Metal-Containing Components in Medicinal Plants. III.[1)] Manganese-Containing Components in Theae Folium as Oral Magnetic Resonance Imaging Contrast Materials

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A manganese-containing component from the water-extract of Theae folium (green tea) was found to exert an augmentative effect in magnetic resonance imaging (MRI) contrast and may possibly be a manganese(II) complex with a pectin-like polysaccharide capable of shortening the spin-lattice relaxation time (T_1) of water protons. Even though only the manganese(II) ion with $S=5/2$ is active in T_1-shortening ability, which should enhance contrast, complexation of this ion with the polysaccharide causes a marked increase in its activity. This manganese-containing pectin-like polysaccharides should prove useful as a low-toxic oral gastrointestinal contrast material in MRI.

Key words Theae folium; MRI; contrast material; manganese; metal-containing component

Magnetic resonance imaging (MRI) has been found to be quite useful for diagnosing many serious diseases such as cancer and cerebral apoplexy.[2-4)] In contrast to other tomographic methods such as X-ray computed tomography (X-ray CT) and positron emission tomography (PET), this method causes no radiation damage and its scope of application should broaden. Hasegawa noted that green tea have an augmentative effect based on the spin-lattice relaxation time (T_1)-shortening action in MRI contrast.[5)] The authors sought the active component of T_1-shortening in Theae folium, and found that the manganese(II) complex with a pectin-like polysaccharide is the responsible factor. Preliminary results of the examination of properties of the Mn(II)-containing component from Theae folium and its usefulness for MRI as a oral gastrointestinal contrast material are discussed in the following.

Experimental

Apparatus The spin-lattice relaxation time (T_1) of protons of HOD of a 1% sample solution was determined by the inversion-recovery method using a Hitachi NMR spectrometer (model R-1500, 60 MHz). The solution for measurement was prepared by adding D_2O (450 μl) to a 1% sample solution (50 μl). The X-band electron spin resonance (ESR) spectra of one of the fractions obtained from the extraction of Theae folium (GT-10) and its related compounds were measured at 25 °C using a JES-FE-3X spectrometer operating at 100 kHz magnetic field modulation. A Chelex 100 (Bio-Rad) was used to prepare Mn-free GT-10. For the ultrafiltration of Mn(II), a Centricon 10 (Amicon) was used (see Fig. 3). Mn concentration was determined with an atomic absorption spectrometer (Shimadzu model AA-670) provided with a Mn hollow-cathode lamp (at 279.5 nm). NMR images were obtained using a Toshiba Medical MRT-50A instrument with a 0.5 T superconducting magnet. HPLC was carried out on a Waters 515 pump and Shodex RI SE-61 detector equipped with several stainless steel columns as follows: TSK gel SCX (H^+) (7.8 mm i.d. × 30 cm) for uronic acids, YMC PA-03 (4.6 mm i.d. × 25 cm) for neutral sugars, and TSK G3000PW (7.5 mm i.d. × 65 cm) + TSK G5000PW (7.5 mm i.d. × 60 cm) or TSK G300PW_{XL} (7.8 mm i.d. × 35 cm) + TSK G5000PW_{XL} (7.8 mm i.d. × 30 cm) for molecular weight distribution.

Fractionation of the Extracts of Theae Folium by Ultrafiltration Theae folium (1.0 kg) was extracted with hot water and all extracts were subjected to a Sepabeads SP-207 (Mitsubishi Chemicals) to remove hydrophobic organic substances such as tannins and caffeine. Lyophilization of the effluent gave a light brown powder (named GT-0; 146 g). GT-0 was successively separated using ultrafiltration membranes (first filter, 100000 fractionated molecular weight (MW) and second, 10000 (MW)) to obtain the following three fractions: GT-10 (> 100000 (MW), 22 g), GT-1-10 (10000—100000 (MW), 2 g), and GT-1 (< 10000 (MW), 118 g).

