

The effects of water extract of Pogostemi Herba on intestinal mucosa of mice with zinc abnormality

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(Received October 7, 1994. Accepted January 19, 1995.)

Abstract

The effects of treatment with water extract of Pogostemi Herba on the pathological changes of the intestinal mucosa and serum zinc content in mice with zinc-deficiency or with zinc-toxicosis were examined. Compared to normal control mice, the mice with zinc-deficiency and with zinc-toxicosis showed increased frequency of irregular array of jejunal villi, and villus heights that were not uniform markedly decreased. Moreover, the frequency of normally shaped villi was decreased in mice with either zinc-abnormality. Pogostemi Herba significantly reduced the frequency of these morphological changes, but it had no effect on the serum zinc concentration, in the zinc-toxicosis and zinc-deficient mice.

Key words Pogostemi Herba, zinc, zinc-deficiency, zinc-toxicosis.

Introduction

Zinc deficiency is either hereditary or acquired. Acquired zinc deficiency can be seen in patients with liver cirrhosis,^{1,2)} total parenteral nutrition,³⁾ alcoholism,^{4,5)} hemodialysis,⁶⁾ or vitamin deficiency.⁷⁾ Zinc deficiency causes severe retardation in growth,⁸⁾ sense disorder of taste and smell,⁹⁾ immune deficiency,^{10,11)} and abnormality of the gastrointestinal tract.¹²⁾ In general, ZnSO₄ solution is used for the treatment of zinc deficiency, with prompt and strong response. However, ZnSO₄ has strong side effects (Zn-toxicosis), and it is difficult to maintain equilibrium of the serum zinc content by the administration of ZnSO₄ solution, especially in patients with disorder of the gastrointestinal tract. In China, an herbal medicinal preparation composed of Pogostemi Herba, Saussureae Radix, Perillae Herba, Galli Stomachichum Corium, Aurantii Nobilis Pericarpium and Anemarrhnae Rhizoma has been administered to the patients with zinc-deficiency instead of ZnSO₄ solution or combined

with it. The administration of the herbal medicine, especially that containing Pogostemi Herba, has been clinically confirmed to be very useful for maintaining the serum zinc content.^{13,14)} However, there has been no pharmacological study of the effect of the Pogostemi Herba preparation in countering zinc abnormality. In the present study, the effects of one of the chief crude drugs in the preparation, Pogostemi Herba, on the pathological changes of the intestinal mucosa and serum zinc content of mice with zinc-deficiency or with zinc-toxicosis were observed.

Materials and Methods

Mouse models of zinc-deficiency and zinc-toxicosis: Thirty female ICR mice (6 weeks) were divided randomly into 5 groups (6 mice each). The control group received standard feed (CE-2, Japanese Clea Company), the mice in the two zinc deficient diet groups received the same CE-2 diet depleted of zinc (5ZnO. 2CO₂. 4H₂O), and those in the two zinc-toxicosis groups were fed normal diet plus 1 ml/day of

1000 ppm ZnSO_4 solution. The administration 1 ml of 1000 ppm ZnSO_4 was confirmed to maintain a 100 % survival rate in spite of disorder of gastrointestinal tract, while the administration of 1 ml of 3000 ppm ZnSO_4 resulted in a 50% survival rate. The mice in one each of the zinc-deficient and zinc-toxicosis groups were fed as described above for each group and were also treated through a gastric tube, with 1 ml/day of water extract of Pogostemi Herba prepared as described below. After 4 weeks of feeding, blood samples were obtained from the heart and the jejunum was histologically observed.

Preparation of water extract of Pogostemi Herba : 50 gms of Huo Xiang (藿香), Pogostemi Herba, *Pogostemon Cablin* BENTH (Sinchuan Province ; grown in Sinchuan Province, China) were soaked in 500 ml distilled water for one hour, which was then boiled and condensed to a total volume of 50 ml. One ml per day was given to each mouse in the two treated groups through a gastric tube for 4 weeks. This dose had been determined in a preliminary experiment, in which it was found that the dose of 1 ml/day resulted in the greatest rate of increase in body weight of zinc-deficient mice.

