Screening of Crude Drugs for Influence on Amylase Activity and Postprandial Blood Glucose in Mouse Plasma

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Screening of crude drugs for inhibitory activity on α -amylase in mouse plasma was performed. Hot water extracts and ethanolic extracts of *Arecae Semen*, *Ephedrae Herba*, *Malloti Cortex*, *Rhei Rhizoma* and ethanolic extract of *Moutan Cortex* inhibited enzyme activity in isolated mouse plasma strongly and dose-dependently. However, the effects of these 9 extracts were not observed in the plasma when they were administered intraperitoneally or orally. Ethanolic extract of *Arecae Semen* also showed a depressive effect on elevation of postprandial blood glucose.

Key words α -amylase; blood plasma; postprandial blood glucose; mouse; crude drug

α -Amylase, one of the digestive enzymes, is secreted in animals by the pancreas and salivary glands. Some of it passes into the blood, and is classified as one of the escape enzymes in clinical analysis.

We earlier reported the influences of crude drugs on the activities of a few enzymes in mouse blood plasma from our studies of these drugs and Chinese medicinal prescriptions.^{1–4)} In this paper, we examine α -amylase as another enzyme in mouse plasma and report a survey of crude drugs that directly influence this enzyme activity. The drugs with depressive effects were further investigated regarding their influence on the increase of postprandial blood glucose in mice.

MATERIALS AND METHODS

Materials Chopped crude drugs were purchased from Nakai-kohshindo (Kobe, Japan), and those tested are listed in Table 1. Of the crude drugs used, 7 were not defined by JP XIII; they were *Aconiti Tuber Praeparata* (tuberous root of *Aconitum* species treated for reduction in virulence, from China), *Clematidis Radix* (root and rhizome of *Clematis chinensis*, from China), *Leonuri Herba* (aerial part of *Leonurus sibiricus*, from China), *Nelumbinis Folium* (leaf of *Nelumbo nucifera*, from China), *Patriniae Rhizoma et Radix* (root and rhizome of *Patrinia villosa*, from Japan), *Phytolaccae Radix* (root of *Phytolacca esculenta*, from Japan) and *Zingiberis Siccatum Rhizoma* (steamed rhizome of *Zingiber officinale*, from China). Glycogen was purchased from Nacalai Tesque (Kyoto) and acarbose was obtained from Bayer Pharmaceuticals (Osaka).

Animals Five to 6 week old male mice of ddY strain were purchased from Nihon SLC, Hamamatsu, Japan. They were housed in plastic cages with free access to food (until 12 h before use) and water, and were kept in a room at $25 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity with a 12 h dark–light cycle.

Test Samples Each crude drug was refluxed (for 1 h) twice with a five-fold volume of water or ethanol (99.5 v/v%) and filtered, followed by removal of the combined solvent *in vacuo*. Each of these extracts was completely dried *in vacuo* and the residue was applied in the assay. A 0.1 ml portion of each test sample adjusted to each final reaction concentration in distilled water was added to mouse plasma for the assay.

Each test solution (1200 mg/kg) was also intraperitoneally or orally given to mice at a constant injection volume of 0.8 ml/30 g or 0.6 ml/30 g body weight, respectively.

Assay Procedure of α -Amylase Activity Cardiac blood was collected from mice with a heparin-treated cylinder and centrifuged to prepare plasma that was used for the assay. The cardiac blood was also collected 5 min or 10 min after intraperitoneal or oral administration of the sample, respectively. The plasma was diluted with an isotonic sodium chloride solution to one-fifth and a 10 μl portion was provided for the amylase activity assay according to the Caraway method,⁵⁾ using a kit (Amylase-Test Wako, Wako Pure Chemical, Japan). The data were calculated to determine the amylase unit. The isotonic sodium chloride solution was given to the control mice. A microplate reader, MPR-A4i (Tosoh, Japan), was used for the measurement of visible absorbance at 650 nm.