Results and Discussion

Table 1 summarizes the fundamental analytical results for the four fractions, GT-0, GT-1, GT-1-10, GT-10, from the water extracts of Theae folium (green tea). Considerable T_1-shortening was observed for the high molecular weight (MW) fractions, GT-1-10 (minor) and GT-10 (major). All four fractions showed high Mn-content, with as much as 3500 ppm (Mn) for GT-1-10. Mn(II) has five unpaired electrons in its *d* orbital, and thus its paramagnetism lessens the T_1 of water signals, as does Gd(III). It is of interest that GT-10 possessed the strongest T_1-reducing activity (0.7 s), even though its Mn content was comparable to that of GT-0 and GT-1, and was only half that of GT-1-10. Thus, this study was conducted on the most active fraction, GT-10. Note that the activity of the water extracts of Theae folium is due not only to GT-10 but also to GT-1 and GT-1-10, because GT-1, having weaker activity based on Mn(II) than that of GT-10, was contained in the extracts at *ca.* five times

Table 1. Fundamental Analytical Results for the Various Fractions of Theae Folium Extract

	GT-0	GT-1	GT-1-10	GT-10
T_1 (s) (1% soln.)	2.3	3.0—3.5	0.7—0.9	0.7
Amounts of powder (g)	146	118	2	22
Ash (550 °C, %)	19.9	23.3	25.1	7.6
Mn (ppm)	1800	1800	3500	1900
Fe (ppm)	60	22	470	88
Zn (ppm)	82	88	130	40
Mg (ppm)	600	640	620	590
Amino acids (ninhydrine)	++	++	−	−
Total proteins (%) (Bio-Rad method)[7)]	0.9	0.7	2.5	0.1
Pectin (official formulary of food additives)	++	−	+	++
Uronic acids (%) (Dische method)[8)]	10	5	24	41

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the weight of GT-10. Table 2 shows the relationship of the Mn concentration to T_1 of the GT-10 fraction. Mn-free GT-10 (Chelex 100-treated) appeared to have no T_1-reducing activity, and T_1 was 6.3 s, the same as that (8.5 s) of H_2O alone. The addition of Mn^{2+} to this Mn-free GT-10 caused almost complete recovery of its activity to 0.6 s, this being very close to 0.7 s (T_1 for GT-10). However, Mn^{2+} at 15 ppm in aqueous solution (T_1: 3.2 s) showed much lower activity than GT-10 (T_1: 0.7 s), although it contained almost the same amount of Mn. The strong T_1-reducing activity of GT-10 is thus due not only to a high Mn-content but to the presence of certain organic substances such as the ligands of the Mn(II) ion. In this connection, it is of interest that the activity of GT-10 is attributed to the multiplicative effect of Mn(II) and organic substances, whereas, in Gd-DTPA, just the paramagnetism of the metal causes the activity.

Table 2. Relationship between Mn Concentration and T_1 for GT-10

Sample (1% soln.)	Mn concentration (ppm)	T_1 (s)
GT-10	16	0.7
GT-10 (Mn free) (Chelex 100)	ND	6.3
GT-10 (Mn free) + Mn	15	0.6
Mn only	15	3.2
H_2O	0	8.5

ND = not detected.

Figure 1 shows high performance gel chromatograms of the GT-10 fraction. Chromatogram A shows that the MW of GT-10 ranges from 10000 to 2000000, and is *ca.*

