicity.

Study objectives

The objective of the *in vitro* study was to determine the efficacy and toxicity on liver activation and liver fat reduction of rapid and synchronized dormant broken ginger rhizome (Awakened Ginger Rhizome^{TM}) in case of oral administration as food. It included the comparison between 6-gingerol and 6-shogaol because these major compounds were candidates for these functions [5,8-10].

Methods (Fig. 1)

Sample

The organic ginger (*Zingiber officinale*) rhizome was grown in Shimabara, Nagasaki Prefecture, Japan. Rapid and synchronized dormancy-breaking was performed by our proprietary Grandir recipe™ using Awakening Liquid™ (based on concentrated malt extract, edible; O'Atari Inc., Japan) to prepare Awakened Ginger Rhizome™. Treated and untreated samples were sliced, steamed, dried and pulverized by a grinder and sieved to pass 60 mesh. Agents of 6-gingerol and 6-shogaol were bought and utilized.

Methods (Fig. 1)

Digestion and absorption treatment

For the use of oral administration, the ginger rhizome powder was digested in the stomach step and duodenum step using a human model system including enzymes and bile extract. Then the fraction of molecular weight of 10,000 or less was filtered as an intestinal absorption fraction for assay [3,11-12].

Methods (Fig. 1)

Liver activation assay

Cell based assay was performed by using our proprietary High Precision-Surface Plasmon Resonance-Three dimension (HP-SPR-3D) system, non-label and –invasive 1h phenotypic screening by mitochondrial membrane potential, as follows [13]. Two-dimensionally cultured viable cells were self-adhered onto an HP-SPR sensor chip, and then collagen was overlaid to obtain *in vivo*-like cell status. The cell response change was measured for about 1h after the sample addition. Human liver cell line Hep G2 was utilized.

Methods (Fig. 1)

Liver fat reduction assay

The collagen gel droplet embedded culture drug sensitivity test (CD-DST) was used as a 3D cell culture method [13, 14]. After 7 days, the fat content was measured after staining with Nile Red [15].

6-Shogaol content determination

The content of 6-shogaol in ginger rhizomes was measured using high performance liquid chromatography (HPLC) [16].

Methods (Fig. 1)