Assay of zinc content of serum and water extract of Pogostemi Herba : Serum samples or water extract of Pogostemi Herba were diluted with 0.5 N HNO_3 solution. The zinc content was assayed by Zeeman atomic spectroscopy (Model 180/80, Hitachi).

Light microscopy : After collection of blood by heart puncture, the abdominal cavity was opened, and a jejunal tissue sample was obtained, fixed with 10 % formalin, dehydrated and embedded using conventional procedures. Five thin sections were prepared from each sample, stained with hematoxylin and eosin (H.E.), observed under a Nikon optic microscope, and photographed.

Morphological classification of intestinal villi : The standard of morphological classification was based on the method reported by Lee and Tonar.¹⁵⁾ All villi seen in 12 photographs ($\times 25$) (2 photographs per sample) were classified as Grade I to V, as follows : Grade I : finger villi only ; Grade II : leaf villi only or leaf and finger villi only ; Grade III : leaf villi with some fusion forming small ridges or convolutions ; Grade IV : convolution or ridging with no recogniz-

able villi, often with some flattening ; and Grade V : notable flattening of the mucosa.

Measurement of the height of villi and crypts : As shown in Figure 1, the following lengths were measured : the total height (TH) from the base of the crypt to the apex of the villus ; the crypt height (CH) from the base of the crypt to the transitional part ; and the villus height (VH), from the transitional part of the crypt to the apex of villus. The ratio of CH/VH was calculated to estimate the turn-over rate of the jejunal mucosal epithelium. Fifty villi were measured in each specimen, and the mean value \pm S.D. was calculated.

Scanning electron microscopy : Specimens were fixed with 3 % glutaraldehyde and 10 % osmium tetroxide, dehydrated, freeze dried, and then coated with gold under an E-1030 type Hitachi ion sputter (JSM-840). Specimens were observed under an S450 scanning electron microscope (Hitachi) and photographed.

Transmission electron microscopy : Intestinal tissues were fixed with 3 % glutaraldehyde and 10 % osmium tetroxide and embedded in resin. Then ultrathin sections were made, stained with uranyl acetate and citric acid and observed under a HS-9

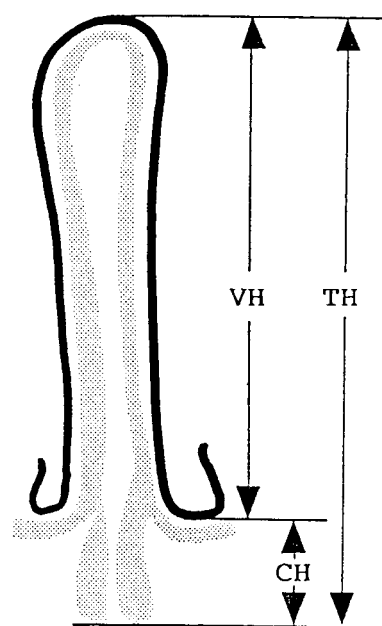


Fig. 1 Method for measurement of villi and crypts.

transmission electron microscope (Hitachi).

Statistical methods : Differences among the groups in serum zinc concentration and in villus and crypt height were examined with Kruskal-Wallis test. The significance test used was Dunnett's post-hoc procedure (two-sided test). Differences among the groups in the frequency of each morphological grade of intestinal villi were examined with Kruskal - Wallis test followed by significance test of difference between pairs of groups using Scheffe type multiple comparison.

Results

Changes of body weight

At the end of the 4-week experimental period, the

Table I The effects of Pogostemi Herba on serum zinc concentrations in mice.

Groups	Serum zinc concentrations $\mu\text{g/dl}$ ($\mu\text{mol/L}$)
Control (n=6)	110.33 ± 14.05
Zinc-toxicosis (n=6)	251.33 ± 9.96^a
Zinc-toxicosis + Pogostemi Herba (n=6)	209.00 ± 6.65^a
Zinc-deficiency (n=6)	59.33 ± 13.66^{aa}
Zinc-deficiency + Pogostemi Herba (n=6)	41.50 ± 10.97^{aa}

Significant difference from control :^a $p < 0.05$, ^{aa} $p < 0.01$.

