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# Study of Shokatsu-cha I. Hypoglycemic effect of Shokatsu-cha in mice

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## Abstract

Shokatsu-cha is a novel Kampo formulation based on the traditional Chinese medicine and proposed for treatment of diabetes. It showed a hypoglycemic effect in experimentally diabetic mice in 10 days of administration at 3 g/kg, *p.o.* in comparison with control (water). It was also effective in the glucose tolerance test with normal and diabetic mice at a single dose of 0.5 or 1 g/kg. Carbohydrate components were suggested mainly to be responsible for the hypoglycemic effect of Shokatsu-cha, although low molecular weight sugar (s) can elevate the basal blood glucose level at high doses of the extract.

**Key words** Kampo, Shokatsu-sho, antidiabetic, Dioscoreae Rhizoma, Rehmanniae Radix, Anemarrhenae Rhizoma.

**Abbreviations** Shokatsu-cha (Xiao-Ke-Ca), 消渇茶; Rokumi-jio-gan (Liu-Wei-Di-Huang-Wan), 六味地黄丸; Byakko-to (Bai-Hu-Tang), 白虎湯; Ikkan-sen (Yi-Guan-Jian), 一貫煎; Byakko-ka-ninjin-to (Bai-Hu-Jia-Ren-Shen-Tang), 白虎加人参湯; Hachimi-gan (Ba-Wei-Wan) 八味丸.

## Introduction

Shokatsu-cha (Xiao-Ke-Ca) is a novel, unique formulation proposed by the Faculty of Pharmacy of the Beijing College of Traditional Chinese Medicine as a remedy for diabetes mellitus, based on the theories and practice of the Kampo. It is originally a dried extract of a mixture of eight crude drugs and tea (oolong tea), packed for each daily dosage in steeping paper bags, for convenience in dispensing to patients and moderating taste.

Three of the eight crude drugs, Sanyaku (山薬), Chimo (知母) and Jio (地黄), composing Shokatsu-cha have individually been proved to have a hypoglycemic effect in animals.[1-3] The rest of the components are also assumed to be effective on the various symptoms of diabetes mellitus in association with the above three.

In the present communication the water extract of Shokatsu-cha has been tested on its effect on the blood glucose level in experimentally diabetic mice and on the glucose tolerance in diabetic and normal mice. Preliminary research for active ingredients in Shokatsu-cha has been attempted on its fractions with different solubility in ethanol and water.

## Materials and Methods

*Preparation of Shokatsu-cha and its fractions*: Shokatsu-cha was prepared by refluxing with boiling water (3000 ml) the mixture (300 g in total) of the crude drugs of Sanyaku (山薬), Dioscoreae Rhizoma, *Dioscorea opposita* THUNB. (河南省, China) (90 g), Jio (地黄), Rehmanniae Radix, *Rehmannia glutinosa* LIBOSCH. (河南省, China) (36 g), Chimo (知母), Anemarrhenae Rhizoma, *Anemarrhena asphodeloides* BGE. (河北省, China) (36 g), Kikyo (桔梗), Platycodi Radix, *Platycodon grandiflorum* A.DE CANDOLLE (河北省, China) (36

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g), Nanshajin (南沙参), Adenophorae Radix, *Adenophora tetraphylla* (THUNB.) FISCH. (江蘇省, China) (36 g), Tanjin (丹参), Salviae Miltiorrhizae Radix, *Salvia miltiorrhiza* BGE. (河北省, China) (22 g), Kukoshi (枸杞子), Lycii Fructus, *Lycium barbarum* L. (守夏省, China) (22 g) and Inyokaku (淫羊雀), Epimedii Folium, *Epimedium grandiflorum* MORR. (四川省, China) (22 g) for 2 h.

The combined filtrate of the thrice extracted mixture was condensed to 1/10 in volume. The concentrated solution was added to the same volume of ethanol and stored at 4°C for 24 h. The filtrate of the supernatant was evaporated *in vacuo* to a dark-colored paste (155 g including 74 g water). It was stored at 4 °C and diluted with sterile distilled water just before use to make solutions of 30 mg net weight of the extract per ml and more depending on the doses described in the results.

Fractions of Shokatsu-cha were obtained by the procedure shown in Fig. 1 according to different solubility to ethanol (EtOH) and water. Amberlite XAD-2 was purchased from Organo Co.