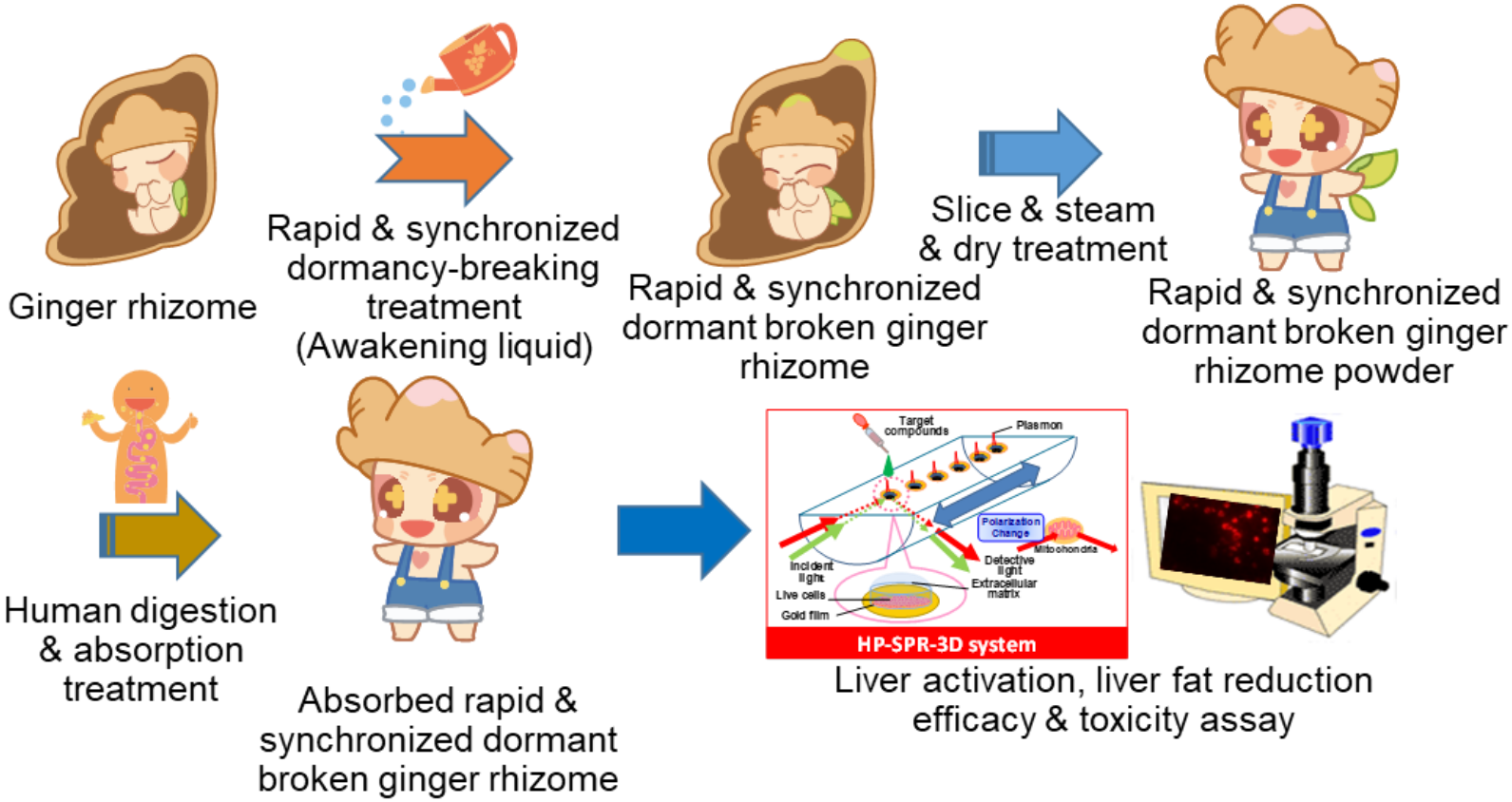


Fig. 1 Experimental scheme.

Results

Effects of liver activation and liver fat reduction by 6-gingerol and 6-shogaol

Ginger's ingredients act directly on liver cells in the liver, and then they are metabolized by the liver, and the metabolized ingredients work on liver cells. For this reason, we monitored mitochondrial activation in liver cells using our proprietary HP-SPR-3D system correlating to clinical results [13].
As a result, at 100nM, 6-gingerol exhibited apoptotic (cell death) toxicity both before and after metabolism in the liver, and 6-shogaol exhibited enhanced metabolic activity in the liver, about 2 times higher after metabolism than before metabolism (Figs. 2 and 3). At 1000nM 6-shogaol, apoptotic toxicity was observed before metabolism, and detoxification was observed after metabolism by promoting metabolic activity. At 100nM, 6-shogaol reduced liver fat (fluorescent area) by approximately 20% (Fig. 4).
From these results, 6-gingerol, a raw ginger ingredient, did not activate liver metabolism or decrease liver fat. Although 6-shogaol, a heated ginger ingredient, activated liver metabolism and decreased liver fat. However, excessive intake of 6-shogaol was found to have adverse effects [7].