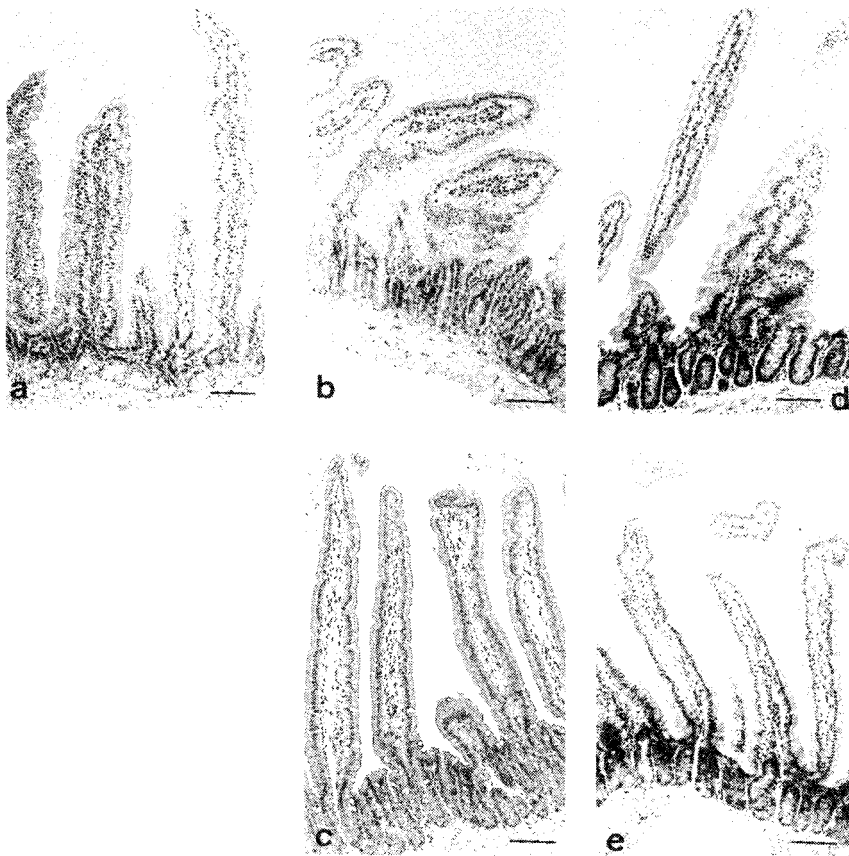


Fig. 2 Light microscopic observation of small intestine (bar 0.1 mm).

a, small intestine of control mouse ; b, zinc-toxicosis mouse ; c, zinc-toxicosis mouse treated with water extract of Pogostemi Herba ; d, zinc-deficient mouse ; e, zinc-deficient mouse treated with water extract of Pogostemi Herba.

control mice showed 9.4 % mean increase of body weight compared to that at the start of the experiment, while the zinc deficiency and zinc-toxicosis groups showed 6.6 % and 8.3 % decrease, respectively. The administration of Pogostemi Herba significantly inhibited the body weight loss in both the zinc-deficiency (mean decrease, 1.9 %) and zinc-toxicosis (mean decrease, 2.6 %) groups.

Alterations of serum zinc concentration

The mean serum zinc concentration in the zinc-toxicosis group was significantly elevated ($p < 0.05$) compared with that in the control group, while that in the zinc deficiency group was significantly decreased ($p < 0.01$). The treatment with Pogostemi Herba had no effect on the significance of the difference from the control group in either the zinc deficiency or zinc-

toxicosis group (Table I).