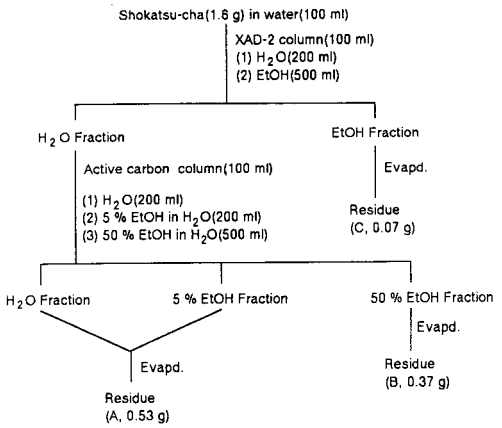


Fig. 1 Fractionation procedure

*Animals and treatment*: Mice (ddY) weighing about 30 g (6 weeks of age) at the start of experiment, male or female as indicated in the results, were fed on conventional pellets and tap water taken *ad libitum*, unless otherwise mentioned. Streptozotocin (150 mg/kg, *i.p.*) was injected as 1.5 % solution in isotonic citrate buffer, pH 4.5, to make experimentally hyperglycemic mice. More than 4/5 of recipient mice developed hyperglycemia in 5 days after the injection. Shokatsu-cha was administered orally via stomach tube at 0.5, 1.0 or 3.0 g/kg/day (as dry weight of the extract) to normal or hyperglycemic mice as mentioned in the results once in a glucose tolerance test and for 10 days in the test of its effect on the blood glucose level. In the glucose tolerance test glucose (2.5 g/kg) was orally administered to test animals 30 min after Shokatsu-cha was given subsequent to 15 h of fasting. Glibenclamide (5 mg/kg/day) was orally administered in a water solution containing dimethyl sulfoxide for 10 days to hyperglycemic mice.

*Determination of the blood glucose level*: Blood samples were collected from the retroorbital sinus just before and 30, 60, 120 and 180 min after glucose administration in the glucose tolerance test. When Shokatsu-cha or glibenclamide was given consecutively, blood samples were taken in 15 h of starvation after the last administration indicated in the results. Glucose concentration was determined by the glucose oxidase method as usual using the kit of glucose test (Wako Pure Chemicals Co., Japan).

## Results

*Consecutive administration to diabetic mice*

Hyperglycemic mice, treated with streptozotocin (150 mg/kg, *i.p.*), were given Shokatsu-cha (1 or 3 g/kg), glibenclamide (5 mg/kg) or water orally for 10 days (Table I). After treatment with the extract the mean value of blood glucose level of mice was greatly lower than that after treatment with water (control), significantly different from that of the control in the group of higher dose. The blood glucose after starvation was almost unchanged after 10 days of Shokatsu-cha treatment from the starting level in either dose. Administration of glibenclamide decreased the blood glucose level of the diabetic mice compared with the control. Average body weight of mice given water or doses of Shokatsu-cha for 10 days was found to be not different (30-33 g)

Table I Effect of consecutive administration of Shokatsu-cha on the blood glucose level of diabetic mice.

| Treatment daily dose, *p.o.* (N) | Blood glucose (mg/dl, mean±S.E.) | |
|---|---|---|
| | Before extract or water | 10 days after extract or water |
| Water (10) | 210 ± 12 | 284 ± 41 |
| Extract 1 g/kg (8) | 192 ± 6 | 202 ± 20 |
| Extract 3 g/kg (7) | 195 ± 7 | 190*± 12 |
| Glibenclamide 5 mg/kg (13) | 195 ± 8 | 215 ± 8 |

Streptozotocin (150 mg/kg, *i.p.*)was given to female mice 10 days before experiment, and 7-13 hyperglycemic mice for each group were treated as indicated in the table with Shokatsu-cha (extract), glibenclamide or water for 10 days (once daily) starting 2 days after termination of streptozotocin injection.
*$p<0.05$ vs. control (water) (ANOVA, Student's $t$-test).

between the groups and unchanged during the experiment in either group. Administration of 3 g/kg/day of the extract to normal mice for 10 days did not affect the blood glucose (data not shown).

*Glucose tolerance test in normal mice*

Glucose tolerance of normal mice was tested by loading them with a high dose of glucose (2.5 g/kg) 30 min after single administration of Shokatsu-cha (0.5-3 g/kg) or water (Table II). The blood glucose level of mice given 0.5 or 1 g/kg of the extract showed significant decrease compared to those given water when measured 30, 60 and 120 min after glucose administration.

It has been found that Shokatsu-cha naturally contains 6 % or more of glucose as a component. The increase of blood glucose before administration of glucose in the group of the highest dose (88±6 mg/dl compared to 37±9 mg/dl in control in Table II) is probably attributed to ingestion of glucose from the extract. It can be deduced therefore that, if such an overestimation of the blood glucose level due to ingestion of the extract itself is corrected, especially in early times after administration of it, greater and significant glucose tolerance could be shown even in the group of the dose 3 g/kg.