Determination of Blood Sugar Immediately after oral administration of test samples, glycogen (2000 mg/kg) was orally given to mice. Sixty minutes later, cardiac blood was collected from the animals with a heparin-treated cylinder, and the plasma was separated as above. A 50 μl 1 portion of plasma was provided for the determination of blood sugar according to the *o*-toluidine–boric acid method,⁶⁾ using a kit (Glucose-Test Wako, Wako Pure Chemical, Japan). The purified water was given to the control mice and the data was calculated to determine glucose concentration (mg/dl). The apparatus described above was used to measure visible absorbance at 650 nm.

Statistics Statistical analysis was performed by Student's *t*-test. Values of $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Some reports have presented influence of crude drugs^{7–11)} and Chinese medicinal prescriptions¹²⁾ on α -amylase activity. However, the influential factor seems to be an inconsistency in the source of enzyme, and amylase in mouse blood plasma has not yet been examined.

Table 1 shows the yields of hot water or ethanol extracts of 40 crude drugs and the changes in activity of the isolated blood plasma amylase at 300 $\mu\text{g}/\text{ml}$ final concentration in the reaction solution. Acarbose, one of the α -glucosidase in-

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Table 1. Change of Amylase Activity in Isolated Plasma of Mice by Addition of Extracts of Crude Drugs

Crude drug	Yield of extract (mg/g)	Activity (amylase unit)		Inhibition (%)
		Water extract	Control	
		Ethanol extract		
Aconiti Tuber Praeparata	260.0	1968.1±28.8	1709.2±22.8	+15.1
	17.0	1559.3±34.2	1738.8±28.3	-10.3
Achyranthis Radix	162.0	1687.2±50.9	1729.7±32.6	-2.5
	92.3	1721.5±18.8	1730.4±41.5	-0.5
Akebiae Caulis	104.6	1839.7±18.8	1716.3±25.8	+7.2
	38.5	1618.3±40.6	1738.8±28.3	-6.9
Alismatis Rhizoma	166.7	1629.7±34.0	1710.4±53.7	-4.7
	48.0	1727.0±33.7	1658.6±39.0	+4.1
Angelicae Radix	420.0	1792.2±74.4	1755.2±54.9	+2.1
	46.7	1450.0±47.9	1669.0±53.6	-13.1
Arecae Semen	98.6	617.0±45.5	1645.3±54.2	-62.5
	52.8	186.0± 4.0	1763.1±15.8	-89.5
Armeniacae Semen	160.0	1638.4±29.3	1721.8±25.0	-4.8
	76.4	1269.7±29.6	1726.4±34.0	-26.5
Asiasari Radix	152.5	1827.3±24.7	1671.8±46.9	+9.3
	87.2	1669.9±26.5	1713.1±60.3	-2.5
Astragali Radix	293.3	1739.3±53.8	1709.6±22.0	+1.7
	120.0	1621.7±54.2	1713.1±60.3	-5.3
Atractylodis Lanceae Rhizoma	460.0	1837.2±18.3	1730.7±22.5	+6.2
	89.0	1396.0±24.3	1641.6±59.6	-15.0
Atractylodis Rhizoma	495.0	1789.3±17.8	1660.5±24.0	+7.8
	84.0	1495.9±38.6	1665.0±56.4	-10.2
Aurantii Nobilis Pericarpium	246.2	1571.3±64.0	1636.1±22.3	-4.0
	219.5	1624.5±42.9	1623.5±25.6	+0.1
Bupleuri Radix	143.2	1740.2±51.4	1648.7±33.9	+5.6
	89.7	1399.0±57.8	1728.8±25.4	-19.1
Carthami Flos	455.0	1919.7±40.1	1741.6±46.8	+10.2
	192.7	1435.9±13.0	1743.0±30.5	-17.6
Clematidis Radix	236.3	1872.2±45.1	1670.3±34.7	+12.1
	61.5	1656.3±33.1	1665.0±56.4	-0.5
Cnidii Rhizoma	317.0	1821.3±41.8	1739.4±18.8	+4.7
	49.0	1616.4±51.4	1680.9±54.3	-3.8
Coptidis Rhizoma	226.7	1712.9±16.0	1758.7±36.7	-2.6
	111.8	1522.8±39.6	1718.8±38.5	-11.4
Cyperi Rhizoma	407.0	1529.0±45.6	1719.4±84.3	-11.1
	37.1	1698.7±19.8	1730.4±41.5	-1.8
Ephedrae Herba	265.0	973.7±25.8	1654.8±26.4	-41.2
	117.5	274.8±16.5	1710.0±31.0	-83.9
Evodiae Fructus	260.0	1699.3± 8.5	1648.8±15.4	+3.1
	313.0	1664.1±24.9	1703.8±37.4	-2.3
Forsythiae Fructus	212.0	1552.5±66.1	1713.9±49.0	-9.4
	57.0	1465.2±22.9	1667.8±34.2	-12.1
Gardeniae Fructus	263.3	1904.5±47.6	1715.1±25.7	+11.0
	182.7	1318.7±30.8	1680.9±54.3	-21.5
Leonuri Herba	152.0	1650.6±70.4	1687.0±43.5	-2.2
	56.5	1728.5±21.7	1705.8±47.2	+1.3
Malloti Cortex	173.0	265.7±65.7	1698.0±38.1	-84.4
	52.1	289.2±23.0	1766.6±35.0	-83.6
Moutan Cortex	341.0	1499.9±23.5	1710.5±48.2	-12.3
	90.2	1043.6±41.3	1711.8±43.3	-39.0
Nelumbinis Folium	198.5	1735.1±36.8	1670.6±39.6	+3.9
	102.0	1367.6±33.6	1646.5±40.1	-16.9
Nupharis Rhizoma	278.8	1707.9±48.0	1633.3±93.9	+4.6
	54.7	1321.6±38.8	1731.8±28.3	-23.7
Paeoniae Radix	340.0	1582.1±29.7	1752.7±30.0	+9.7
	156.0	1580.0±54.1	1684.7±58.2	-6.2
Patriniae Rhizoma et Radix	126.5	1774.4±29.5	1752.3±44.2	+1.3
	50.8	1447.8±18.4	1682.7±33.1	-14.0
Persicae Semen	174.5	1666.0±63.8	1723.4±47.7	-3.3
	135.5	1342.7±21.3	1735.8±14.8	-22.6
Phytolaccae Radix	366.5	1532.0±28.8	1635.3±35.0	-6.3
	71.0	1736.8±44.5	1721.7±39.5	+0.9
Pinelliae Tuber	304.7	1479.8±46.4	1651.3±43.7	-10.4
	9.4	1679.6±28.7	1690.0±28.2	-0.6
Plantaginis Semen	68.0	1759.2±60.2	1647.9±66.2	+6.8
	38.5	1578.5±42.8	1724.9±31.5	-8.5