Histological changes and morphological classification of intestinal villi

In the normal control group, the intestinal villi were arrayed regularly, with uniform VH, and the most frequent grade of villi was Grade I, and 85.51 % of all the villi were normal shape (Grade I-III). In the zinc-deficiency and zinc-toxicosis groups, the jejunal villi were arrayed irregularly, and the VH were not uniform. The frequency of villi of Grade I-III in the zinc-deficiency and zinc-toxicosis groups was markedly decreased to 44.0 and 45.59 %, respectively. In contrast, in the mice treated with Pogostemi Herba, the jejunal villus array was prominently improved, the frequency of normal shaped villi was increased, and that of abnormal villi (Grade IV-V) was significantly

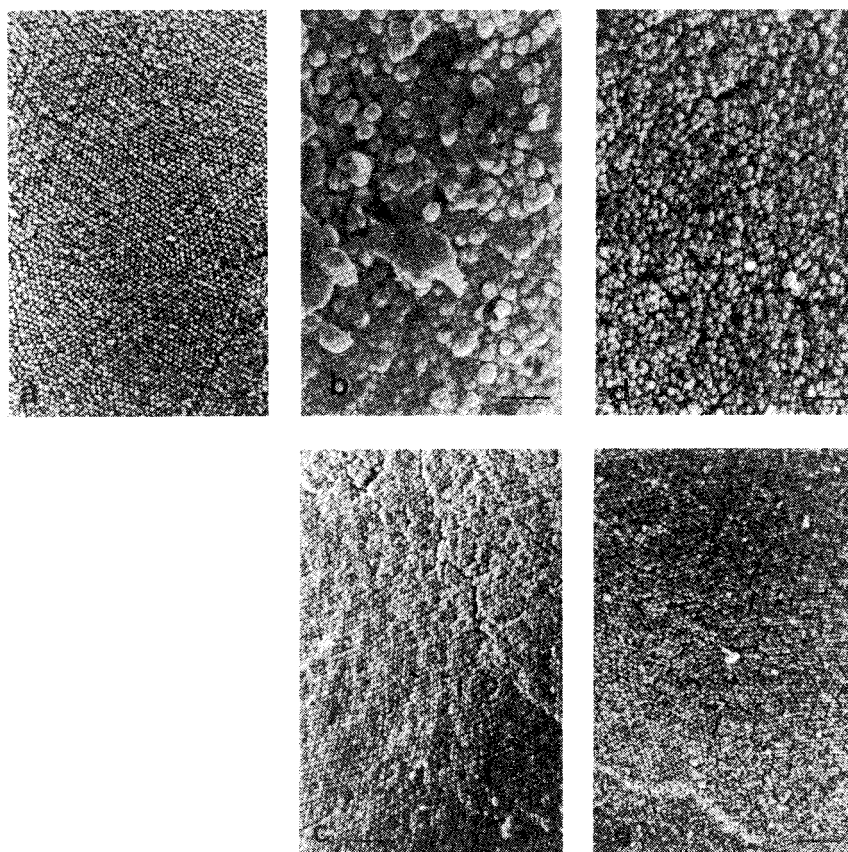


Fig. 3 Scanning electron microscopic observation of small intestine (bar 0.5 μm).
 a, intestine of control mouse ; b, zinc toxicosis mouse ; c, zinc toxicosis mouse treated with water extract of Pogostemi Herba ; d, zinc deficient mouse ; e, zinc deficient mouse treated with water extract of Pogostemi Herba.

decreased ($p < 0.01$) (Table II). A significant difference ($p < 0.01$) in the distribution of these five grades was confirmed between zinc-toxicosis mice with and without Pogostemi Herba treatment and between the zinc-deficiency mice with and without Pogostemi Herba treatment. Light microscopically, hypertrophy of the crypts and lymphangiectasis were very rarely seen in any group (Fig 2).

Alteration of the intestinal villous height

(i) *TH and VH* : Compared to those in the control group, the TH and VH values were significantly ($p < 0.01$) decreased in both the zinc deficient and zinc-toxicosis mice. In the zinc deficient mice treated with Pogostemi Herba, however, the TH and VH values were significantly ($p < 0.01$) increased compared to those in the zinc deficiency group. The VH value in the zinc-toxicosis group treated with

Pogostemi Herba was significantly increased ($p < 0.05$) compared to that in the zinc-toxicosis group (Table III).

(ii) *CH and CH/VH ratio* : In the zinc-toxicosis and zinc deficiency groups, the values of CH and the CH/VH ratio were higher ($p < 0.01$) than those in the control group, but the CH value and CH/VH ratio in the two groups treated with Pogostemi Herba, were lower ($p < 0.01$) than those in the corresponding with either zinc-deficiency or zinc toxicosis alone (Table III).