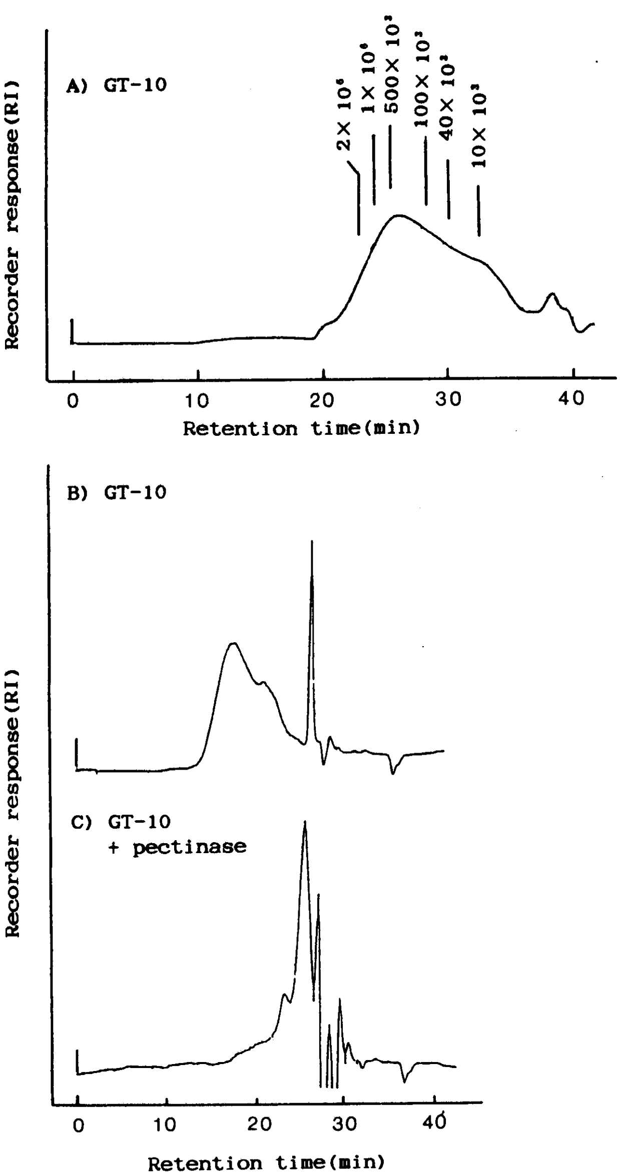


Fig. 1. High Performance Gel Chromatography of the Fraction GT-10

A) Sample concentration, 0.5%; sample size, 250 μl; column, TSK G3000PW (7.5 mm i.d. × 65 cm) + TSK G5000PW (7.5 mm i.d. × 60 cm); flow rate, 1.0 ml/min; detector, RI × 16; mobile phase, 0.2 M phosphate buffer (pH 6.9). B, C) Sample concentration, 0.1%; sample size, 200 μl; column, TSK G3000PW$_{XL}$ (7.8 mm i.d. × 35 cm) + TSK G5000PW$_{XL}$ (7.8 mm i.d. × 30 cm); flow rate, 0.8 ml/min; detector, RI × 8; mobile phase, 0.15 M phosphate buffer (pH 6.8).

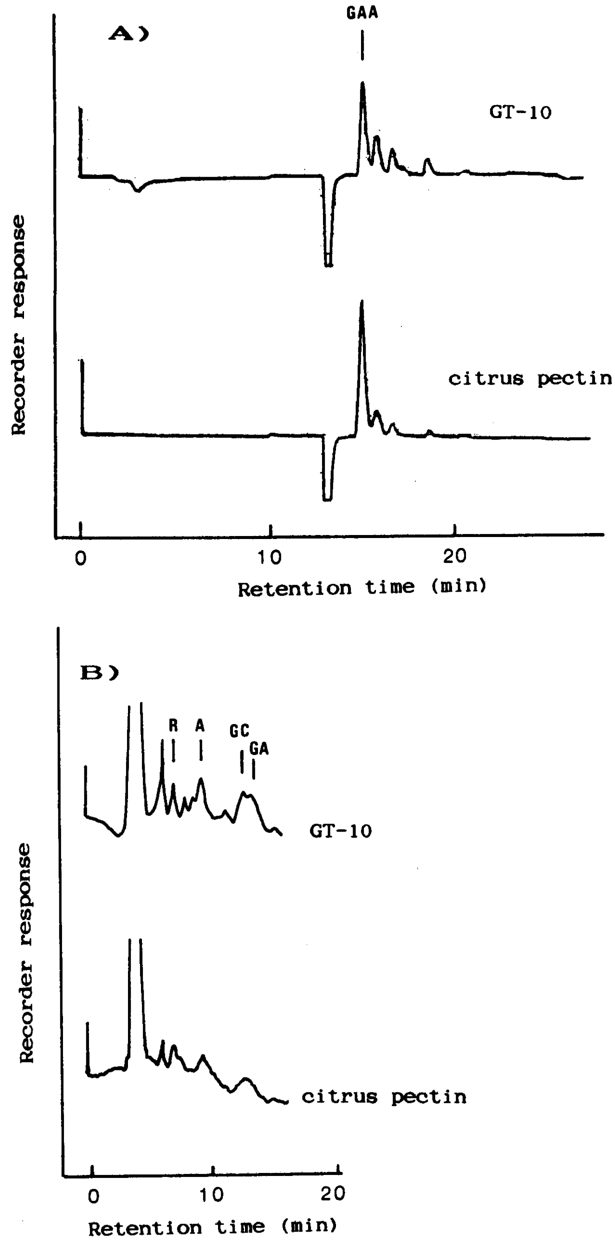
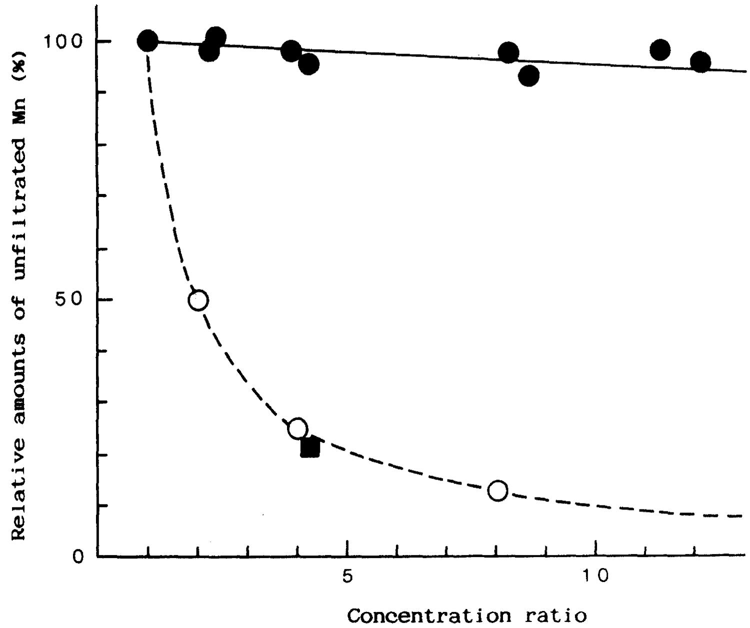