Table 1. (continued)

Crude drug	Yield of extract (mg/g)	Activity (amylase unit)		Inhibition (%)
		Water extract	Control	
		Ethanol extract		
Polyporus	18.0	1900.9±31.7	1724.2±24.4	+10.2
	6.8	1808.0±22.3	1721.7±39.5	+5.0
Poria	14.0	1600.2±24.7	1721.8± 6.7	-7.1
	11.8	1638.1±29.7	1690.0±28.2	-3.1
Rhei Rhizoma	376.0	327.2±12.5	1750.2±26.9	-81.3
	207.3	468.8±15.4	1735.8±14.8	-73.0
Scutellariae Radix	340.0	1606.2±28.4	1748.8±29.6	-8.2
	148.8	1514.3±29.5	1712.1±28.5	-11.6
Sinomeni Caulis et Rhizoma	75.0	1753.0±37.3	1632.7±37.2	+7.4
	42.7	1420.5±44.1	1641.6±59.6	-13.5
Zingiberis Rhizoma	116.2	1442.9±80.5	1741.4±17.7	-17.1
	43.0	1698.2±18.9	1670.9±20.2	+1.6
Zingiberis Siccata Rhizoma	83.4	1574.1±14.4	1732.9±36.1	-9.2
	49.5	1681.4±35.6	1726.4±34.0	-2.6
Acarbose ^{a)}		344.8±24.3	1648.5±66.1	-79.1

Each value represents the mean±S.E. (n=8—10). Concentration of test samples in reaction solution, 300 µg/ml. a) Concentration in reaction solution, 100 µg/ml.