Results

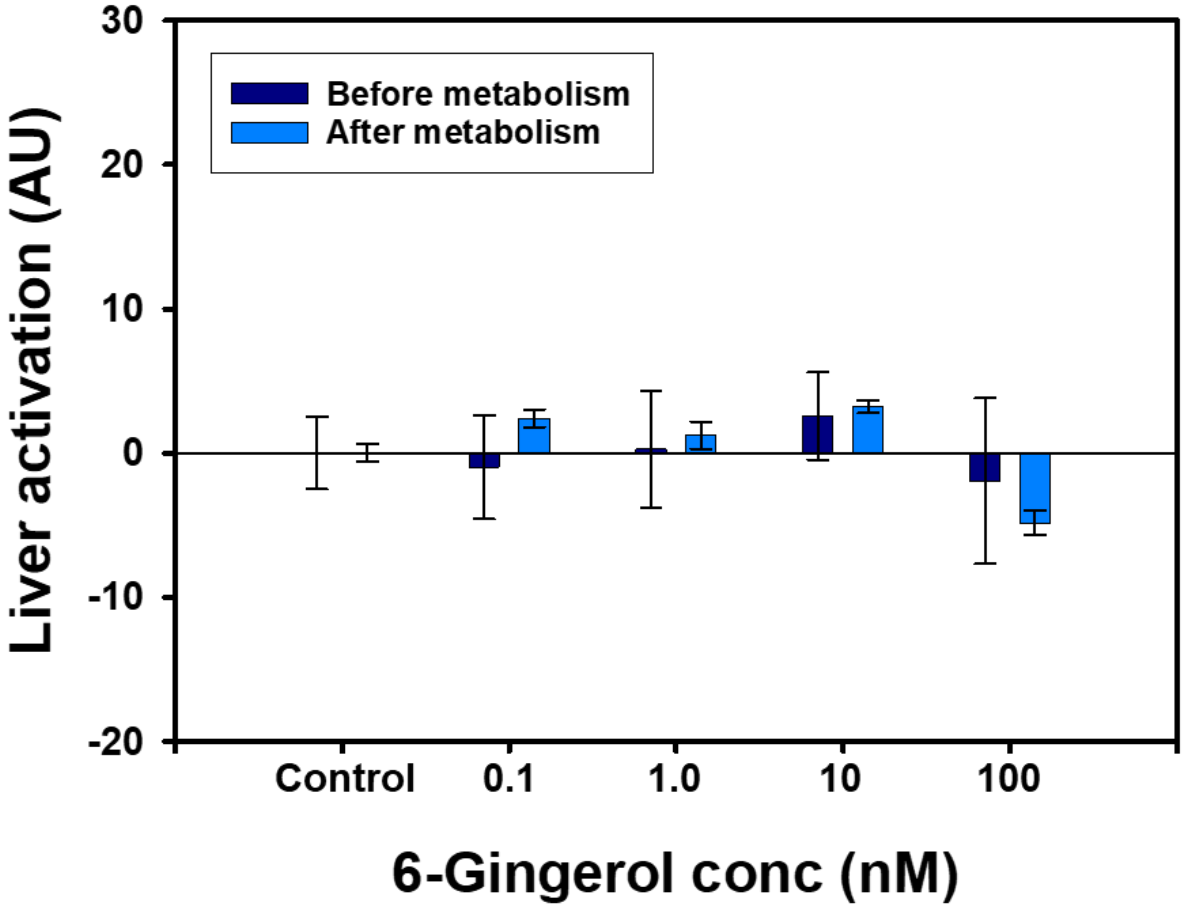


Fig. 2 Liver activation effect by 6-gingerol.