Scanning electron microscopy

Compared with that in the control groups, the surface of the intestinal epithelium in the zinc-deficient and zinc-toxicosis groups was irregular and swollen and the apices of the villi were swollen and scattered unevenly. These alterations were more

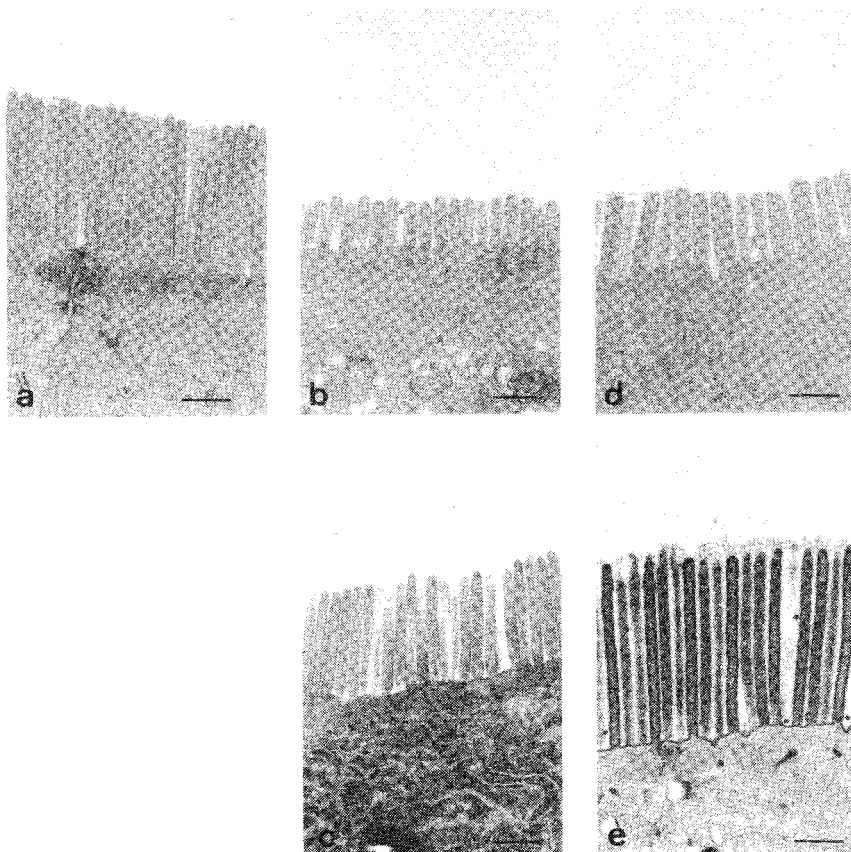


Fig. 4 Transmission electron microscopic observation of microvilli of epithelial cells (bar 0.5 μ m). a, microvilli of control mouse ; b, zinc toxicosis mouse ; c, zinc toxicosis mouse treated with water extract of Pogostemi Herba ; d, zinc deficient mouse ; e, zinc deficient mouse treated with water extract of Pogostemi Herba.

Table II The effects of Pogostemi Herba on the frequencies of morphological grades of the intestinal villi of mice.

Groups	Grade					Normal (I + II + III)	Abnormality (IV + V)
	I	II	III	IV	V		
Control (n=6)	71 (51.45%)	22 (15.94%)	25 (18.12%)	19 (13.77%)	1 (0.72%)	118 (85.51)	20 (14.49)
Zinc toxicosis (n=6)	32 (23.53%)	8 (5.90%)	22 (16.17%)	59 (43.38%)	15 (11.03%)	62 (45.59%)	74 ^{aa} (54.41)
Zinc-toxicosis+ Pogostemi Herba (n=6)	71 (40.57%)	17 (9.71%)	40 (22.86%)	41 (23.43%)	6 (3.43%)	128 (73.14)	47 ^{aa} 47 ^{bb} (26.86)
Zinc-deficiency (n=6)	38 (21.71%)	5 (2.86%)	34 (19.43%)	81 (46.29%)	17 (9.71%)	77 (44.00)	98 ^{aa} (56.00)
Zinc-deficiency + Pogostemi Herba (n 6)	97 (49.74%)	21 (10.77%)	36 (18.46%)	34 (17.44%)	7 (3.59%)	154 (78.97)	41 ^{cc} (21.03)

* Values represent numbers of villi and values in parentheses represent % of the total in each group.

