

High temperature manufactured *Corbicula japonica* shell powder (Carcite type crystal structure) improved diabetes in mouse

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Abstract

The Carcite type of *Corbicula japonica* shell powder developed at high temperature 500°C had improved mal-function of liver in the LEC rat liver disorder model, and restored its function. Additionally a new bio-function of the fired shell powder was lately found to lower the blood sugar level in the Type 2 diabetes model mouse (KK/TaJcl). These mice were raised with 2.5% shell powder for a month showed the significant lower level of blood sugar at 81 ± 23 mg to that of the control raised without shell powder at 208 ± 86 mg ($P = .05$). Fat globules deposition and vacuoles formation in the liver were clearly less in the shell powder-fed group compared to those of the control group in histo-pathological inspections. Anti-diabetes efficiency of the Carcite type shell powder prepared at 500°C 2hrs treatment acquired a blood sugar reduction ability suggesting applicability against the human diabetes patients.

Keywords: Multi-functional calcium; *Corbicula japonica* calcite; Diabetes; Blood sugar level improvement; Type 2 diabetes mouse model

1 Introduction

For a long period the Japanese had empirically believed a beneficially medicinal bio-function of *Corbicula japonica* meat to restore mal-function of liver based on their experiences without scientific endorsement (1). Moreover, another fascinating traditional story in a small fishing village was that the fired shell of the *Corbicula japonica* (not meat) had similar works against the liver disorders. By requirement of the village council we designed the experiments to confirm the traditional village talk, and the results obtained was positive, and proved improvement of mal-function of the liver disorder in the LEC rat test that was the liver disorder animal model (2).

In a present society, Japanese daily-food style is greatly changed after World War II by introduction of the western lifestyle (food style) cultures that is considered to be main cause of increasing the lifestyle related diseases as the heart disease, stroke, obesity, type 2 diabetes and atherosclerosis.

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In the studies of the fired *Corbicula japonica* shell powder (Calcite) a new bio-function was found to be effectively working against diabetes type 2 in the mouse model by lowering blood sugar level. This unexpected bio-functional finding of the shell powder was focused on in this paper aiming at a clinical application against the human diabetes to restore normal blood sugar level by taking the oral ingestion.

2 Material and methods

Corbicula japonica shells without meat were extensively washed out in pure water first, then shells were heated at 500°C 2hrs after drying to prepare the Calcite type of shell powder. Then, the fired samples were milled to powder by the machine (T-100, Kawasaki Heavy Industry, Japan, thereafter they were filtrated by mesh with 45 um pore size. Crystal structure of the fired shell powder was analyzed by the X-ray diffraction apparatus (XD-610, Shimadzu, Japan). Under these treating conditions shell powder completely changed the crystal structure from Aragonite to Calcite. Additionally increasing temperature made shells to the Lime CaO at 750°C 2hrs treatment. Aragonite and Calcite type shell powder were examined their liver improvement efficacy using the LEC rat liver disorder model, and efficacy was admitted in Calcite type powder (2).

2.1 Diabetes mouse model

Seven weeks aged male KK/TaJcl mice (3) were obtained from the CLEA Japan Inc. This mouse strain develops diabetes at 80% in 17-18 weeks old. They were raised in the air conditioned Animal House feeding solid food (CE-2) and bottle water feeding ad libitum.

2.2 Experiment design

Two cages were set with 5 mice for control fed only HFD32 (High Fat Diet; fat volume 32%) and 6 for 2.5% Calcite-HFD feeding group; Mice were fed with the above food for a month, and animals were each day checked conditions. Weight was measured twice at the beginning and at end of the tests.

2.3 Blood collection and histo-pathological inspection of liver

Animals were abstained from food a day before sacrifice for blood collection from abdominal aorta and measuring fat weight around liver and testes. Histo-pathological inspection was done by the standard method using HE and Oil Red O Stain (COSMOBIO, Japan) to detect the oil drop in liver cells. Animals were anesthetized for these treatments to avoid excess burden. Animal experiments were carried out under an approved by the University Animal Ethic Regulation to avoid excess burden to the animals.

2.5 Statistical analysis

GraphPad Prism (GraphPad Software, USA) was employed for evaluation of statistical significance ($P = 0.05$).

3 Results

No statistical significance of body and fat weight around testis were observed between Calcite fed and non fed control group (Table 1).

