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European Journal of Pharmacology 387 (2000) 337–342

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Enhancement by escins Ib and IIb of Mg^{2+} absorption from digestive tract in mice: role of nitric oxide

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Received 21 June 1999; received in revised form 26 October 1999; accepted 29 October 1999

Abstract

The effects of escins Ib and IIb isolated from horse chestnuts on Mg^{2+} absorption from the digestive tract and the role of endogenous nitric oxide (NO) were investigated in mice. Test samples were given orally to fasted mice 30, 120, 180, 240 and 300 min before administration of 0.5 M $MgSO_4$ (10 ml/kg, p.o.). The serum Mg^{2+} levels were determined 30, 60, 120 and 180 min after administration of $MgSO_4$. Escins Ib and IIb (12.5 and 25 mg/kg) significantly increased the serum Mg^{2+} by 10.0–27.3%, 30, 120 and 180 min after administration of the samples, and 30, 60, 120 and 180 min after administration of $MgSO_4$. Escins Ib and IIb (12.5 mg/kg) significantly decreased the Mg^{2+} content in the small intestinal fluid in $MgSO_4$-loaded mice, but did not increase the serum Mg^{2+} levels in normal mice. The effects of escins Ib and IIb (12.5 mg/kg) on serum Mg^{2+} levels were attenuated in a dose-related manner by the pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME, 3–20 mg/kg, i.p., an inhibitor of constitutive and inducible NO synthase), but not with D-NAME (10 mg/kg, i.p., the inactive enantiomer of L-NAME) or dexamethasone (0.05 and 0.5 mg/kg, s.c., an inhibitor of inducible NO synthase). The effect of L-NAME was reversed by L-arginine (600 mg/kg, i.p., a substrate of NO synthase), but not by D-arginine (900 mg/kg, i.p., the enantiomer of L-arginine). These results suggest that escins Ib and IIb enhance Mg^{2+} absorption from the digestive tract in mice, in which the constitutive, but not the inducible, NO synthase plays an important role.

Keywords: Escin Ib; Escin IIb; Mg^{2+} absorption; Nitric oxide (NO)

1. Introduction

Magnesium is an intracellular cation. It is an essential element that catalyzes more than 300 enzymatic reactions involving energy metabolism and protein and nucleic acid synthesis. Mg^{2+} is absorbed uniformly from the small intestine. Despite the ubiquitous nature of Mg^{2+}, low serum Mg^{2+} occurs due to either decreased absorption or increased excretion. Hypomagnesemia is surprisingly common in hospital populations. Low Mg^{2+} concentration may be a factor in a wide variety of clinical conditions (Ahsan, 1997). Magnesium deficiency may cause weakness, tremors, seizures, cardiac arrhythmias, hypokalemia, and hypocalcemia. The causes of hypomagnesemia are reduced intake (poor nutrition or intravenous fluids without Mg^{2+}), reduced absorption (chronic diarrhea, malabsorption, or bypass/resection of bowel), redistribution (exchange transfusion or acute pancreatitis), and increased excretion (medication, alcoholism, diabetes mellitus, renal tubular disorders, hypercalcemia, hyperthyroidism, aldosteronism, stress, or excessive lactation) (Elin, 1988; Garland, 1992). A large segment of the US population may have a chronic latent magnesium deficiency that has been linked to atherosclerosis, myocardial infarction, hypertension, cancer, kidney stones, premenstrual syndrome, and psychiatric disorders (Elin, 1988).

Absorption of water and electrolytes in the small intestine is a very important process in the maintenance of normal digestion (Spiller, 1994). Nitric oxide (NO), a free radical produced from the terminal guanidino nitrogen of L-arginine (Palmer et al., 1988) by the enzyme NO synthase, has a wide variety of physiological and pathophysiological functions (Whittle, 1994). NO has been identified as a nonadrenergic, noncholinergic neurotransmitter causing smooth muscle relaxation, including in many gastrointestinal tract locations (Garthwaite, 1991; Murray et al., 1991). The intestinal epithelium may be exposed to NO

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0014-2999/00/$ - see front matter
PII: S0014-2999(99)00772-4

released from macrophages and mast cells of the lamina propria, enteric nerves, smooth muscle, and endothelium. Therefore, NO may be an important mediator of processes affecting intestinal epithelial function. Endogenous NO seems to be a proabsorptive molecule, based on the findings that NO synthase inhibitors reverse net fluid absorption to net secretion in mice, rats, guinea pigs, rabbits, and dogs (Izzo et al., 1998). It has been shown that NO maintains a proabsorptive tone in the intestine (Barry et al., 1994; Mailman, 1994; Rao et al., 1994; Hällgren et al., 1995; Maher et al., 1995; Schirgi-Degen and Beubler, 1995). However, NO also has been reported to have a prosecretory effect (McNaughton, 1993; Wilson et al., 1993; Gaginella et al., 1994).