Results

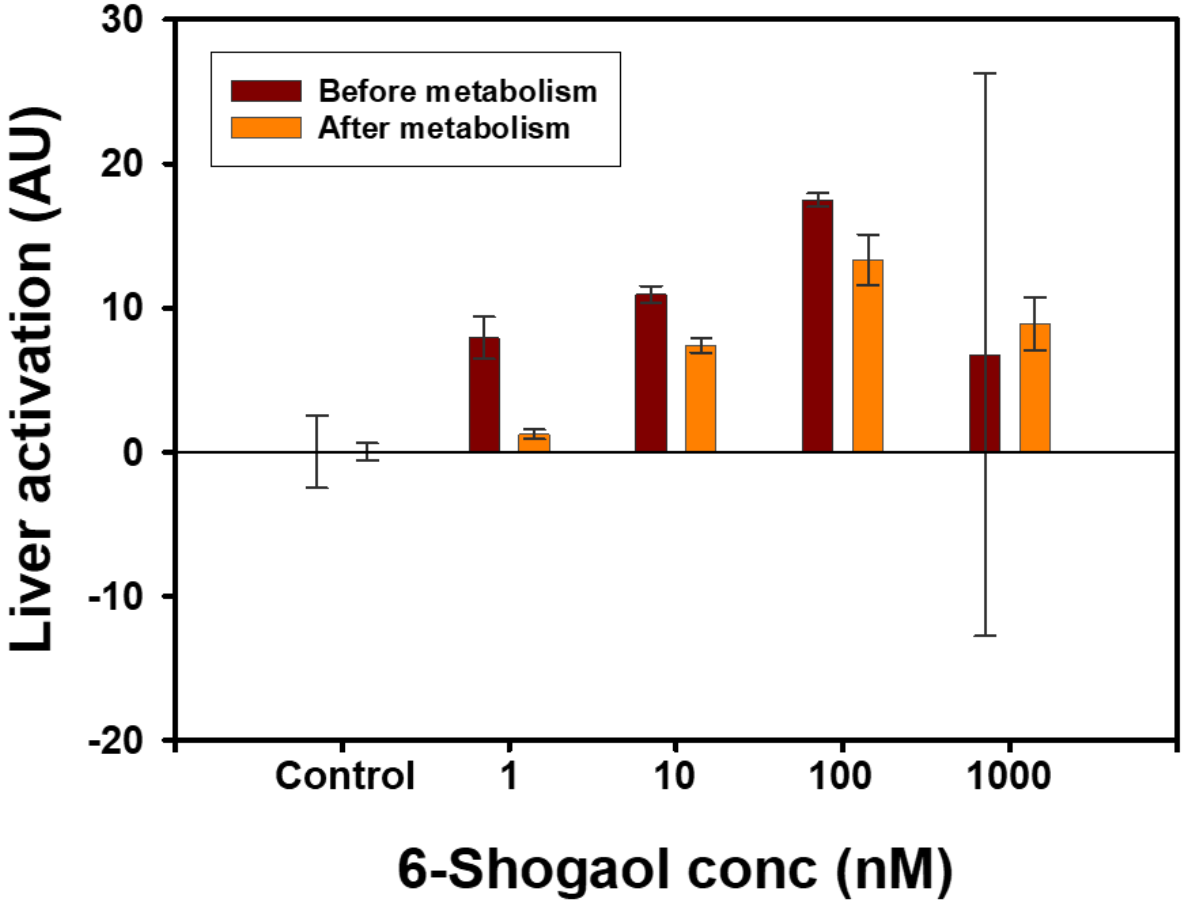


Fig. 3 Liver activation effect of 6-shogaol.

Results

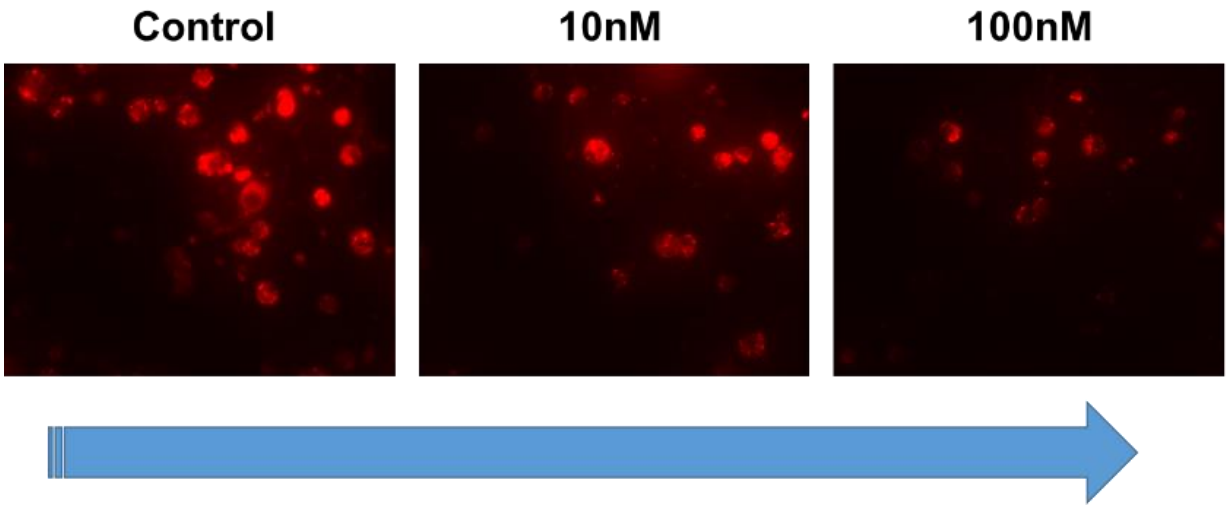


Fig. 4 Effect of liver fat reduction by 6-shogaol.