*Glucose tolerance test in diabetic mice*

Hyperglycemic mice (in 6 days after streptozotocin injection) were then tested for their glucose tolerance after single administration of Shokatsu-cha (0.5-3 g/kg) (Table III). In this experiment, administration of 0.5 g/kg of the extract was significantly effective in the decrease of the blood glucose level in 120 and 180 min following a load of glucose compared to control mice. In the groups of higher doses of the extract glucose tolerance was apparently not demonstrated over control. The high blood glucose level before administration of glucose (e.g. 168±18 mg/dl glucose in the highest dose) would explain the controversial effect.

*Glucose tolerance test with fractions of Shokatsu-cha*

Shokatsu-cha was fractionated by differential solubility into water and ethanol so as to separate saccharides and saponins in the extract (Fig. 1). Fraction A is assumed to retain com-

Table II Effect of single administration of Shokatsu-cha on the glucose tolerance of normal mice.

| Treatment dose, *p.o.* | Blood glucose (mg/dl, mean±S.E.) | | | | |
|---|---|---|---|---|---|
| | Before glucose | Min after glucose administration | | | |
| | | 30 | 60 | 120 | 180 |
| Water | 37± 9 | 442 ±28 | 338 ±23 | 189 ±17 | 142±19 |
| Extract 0.5 g/kg | 57±11 | 378*±16 | 279*±11 | 149*± 8 | 121±10 |
| Extract 1g/kg | 35± 8 | 400 ±28 | 276*±22 | 129*±12 | 95± 8 |
| Extract 3 g/kg | 88± 6 | 423 ±27 | 289 ±20 | 163 ±12 | 130±11 |

Doses of Shokatsu-cha (extract) or water were orally given to groups of 10 male mice as shown in the table 30 min before single administration of a high dose of glucose (2.5 g/kg). Periodically after glucose loading, blood samples were taken from the retroorbital sinus as indicated and glucose concentration was determined as described in Materials and Methods. *$p<0.05$ vs. control (water) values of corresponding min after glucose (Student's $t$-test).

Table III Effect of single administration of Shokatsu-cha on the glucose tolerance of diabetic mice.

| Treatment dose, *p.o.* | Blood glucose (mg/dl, mean±S.E.) | | | | |
|---|---|---|---|---|---|
| | Before glucose | Min after glucose administration | | | |
| | | 30 | 60 | 120 | 180 |
| Water | 140±12 | 452±20 | 399±27 | 318 ±29 | 266 ±24 |
| Extract 0.5 g/kg | 116±13 | 424± 9 | 345±14 | 237*±20 | 180*±17 |
| Extract 1g/kg | 123±14 | 430±22 | 363±21 | 299 ±27 | 247 ±29 |
| Extract 3 g/kg | 168±18 | 412 ±28 | 370±21 | 319 ±29 | 215 ±18 |

Doses of Shokatsu-cha (extract) or water were orally given to groups of 10 male diabetic mice as shown in the table 30 min before single administration of a high dose of glucose (2.5 g/kg). Diabetic mice were prepared by injection of streptozotocin (150 mg/kg) 6 days before experiment. See the legend to Table II for other comments.

pounds of relatively small molecular weight such as mono- and disaccharides. Fractions B and C are proposed to be repleted with polysaccharides and compounds other than sugars such as saponins, respectively. Fractions A (2.0 g/kg) and B (1.4 g/kg) significantly elevated the blood glucose level after administration of them, the former more explicitly (Table IV). But fraction B depressed elevation of the blood glucose 30 and 60 min after loading a high dose of glucose compared with control (water), longer than fraction A did (30 min after glucose administration only). The

Table IV Effect of the fractions from Shokatsu-cha on the glucose tolerance of normal mice.

| Treatment dose, *p.o.* | Blood glucose (mg/dl, mean±S.E.) | | | | |
|---|---|---|---|---|---|
| | Before glucose | Min after glucose administration | | | |
| | | 30 | 60 | 120 | 180 |
| Water | 104.1 ±6.1 | 504.2 ±15.9 | 395.9 ±21.1 | 215.5±12.0 | 163.2±9.2 |
| Fraction A 2.0 g/kg | 141.3*±11.3 | 414.5* ±30.7 | 334.2 ±33.3 | 200.4±17.6 | 166.7±16.8 |
| Fraction B 1.4 g/kg | 126.6*±5.7 | 402.3**±18.0 | 328.3*±19.9 | 208.7±9.0 | 169.0±10.6 |
| Fraction C 0.26 g/kg | 120.0 ±7.6 | 447.2 ±30.9 | 400.3 ±19.6 | 272.4±24.2 | 196.8±21.3 |

Doses of fractions A,B and C or water were orally given to groups of 8 normal male mice as shown in the table 30 min before single adminstration of a high dose of glucose (2.5 g/kg). $^{**}p<0.01$ and $^{*}p<0.05$ vs. each control (water) (ANOVA, Student's *t*-test).

elevation of the blood glucose by fraction A itself was probably due to the intestinal absorption of a significant amount of monosaccharides concentrated in the fraction, which would have disturbed the apparent glucose tolerance effect of fraction A. The C fraction (0.26 g/kg), which is expected to be enriched with saponins, did not show any significant effect in the test. Therefore the carbohydrate components of Shokatsu-cha would mainly participate in the hypoglycemic effect of the formulation.