Table 1 Body Weight and Fat Weight around Testis (gr)

Group	Body weight	Fat weight around testis
Control (n=5)	35.5±4.1	1.1±0.5
Calcite-Fed (n=6)	31.4±3.2	0.9±0.5

No statistical significance on body weight and fat weight around testis between Calcite-fed mice and Control group.

3.1 Calcite Shell Powder Efficacy against Diabetes Improvement in KK/TaJcl Mouse Model

Calcite-fed mice showed drastically low level in blood sugar ($p = .05$), and no statistical significances were there in other blood components.

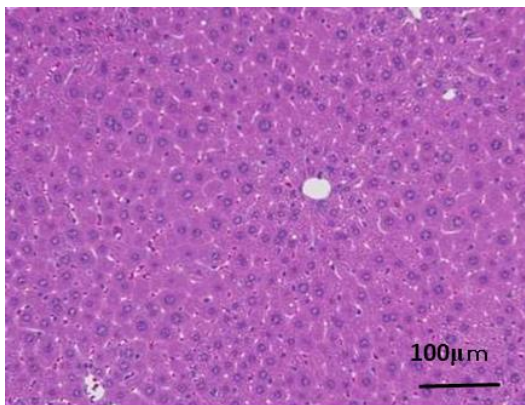
Table 2 Blood Constituents in Diabetes Mouse Model

Group	AST (GOT, IU/L)	ALT (GPT, IU/L)	BUN (mg/dl)	GLU (mg/dl)
Control	128.0±22.5	24.0±6.0	25.4±3.0	208.0±86.1
Calcite-Fed	243.8±121.8	35.3±9.9	30.2±3.1	81.0±23.4

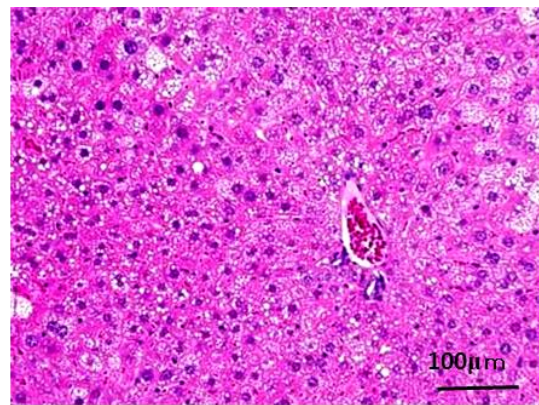
AST: glutamic oxaloacetic transaminase; ALT: glutamic pyruvic transaminase; BUN: blood urea nitrogen; GLU: glucose

3.2 Histo-Pathological Findings

Large vacuoles in the control mouse liver were numerous along with degenerated tissue structures (left), and those of the calcite-fed mouse liver were less or no vacuole keeping well tissue structure (right).



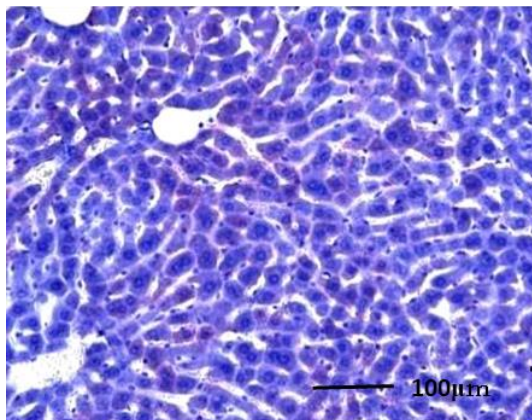
Control mouse liver tissue (HE stain)



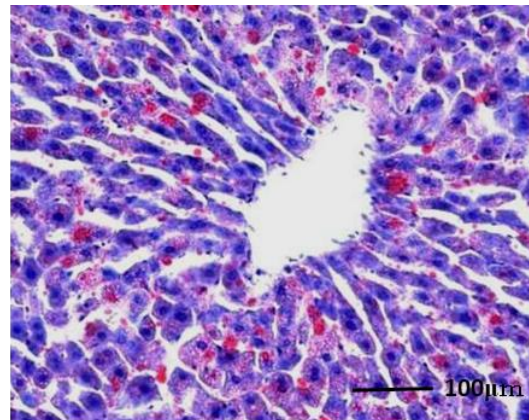
Calcite-fed mouse liver tissue (HE stain)

The Oil Red O staining (detection of neutral fat or polyethylene granula in liver tissue) showed numerous fat deposits (red in color) in control tissues (left), but no (less red granule) was visible in Calcite-fed mouse liver (right).