Fig. 2. HPLC of the Acid-Hydrolysis Products of GT-10 or Citrus Pectin

A) Sample concentration, 0.5%; sample size, 20 μl; column, TSKgel SCX (H^+) (7.8 mm i.d. × 30 cm); flow rate, 0.5 ml/min; detector, RI × 16; mobile phase, 0.1% H_3PO_4. B) Sample concentration, 2.5%; sample size, 20 μl; column, YMC PA-03 (4.6 mm i.d. × 25 cm), flow rate, 1.0 ml/min; detector, RI × 8; mobile phase, $CH_3CN:H_2O$ (4:1). GAA, galacturonic acid; R, rhamnose; A, arabinose; GC, glucose; GA, galactose.



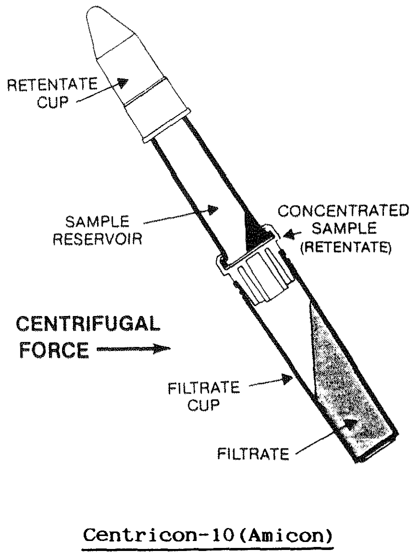


Fig. 3. Unfiltrated Mn in the Ultrafiltration of GT-10 Using Centricon-10

●, GT-10; ○, calculated value for free Mn^{2+}; ■, free Mn^{2+}.

500000 on the average (see Fig. 1A). As shown in Fig. 1B, incubation of the GT-10 fraction with pectinase caused drastic changes in the chromatogram: the high-MW substance degraded readily to low-MW substances, indicating that GT-10 consists mainly of pectin-like polysaccharides. This suggestion is supported by the fundamental properties of GT-10, as shown by positive pectin test results and high uronic acid content (41%). In Fig. 2, chromatograms of HPLC for the acid-hydrolysis products of GT-10 and citrus pectin, a typical pectin, are compared. GT-10 consisted mainly of galacturonic acid as the major acid sugar, with several neutral sugars such as rhamnose, arabinose, glucose, and galactose as minor components. Essentially the same was observed for citrus pectin, which was used as a reference substance.