Table 2. Effect of Extracts of Crude Drugs on Amylase Activity in Isolated Mouse Plasma

Crude drug	Dose of crude drug ^{a)}	Activity (amylase unit)		Inhibition (%)
		Extract	Control	
Arecae Semen (W)	300	617.0±45.5	1645.3±54.2	-62.5
	150	891.7±24.4	1692.4±56.3	-47.3
	75	1231.7±46.7	1540.0±35.0	-20.0
	38	1391.8±40.0	1605.8±26.5	-13.3
Arecae Semen (E)	300	186.0± 4.0	1763.1±15.8	-89.5
	150	396.9±25.3	1750.2±15.9	-77.3
	75	793.0±40.3	1750.2±15.9	-54.7
	38	1422.6±31.6	1750.2±15.9	-18.7
Ephedrae Herba (W)	300	973.7±25.8	1654.8±26.4	-41.2
	150	1120.6±13.5	1713.6±20.0	-34.6
	75	1473.2±26.6	1720.0±13.1	-14.3
	38	1473.2±26.6	1720.0±13.1	-14.3
Ephedrae Herba (E)	300	274.8±16.5	1710.0±31.0	-83.9
	150	523.6± 9.4	1720.2±16.1	-69.6
	75	824.1±41.3	1674.6±23.0	-50.8
	38	1389.5±45.2	1713.6±33.4	-18.9
Malloti Cortex (W)	300	265.7±65.7	1698.0±38.1	-84.4
	150	1051.6±47.9	1692.4±56.3	-37.9
	75	1543.8±45.7	1540.0±35.0	+0.2
	38	289.2±23.0	1766.6±35.0	-83.6
Malloti Cortex (E)	300	937.6±26.0	1837.3±29.6	-49.0
	150	1304.6±29.3	1837.3±29.6	-29.0
	75	1472.1±14.5	1837.3±29.6	-19.9
	38	1472.1±14.5	1837.3±29.6	-19.9
Moutan Cortex (E)	300	1043.6±41.3	1711.8±43.3	-39.0
	200	1293.6±21.1	1673.1±20.9	-22.7
	150	1564.8±21.2	1665.3±41.3	-6.0
	38	327.2±12.5	1750.2±26.9	-81.3
Rhei Rhizoma (W)	300	644.9±15.7	1727.0±28.9	-62.7
	150	1077.4±19.0	1717.7±27.2	-37.3
	75	1645.9±15.4	1733.9±16.7	-5.1
	38	468.8±15.4	1735.8±14.8	-73.0
Rhei Rhizoma (E)	300	892.6±45.3	1635.6±36.2	-45.4
	150	1255.2±27.4	1689.6±32.2	-25.7
	75	1553.4±19.7	1705.6±39.2	-8.9
	38	1553.4±19.7	1705.6±39.2	-8.9

Each value represents the mean±S.E. (n=8—10). a) Concentration of test samples in reaction solution, µg/ml. W, hot water extract; E, ethanolic extract.

inhibitors, was employed as a positive control (final concentration in reaction solution, 100 µg/ml). Hot water and ethanol extracts of Arecae Semen, Ephedrae Herba, Malloti Cortex and Rhei Rhizoma and ethanol extract of Moutan Cortex exhibited inhibitory activities strongly by more than 40%.

Among them, it was reported earlier that Rhei Rhizoma inhibits the α -amylase derived from *Bacillus* species and porcine pancreas.¹⁰⁾

As shown in Table 2, these activities of 9 extracts are almost dose-dependent.