Figure 1 No (less) Vacuoles Formation in Calcite-fed Mouse Liver Section (Right)



Calcite-fed mouse liver tissue



Control mouse liver tissue

(Red spots (presence of fat) were numerous in the control liver, but no red spot(s) was visible in the Calcite-fed mouse liver)

Figure 2 Fat Deposits Detection (Red Spots) in Control (Left) And Calcite-fed Mouse Liver Tissue (Right)

4 Discussion

Our previously description was on the medicinal efficiency of Calcite type of shell powder prepared from the bivalve *Corbicula japonica* by heating at 400-500 °C against liver disorders (2). This material was surely demonstrated the anti-hepatitis activity in the LEC rat hepatitis model. Later similar activity was confirmed in the liver disorder patients by restoring abnormal level of liver enzyme marker (AST, ALT) to normal level (4). Additional bio-activities of Calcite shell powder were proved by lipid lowering, immune enforcement potency, liver cell proliferation activity, thereafter it makes us name 'Multi-functional Calcite'.

Recent findings indicate that the liver disease(s) precedes development of type 2 diabetes. In prospective cohort studies, cirrhosis accounted for 12.5% of death in patient with diabetes, and in U.S. Diabetes is now the most common cause of liver disease (5).

Moreover it is lately pointed out that the impaired calcium homeostasis in diabetes associates the development of diabetes (6). Calcium is one of the key elements to associate with biological processes in body, such as cell division and growth, blood coagulation, cardiovascular homeostasis, hormone responses, neural electrical activity, and bone formation. The total plasma calcium must be maintained within a narrow range, because a big range of plasma calcium concentration is lethal.

Variety of diseases like hepatitis, diabetes, arteriosclerosis and immune system disorder are known caused to the calcium deficiency (7), and we could confirm a direct association of the calcium to control blood sugar level in diabetes mouse model. Essential role of calcium is the use of calcium ions (Ca^{2+}) to communicate and drive intracellular processes as a step in a signal transduction *in vivo*, therefore Ca^{2+} concentration is maintained at ratio 1:10000 between intracellular and extracellular fluid, then Ca^{2+} can act in signal transduction resulting from activation of ion channels (8). Diabetes is associated with profound deterioration of calcium and bone metabolism, partly from impaired intestinal calcium absorption, and caused a large number of liver disorder including elevated liver enzymes, fatty liver disease, cirrhosis, hepatocellular carcinoma, and acute liver failure (9).

Previous our experiments proved security/safety of the *Corbicula japonica* shell Calcite that did not contaminate toxic mineral as Pb, As, Cu in chemical analysis (2), and effectively reduced the fat in liver in animal hepatitis model. The *Corbicula japonica* shell Calcite therefore is one of the leading candidates to restore both human diabetes and liver disorder.

Our present concern in the food sciences is the 'foods and heating relation', because the foods-heating occasionally a new potential tool to create (induce) a new ingredient (s) with new bio-functions. We will soon start an kind of investigations to find new bio-functions to fight against diseases and maintain human health conditions (10).

5 Conclusion

Bivalve *Corbicula japonica* shell powder Calcite showed anti-diabetes activity in the mouse diabetes model, showing reduction of blood sugar level. Histological observation also demonstrated no (less) vacuoles formation and fat deposits in liver tissue inspection. Calcite type of shell powder created from the *Corbicula japonica* shell is one of powerful candidate for treatment of type 2 diabetes. Heating ingredients occasionally develops and bestows a new bio-function to the average food stuffs, and should try to create new bio functions.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared no presence of competing interests in the present studies.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Authors' contributions

Author 'Sasaki' designed the whole studies, evaluated the results, and prepared the first draft of manuscript, Authors 'Okamoto' and 'Amba' managed the animal experiments and the analyses of data, Authors 'Someya' and 'Minegishi' prepared the experiments materials as collecting shell fish, heat treatment of shells for preparation of the fired shell powder. Their Affiliated Companies contributed to support financially to continue present researches. All authors herein approved the final manuscript, and publication.

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