Results

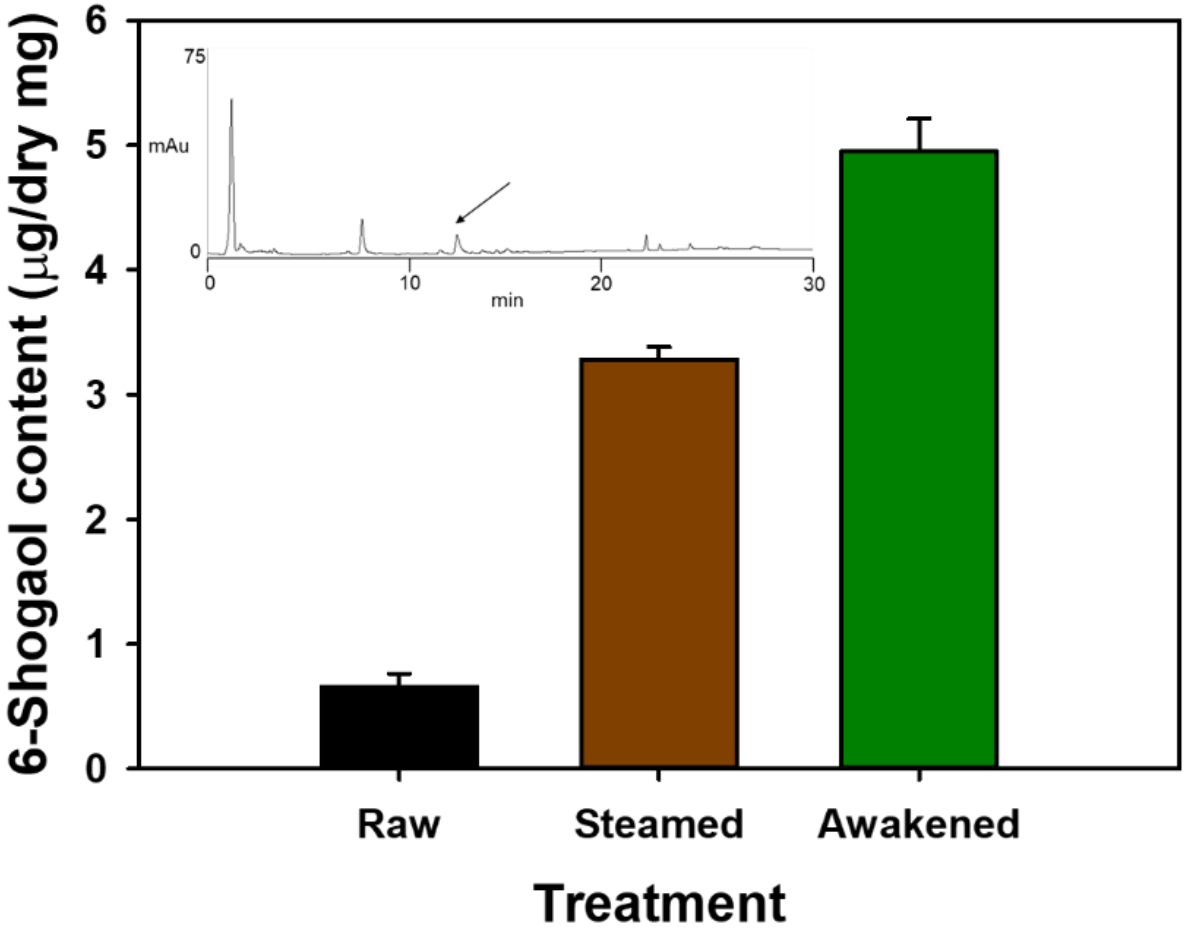


Fig. 5 Content change of 6-shogaol in ginger rhizomes by various treatments.

Results

Effects of liver activation and liver fat reduction by rapid and synchronized dormant broken ginger rhizome (Awakened Ginger Rhizome™)

The effect of liver activation was observed as mitochondrial activation using HP-SPR-3D by Awakened Ginger Rhizome™, assuming oral administration (eating) (Fig. 6). The activation was observed in a concentration-dependent manner. There was a concentration-dependent decrease in liver fat, which was about 27% at 2.0 mg/ml (Fig. 7). However, apoptotic toxicity was observed at 3.0 mg/ml and above. Similarly, untreated, 1.0 mg/ml had little effect, while apoptotic toxicity was observed at 2.0 mg/ml and above.