Significant difference from control : ^{aa} $p < 0.01$;

Significant difference from Zinc toxicosis : ^{bb} $p < 0.01$;

Significant difference from Zinc-deficiency : ^{cc} $p < 0.01$.

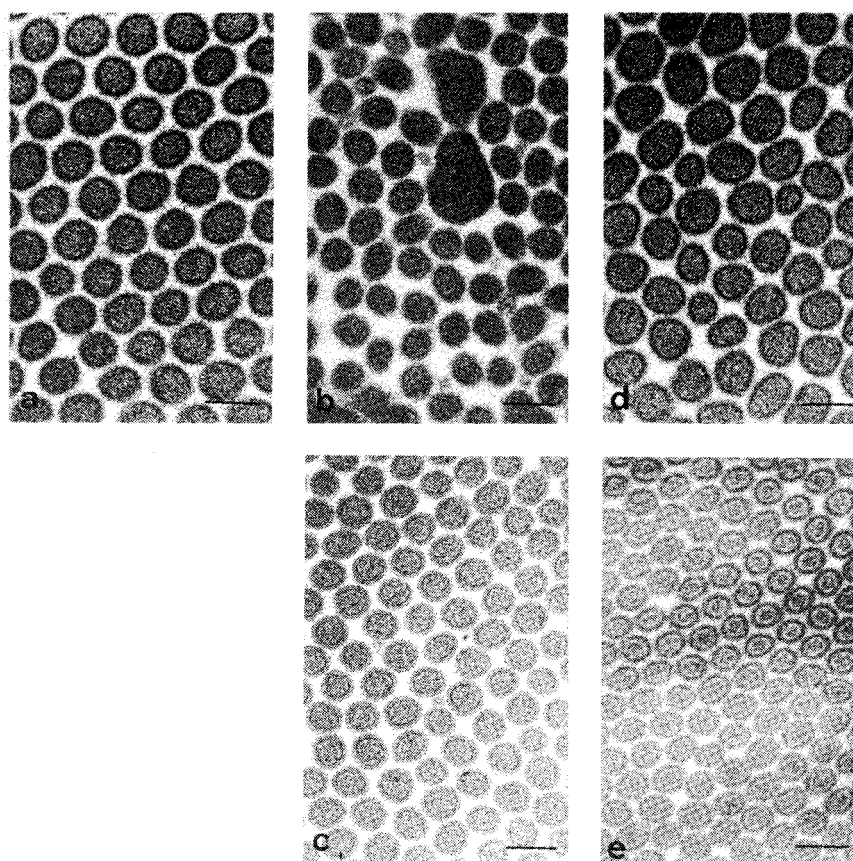


Fig. 5 Cross section of microvilli of epithelial cells (bar 0.2 μ m).

a, control mouse ; b, zinc-toxicosis mouse ; c, zinc-toxicosis mouse treated with water extract of Pogostemi Herba ; d, zinc-deficient mouse ; e, zinc-deficient mouse treated with water extract of Pogostemi Herba.

Table III The effects of Pogostemi Herba on the height of intestinal villi of mice.

Groups	TH(μm)	VH(μm)	CH(μm)	CH/VH
Control	440.36 \pm 50.09	377.86 \pm 20.79	90.63 \pm 8.03	0.290 \pm 0.046
Zinc toxicosis	368.58 ^a \pm 57.85	234.42 ^{aa} \pm 37.15	134.62 ^{aa} \pm 20.15	0.564 ^{aa} \pm 0.082
Zinc toxicosis + Pogostemi Herba	383.63 ^a \pm 23.32	285.57 ^{aa} \pm 20.15	98.32 ^{bb} \pm 4.24	0.371 ^{bb} \pm 0.011
Zinc deficiency	303.40 ^{aa} \pm 32.22	180.24 ^{aa} \pm 51.39	126.72 ^{aa} \pm 25.70	0.676 ^{aa} \pm 0.024
Zinc deficiency + Pogostemi Herba	443.08 ^{cc} \pm 14.58	343.97 ^{cc} \pm 26.40	100.48 ^{cc} \pm 2.45	0.387 ^{cc} \pm 0.034