To elucidate the chemical species of Mn in GT-10, ultrafiltration and ESR spectrometry were conducted. Figure 3 shows the behavior of the Mn of GT-10 in ultrafiltration using Centricon-10. If Mn, like free Mn^{2+}, can pass through the ultrafiltration membrane easily, Mn in the concentrated sample should decrease with the concentration ratio (as shown by an open circle). In fact, free Mn^{2+} (in $MnCl_2 \cdot 4H_2O$ aqueous solution) passed through the membrane with water; at a concentration ratio of four only *ca.* 25% of the origin (closed square) was retained, consistent with the calculated value. On the other hand, Mn in GT-10 did not pass through the membrane; almost all of the Mn was retained in the concentrated sample containing pectin-like polysaccharides. The Mn thus appears not to be present as free ions but to bind to pectin-like polysaccharides, possibly through coordination bonds. This consideration was confirmed by a comparison of the ESR spectra of GT-10 and $MnCl_2 \cdot 4H_2O$ containing Mn at the same content level (Fig. 4). Although the spectra exhibited an obviously typical Mn(II) ($S=5/2$) hyperfine pattern, GT-10, in spite of no change in content, showed less than one-tenth the free Mn^{2+} in signal height. This reduction in the amplitude of the GT-10 spectrum was due to the restricted motion of bound Mn(II) ions, as was also the case with Mn(II)-concanavalin A.[6] The addition of Mn-free GT-10 to Mn^{2+} markedly decreased the height of the signals arising from Mn(II) ($S=5/2$), and the height did not change significantly in the range over a ten-fold mole ratio (assumed 194 (galacturonic acid–H_2O) as MW of GT-10) (Fig. 5). Nearly all Mn^{2+} may be barely bonded by ten times the number of moles of the polysaccharide. These results indicate that the Mn(II) forms complexes with a pectin-like polysaccharide in GT-10, though not strongly.

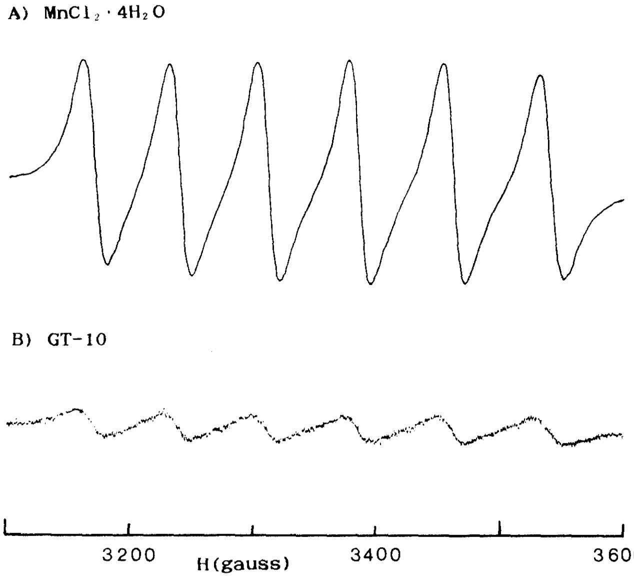


Fig. 4. ESR Spectra for GT-10 at 25°C

Table 3 summarizes the T_1-reducing activity of GT-10 and its related compounds. No activity was observed for galacturonic acid (the main constitutive sugar of GT-10), galactan (a polymer of galactose), or dextran (a polymer of glucose). In contrast with these, significant activity was seen for pectin, pectic acid (a polymer of galacturonic

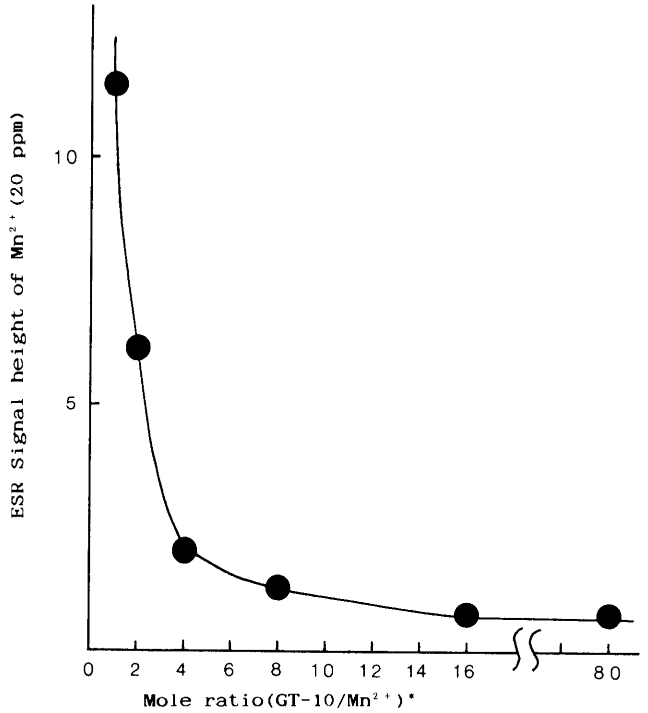


Fig. 5. Relationship between ESR Signal Height (Mn^{2+}) and Mole Ratio (GT-10/Mn^{2+})

*Calculated using a value of 194 (galacturonic acid–H_2O) as MW of GT-10.