Results

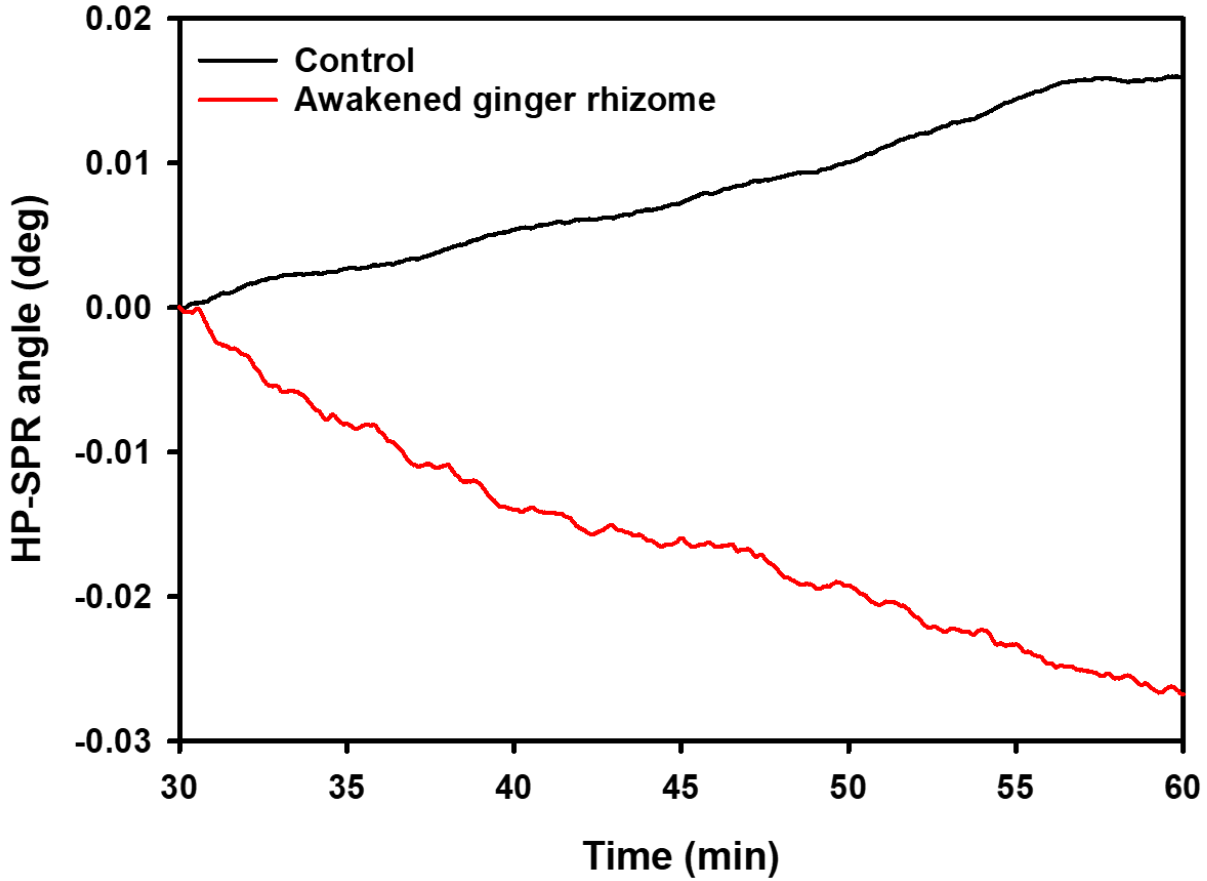


Fig. 6 Effect of liver mitochondrial activation by Awakened Ginger Rhizome™ (rapid and synchronized dormant broken ginger rhizome).