Significant difference from control : ^a $p < 0.05$, ^{aa} $p < 0.01$;
Significant difference from excess zinc : ^b $p < 0.05$, ^{bb} $p < 0.01$;
Significant difference from zinc deficient : ^c $p < 0.05$, ^{cc} $p < 0.01$.

prominent in the zinc-deficient mice than in the zinc-toxicosis mice. The apices of microvilli were swollen and presented the appearance of round beads, and leakage of the granules of the Goblet cells out of the cracks on the cell membrane was observed. In contrast, in the zinc-deficient and zinc-toxicosis mice treated with Pogostemi Herba, the surface of the cellular membrane was smooth and even, and the microvilli were arrayed regularly as in the control group (Fig 3).

Transmission electron microscopy

The microvilli of the intestinal mucosal epithelium in the zinc-deficient or zinc-toxicosis mice were shorter and thicker than those in the control mice (Fig. 4). The microvilli were arrayed irregularly, especially in the zinc-toxicosis mice, and the diameters of

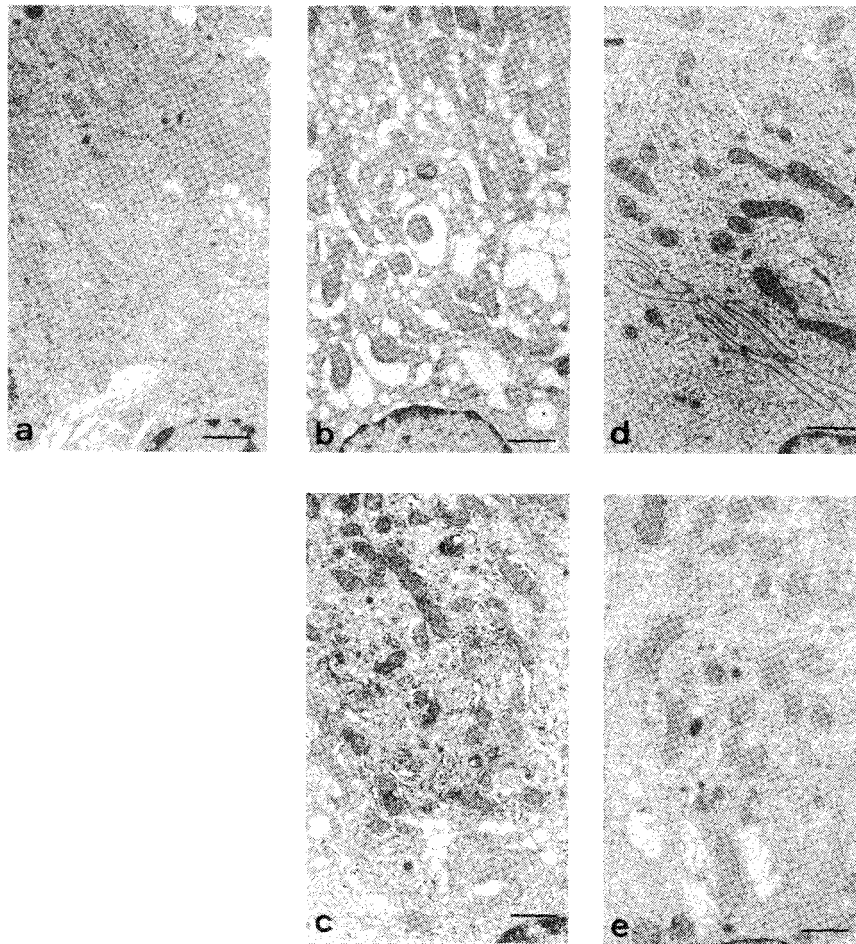


Fig. 6 Transmission electron microscopic observation of epithelial cells of small intestine (bar 0.5 μm).