## Discussion

Shokatsu-cha was shown in experimentally diabetic mice to be capable of hypoglycemic action comparable to that of glibenclamide in efficacy (Table I). A single dose (0.5, 1 or 3 g/kg) of it was effective in glucose tolerance tests of normal and diabetic mice (Table II and III).

Hypoglycemic action of several formulae of traditional Chinese medicine has been reported in experimental animals. Hachimi-gan (Ba-Wei-Wan), which is comparable to Shokatsu-cha in its major components (Jio and Sanyaku), was demonstrated in rats with cyproheptadine-induced diabetes to depress the elevation of blood glucose.[4] Shokatsu-cha is a novel formula proposed for treatment of diabetes mellitus, derived from and based on formulae of Rokumi-jio-gan (Liu-Wei-Di-Huang-Wan), 六味地黄丸, Byakko-to (Bai-Hu-Tang), 白虎湯, and Ikkan-sen (Yi-Guan-Jian), 一貫煎,[5] which are all clinically used for treatment of diabetes or other metabolic diseases accompanied by profound and durable thirst. Sanyaku (山薬) is a principal material in Shokatsu-cha, proved to be effective on some of the diabetical symptoms or "shokatsu-sho (消渇証)"[6] according to traditional Chinese medicine: extraordinary thirst and appetite (stomach congestion), heavy diuresis, depressed metabolism or progressive consumption and hyperglycemia.

Jukujio (熟地黄) in Rokumi-jio-gan was replaced by Jio (地黄) in Shokatsu-cha, because the latter is thought to have febrifuge[7] as well as hypoglycemic effects,[3,6] whereas the former is rather pyrogenetic or hyperemic.[7] Hypothermic activity of Jio is thought to work synergistically with the hypoglycemic one in treatment of shokatsu-sho. Chimo (知母) is also hypoglycemic[2,8] and febrifuge.[9] Kikyo (桔梗) is hypoglycemic[2] and accelerates trachial expectoration[10] and hypothermia.[11] Tanjin (丹参) is antianemic, febrifuge and stimulatory on the cardiovascular system.[12]

Therefore the ingredients of Shokatsu-cha are all assumed to act synergistically and

advantegeously in the amelioration of diabetic symptoms. In fact, single administration of each extract of Sanyaku, Jio and Chimo at 0.5 g/kg was not significantly effective in the glucose tolerance test in our experiments (unpublished data), whereas that of Shokatsu-cha was shown to be effective even at 0.5 g/kg. The combined effect of the components of the Wakan-yaku was discussed by Kimura [13] on the hypoglycemic formulation, Byakko-ka-ninjin-to (Bai-Hu-Jia-Ren-Shen-Tang). It has been demonstrated that characteristics in pharmacological effects of constitutive drugs in the formulation totally work synergistically, even if there is a rather antagonistic relation between any two of those, e.g. between Chimo and Ninjin.

The doses (0.5-3 g/kg) of Shokatsu-cha used are so high that they correspond to some 30 g/man or more a day. But it is often the case that pharmacologically effective doses within a limited period of administration of a Kampo formula or its extract in animals are over 10 times those of therapeutic use. Anyway it is disadvantageous for evaluation of the pharmacological effect of Shokatsu-cha that plenty of sugars of low molecular weight contained in it could disturb the hypoglycemic effect of it by raising the basal blood glucose level, when it is applied to animals in such high doses (cf. Table II-IV).

Hikino *et al.*[1] identified several glycans, and Tomoda *et al.*[14] mucilage B, as hypoglycemic components in the water extracts of Sanyaku. Takahashi *et al.*[8] found hypoglycemic anemarans in the water extract of Chimo. Recently hypoglycemic effect of several saponins from Chimo has been demonstrated in diabetic mice by Kimura *et al.*[15] Our experimental result on the separated fractions from Shokatsu-cha suggests carbohydrate components as major effective ingredients on hyperglycemia. It may be reasonable therefore to seek identification of essential components of Shokatsu-cha effective in its hypoglycemic activity.

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## 和文抄録

消渇茶は中医学に基づき，糖尿病治療薬として作られた新しい漢方処方である。実験的糖尿病マウスにその水性エキスの3 g/kgを経口で10日間連続投与したところ，対照動物（水投与）よりも血糖が低下した。また正常，あるいは糖尿病マウスに0.5または1 g/kgを1回投与後の耐糖力テストでエキスは有効と認められた。消渇茶の炭水化物が主な血糖低下成分であることが示唆されたが，一方その低分子の糖成分は高用量では却って血糖を上げることが示された。

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