Table 3. Influence of Intraperitoneally or Orally Administered Crude Drugs on Amylase Activity in Plasma of Mice

Sample	Activity (amylase unit)		Inhibition (%)
	Extract	Control	
<i>(i.p.)</i>			
Arecae Semen (W)	1576.7±105.1	1424.5±48.2	+10.7
Arecae Semen (E)	1554.4±74.6	1574.7±41.4	-1.3
Ephedrae Herba (W)	1454.7±50.4	1420.7±33.4	+2.4
Ephedrae Herba (E)	1470.4±84.8	1518.4±88.0	-3.2
Malloti Cortex (W)	1424.5±227.0	1424.5±48.2	0.0
Malloti Cortex (E)	1416.4±97.6	1362.6±110.8	+4.0
Moutan Cortex (E)	1283.9±43.7	1382.9±67.1	-7.2
Rhei Rhizoma (W)	1555.3±51.2	1606.8±30.5	-3.2
Rhei Rhizoma (E)	1224.2±73.4	1302.5±56.3	-6.0
Acarbose	607.3±37.2	1311.2±78.1	-53.7
<i>(p.o.)</i>			
Arecae Semen (W)	1474.3±52.8	1385.8±124.6	+6.4
Arecae Semen (E)	1513.2±63.0	1606.6±77.8	-5.8
Ephedra Herba (W)	1497.9±49.0	1396.8±95.6	+7.2
Ephedra Herba (E)	1554.0±9.2	1510.1±100.9	+2.8
Malloti Cortex (W)	1474.3±81.5	1385.8±124.6	+6.4
Malloti Cortex (E)	1286.7±87.8	1429.1±55.1	-10.0
Moutan Cortex (E)	1415.9±21.9	1394.9±27.7	+1.5
Rhei Rhizoma (W)	1225.8±59.9	1335.3±42.6	-8.2
Rhei Rhizoma (E)	1287.9±30.2	1362.1±78.5	-5.4
Acarbose	1300.7±65.5	1295.0±36.1	+0.4

Each value represents the mean±S.E. ($n=5-6$). Dose of test samples, extract, 1200 mg/kg; acarbose, 400 mg/kg. W, hot water extract; E, ethanolic extract.

When these 9 extracts showing inhibitory activities were given intraperitoneally and orally (1200 mg/kg) 5 and 10 min before blood collection, respectively, no effect was found against amylase activity in blood plasma. The results are shown in Table 3. Acarbose (400 mg/kg), the positive control, exhibited the activity only when intraperitoneally given and was not effective with oral administration.

Because digestive enzymes are included in gastroenteric drugs, the influence of stomachic crude drugs blended with them must be considered. Examination from the standpoint described above was attempted.^{7-9,11} Nine extracts of crude drugs, which showed inhibitory activities on α -amylase in isolated blood plasma *in vitro* and no activities in blood after intraperitoneal or oral administration, still have the possibility to inhibit the amylase activity in the mouse gastrointestinal tube. Accordingly, the depressive effect on the rise of blood glucose level in glycogen loaded mice was investigated.

Figure 1 shows the alteration in blood glucose level 60 min after oral administration of each extract (1200 mg/kg) and glycogen (2000 mg/kg). Ethanolic extracts of Arecae Semen, Ephedrae Herba and Malloti Cortex repressed increase in this level significantly, while ethanol extract of Arecae Semen had an especially strong depressive effect to the extent of about 80% of acarbose (positive control, 400 mg/kg, *p.o.*). Therefore, it was suggested that Arecae Semen inhibits the α -amylase activity in the gastrointestinal tube and is expected to improve postprandial hyperglycemia^{13,14} in dia-

Fig. 1. Variation of Blood Glucose Level in Glycogen Loaded Mouse Plasma after Oral Administration of Extracts of Crude Drugs

Closed columns, crude drug extracts (1200 mg/kg, *p.o.*) and glycogen (2000 mg/kg, *p.o.*); shaded columns, glycogen alone (2000 mg/kg, *p.o.*). Each value represents the mean of 5-8 mice per group. Horizontal lines show the standard error of the mean. * Significantly different from control, $p < 0.01$. W, hot water extract; E, ethanolic extract. The average of blood glucose level without administration of sample or glycogen was about 154 mg/dl.

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