Table 3. T_1 Values for a 1% Solution of GT-10 or Its Related Compounds

Compound	Mn concentration (ppm)	T_1 (s)
GT-10	16	0.7
Galacturonic acid	9.9	3.8
Galactan	9.6	3.5
Dextran	9.6	3.8
Pectin (BNF)	7.6	1.3
Pectic acid (poly-GA)	9.6	1.0
CMC (1150)	9.6	1.3
Sodium alginate	9.6	1.4
$MnCl_2 \cdot 4H_2O$	9.9	3.8
H_2O	0	8

acid), CMC (carboxymethylcellulose), and sodium alginate (polymer of mannuronic acid). These results strongly indicate the importance of having the carboxylate group and of being a high-MW substance for T_1-reducing activity.

An MRI tomograph obtained, following the administration of GT-10 (*ca.* 1.0 g/200 ml), was compared with that of the control (Fig. 6). The inside of the stomach was lighter than the control due to T_1-reducing. The dark part was due to air in the stomach. Light imaging for the digestive tube is required to distinguish organs whose boundaries are vague, as in the case of the duodenum and pancreas. The tomographs strongly indicate the Mn-containing polysaccharide from Theae folium to be very useful for MRI as an oral gastrointestinal contrast material. Pharmaceutical studies on the pharmacokinetics, effective dose, side effects, *etc.* of the GT-10 should be performed in the near future. The structure of the pectin-like polysaccharide is now being determined.

In summary, preliminary studies on the GT-10 fraction having T_1-reducing activity in Theae folium indicate the following:

1. T_1-shortening activity in Theae folium is mainly due to a Mn(II) complex with the pectin-like polysaccharide. Even Mn(II) ions alone have significant activity, but coordination bonds with the polysaccharide cause the activity to become to much stronger.
2. An organic compound capable of reducing T_1 by bonding with a Mn(II) ion must possess many carboxylate groups and be a high-MW substance.
3. The pectin-like polysaccharide containing Mn(II), a component of Theae folium, is a popular beverage and its safety has been confirmed. It should prove useful as a low-toxic oral gastrointestinal contrast material in MRI.

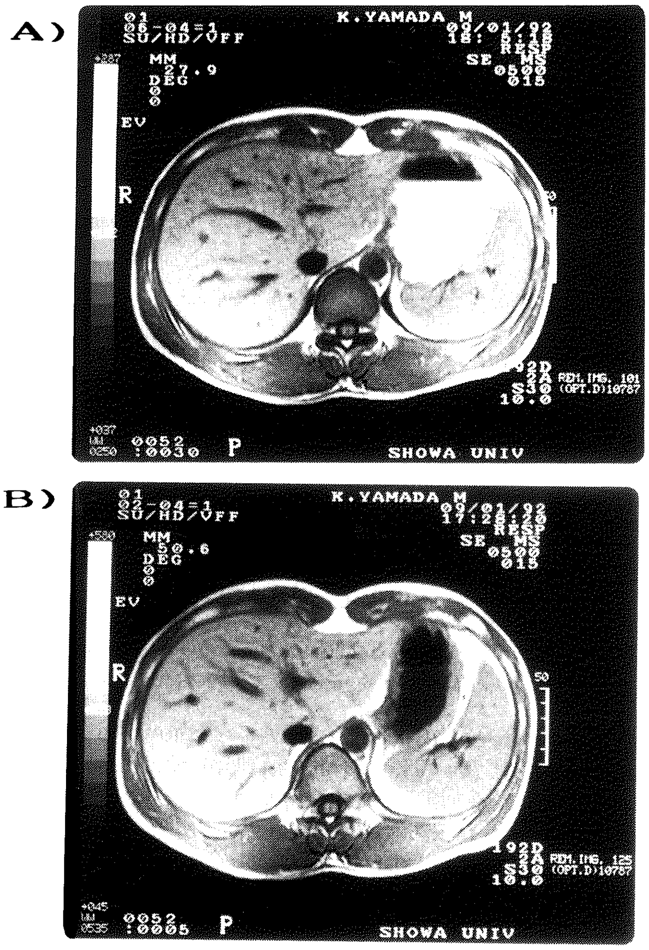


Fig. 6. Cross-Sectional Photographs Taken through the Body Just at the Stomach in MRI Computed Tomography

A) 20 min after the administration (15 mg/kg, *p.o.*) of GT-10 aqueous solution (1 g/200 ml). B) Before the administration of the same solution. T_1-Emphasized image, spin echo method (SE 500/15).

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