a, epithelial cell of control mouse ; b, zinc-toxicosis mouse ; c, zinc-toxicosis mouse treated with water extract of Pogostemi Herba ; d, zinc-deficient mouse ; e, zinc-deficient mouse treated with water extract of Pogostemi Herba.

the villi were not uniform (Fig. 5). In addition, the Golgi apparatus and rough endoplasmic reticulum were swollen and degenerated. Compared to those in the zinc-deficiency and zinc-toxicosis groups, the height, diameters and arrangement of microvilli in the two groups treated with Pogostemi Herba were all more similar to those in the control mice. The enlargement of the Golgi apparatus and rough endoplasmic reticulum was also diminished, and degeneration of the cristae of mitochondria was seldom seen (Fig 6).

Discussion

Our experiment demonstrated that the morphological changes of the intestinal mucosa in zinc-deficient mice are quite similar to those in zinc-toxicosis mice. This is the first report to our knowledge that the morphological changes of intestinal mucosa are similar in zinc-deficient and zinc-toxicosis mice. Although we cannot account for this similarity, we suspect that zinc deficiency may share common features with zinc toxicosis. The amelioration by the water extract of Pogostemi Herba of the pathological lesions of the intestinal mucosa found in both zinc-deficient and zinc-toxicosis mice also supports this speculation. It was confirmed that the zinc content of the water extract of Pogostemi Herba was only 1.07 ppm, and the serum zinc concentration of the mice treated with Pogostemi Herba showed no prominent alteration. This result indicates that the actions of Pogostemi Herba have no relation to the regulation of serum zinc level; accordingly, the water extract of Pogostemi Herba might inhibit the formation of the lesions of the intestinal epithelium in zinc abnormality by its direct action on the intestinal mucosa. That is, Pogostemi Herba might have a non-specific action countering these lesions. We previously found that Chinese herbal medicine containing Pogostemi Herba improved the morphological changes associated with age (unpublished data). If Pogostemi Herba is effective in countering non-specific abnormality of intestinal mucosa, it is not unreasonable to speculate that it would also be effective in diminishing the lesions of intestinal mucosa in both zinc-deficient and zinc-toxicosis mice.

In Chinese medicinal practice, Pogostemi Herba

is recognized as effective for digestive system disorders. Its antifungal¹⁶⁾ and antispirechete effects¹⁷⁾ have been demonstrated experimentally, although an action against intestinal disorders has not. The present study clarified that Pogostemi Herba has a marked inhibitory action on intestinal disorders caused by zinc abnormality. This preparation may improve the condition of patients with zinc-deficiency through its protective effects on intestinal mucosa. In Japan, zinc-deficiency is seen in patients with such acquired conditions as liver cirrhosis, alcoholism, hemodialysis and total parenteral nutrition, while in China the role of hereditary zinc-deficiency is not inconsiderable. In these zinc-deficiencies of various etiologies, the disturbance of absorption of zinc in intestine is thought to be present a common and basic factor in the induction of zinc-deficiency. Zinc-deficiency adversely affects the gastrointestinal tract, in a vicious circle in which the abnormality of the gastrointestinal tract induces abnormal zinc metabolism. Accordingly, the improvement of gastrointestinal tract function is the most important and effective aspect of any treatment for zinc-deficiency. In addition, the serum zinc concentration may easily be regulated after the administration of ZnSO₄ solution, disrupting the vicious circle, if the disorder of the gastrointestinal tract is first treated with Pogostemi Herba. The detailed action mechanism of Pogostemi Herba is now under investigation.

和文抄録

亜鉛欠乏食を投与して作成した亜鉛欠乏マウスおよび過剰亜鉛を投与したマウスの空腸絨毛は、いずれも配列が乱れ、絨毛の長さは短く、不均一となる。また絨毛の形態も葉状あるいは指状の正常形態を示すものが著しく減少する。ところが藿香の煎液を投与したマウスでは、血清亜鉛に変化はみられないが、小腸粘膜の障害が明らかに抑制されることが見出された。

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