Results

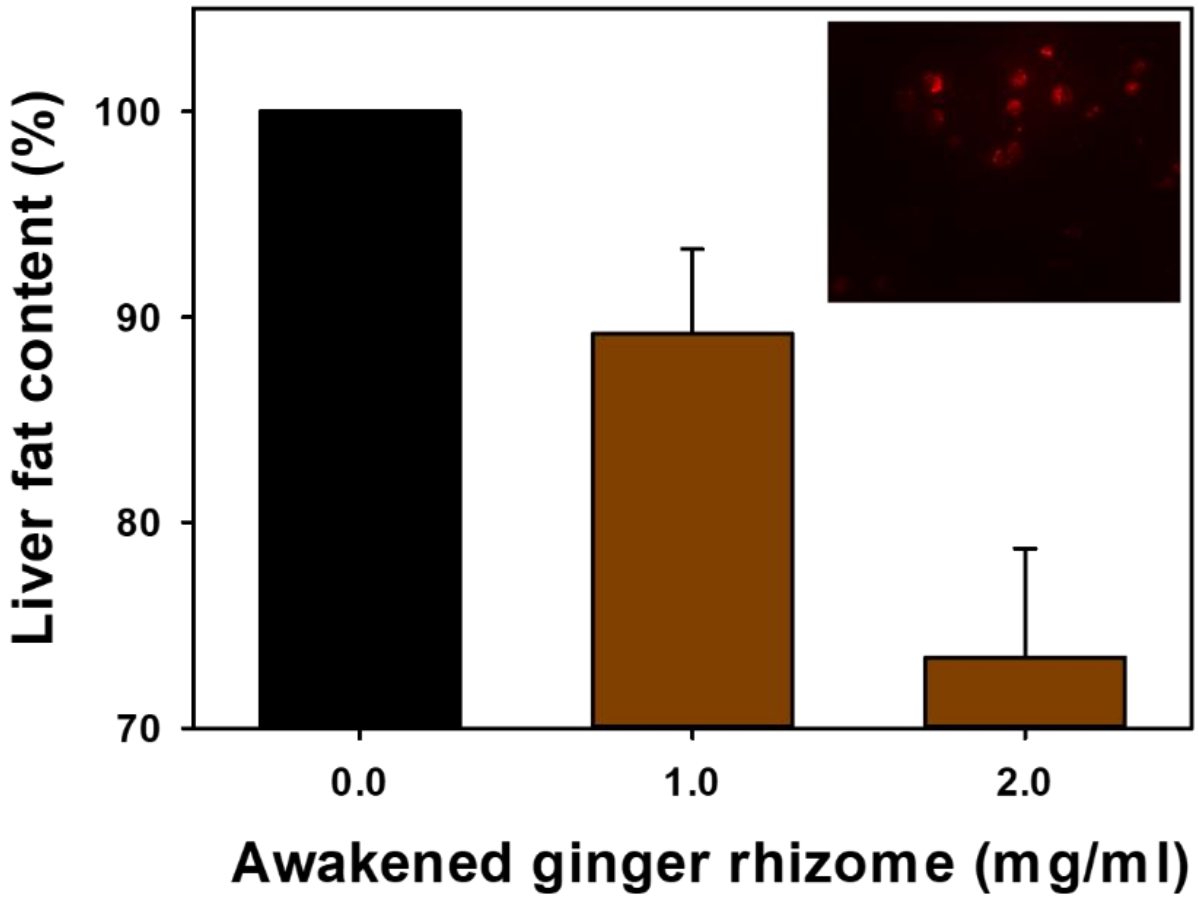


Fig. 7 Effect of liver fat reduction by Awakened Ginger Rhizome™ (rapid and synchronized dormant broken ginger rhizome).

Discussion

Assuming oral administration, Awakened Ginger Rhizome™ showed significant efficacies in liver activation and liver fat reduction. The efficacy was expressed by rapid and synchronized dormancy-breaking using Grandir recipe™. This process gave the liver activation as a dose-dependent manner. Additionally, it changed the rhizome ingredients to lead significant 6-shogaol content increase. From these, significant fat reduction was achieved because 6-shogaol triggered to decompose fat (lipid) and to promote β-oxidation of lipids. However, the magnitude of fat reduction by 6-shogaol was about 20%. This 20% is smaller than the number by Awakened Ginger Rhizome™ of 27%. Compounds other than 6-shogaol will involve in fat reduction in case of Awakened Ginger Rhizome™

Discussion

The toxicity of the ginger rhizome was also lowered into half by rapid and synchronized dormancy-breaking. The Grandir recipe™ was proven as effective process to increase or generate efficacy with minimizing toxicity.
Furthermore, it showed liver activation. Thus, it will contribute to health maintenance by protecting liver from any damages by drinking, stress, smoking and even by drugs.
Consequently, Awakened Ginger Rhizome™ was successfully developed as effective and safe functional food supplement.

Conclusions

Awakened Ginger Rhizome™ showed two things:
Suitable for therapy and maintenance against liver fat reduction with minimized toxicity by oral administration.
Suitable for fight and prevention against liver damages with minimized toxicity by oral administration in view of liver activation.
Consequently, Awakened Ginger Rhizome™ is suitable for functional food supplement.

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Acknowledgments

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