We have reported that saponin fraction and its principal saponins, escins Ia, Ib, IIa, and IIb, isolated from the seeds of *Aesculus hippocastanum*, inhibited the increase of serum glucose or ethanol levels in oral glucose or ethanol-loaded rats (Yoshikawa et al., 1994, 1996), reduced the rate of gastric emptying in mice and rats (Matsuda et al., 1998, 1999a), accelerated gastrointestinal transit and prevented experimental ileus in mice (Matsuda et al., 1999b), and prevented ethanol-induced gastric mucosal lesions in rats (Matsuda et al., 1999d). Further studies demonstrated that endogenous NO was involved in both gastroprotection and accelerative effects on gastrointestinal transit (Matsuda et al., 1999c,d). In this study, we investigated the effects of escins Ib and IIb on Mg^{2+} absorption from gastrointestinal tract in $MgSO_4$-loaded mice. The role of endogenous NO is also discussed.

2. Materials and methods

2.1. Chemicals

Escins Ib and IIb (Fig. 1) were isolated from the seeds of European *A. hippocastanum* as in our previous report (Yoshikawa et al., 1994, 1996). N^G-nitro-L-arginine methyl ester (L-NAME), D-NAME, L-arginine and D-arginine were purchased from Sigma (USA). Dexamethasone, magnesium sulfate ($MgSO_4 \cdot 7H_2O$) and other reagents were purchased from Wako (Japan).

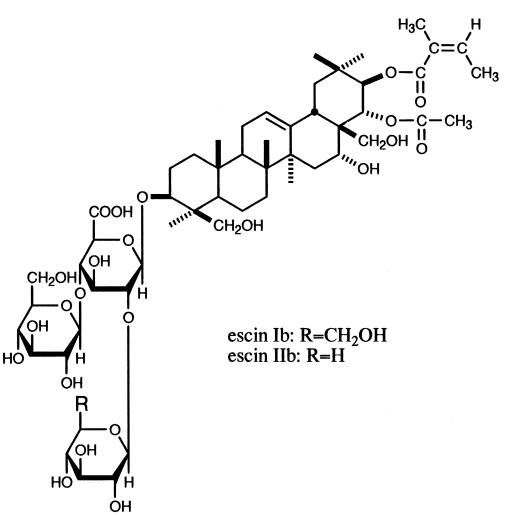

Fig. 1. Chemical structures of escins Ib and IIb.

2.2. Animals

Male ddY mice, weighing 27–30 g, were purchased from Kiwa Laboratory Animal (Japan). The animals were maintained under a constant temperature of 23 ± 2°C and were fed standard laboratory chow (MF, Oriental Yeast, Japan) for a week. The animals were fasted for 18–20 h prior to experiments, but were given water ad libitum. Each test sample was dissolved in phosphate-buffered saline solution, and the solution was administered orally at 10 ml/kg in each experiment, while the vehicle was administered orally at 10 ml/kg to the corresponding control group.

2.3. Measurement of serum Mg^{2+}

Blood samples were collected from the retro-orbital sinus in order to measure serum Mg^{2+}. Serum Mg^{2+} levels were determined by the xylidyl blue method (reagent kit: Magnesium B-test Wako; Wako).

2.4. Mg^{2+} absorption from the gastrointestinal tract in $MgSO_4$-loaded mice

The fasted mice were placed in separate cages for 2 h. The test samples were given orally by means of a metal orogastric tube, and 0.5 M $MgSO_4$ solution (10 ml/kg) was administered orally 30, 120, 180, 240 or 300 min later. Serum Mg^{2+} levels were determined 30, 60, 120 or 180 min after administration of the $MgSO_4$.

2.5. Mg^{2+} measurements of small intestinal fluid in $MgSO_4$-loaded mice

The fasted mice were placed in separate cages for 2 h. The test samples were given orally by means of a metal orogastric tube, and 0.5 M $MgSO_4$ solution (10 ml/kg) was administered orally 30 min later. An hour thereafter, the mice were killed by cervical dislocation. The abdominal cavity was opened, both the conjunctions between the pylorus and the small intestine, and the ceacum and the small intestine were clamped, the small intestine was dissected out, and the intralumen was washed carefully with 2 ml saline. The Mg^{2+} level of the washed intestinal fluid was determined as described above.

2.6. Serum Mg^{2+} levels in normal mice

The fasted mice were placed in separate cages for 2 h. The test samples were given orally. The serum Mg^{2+}

Table 1
Effects of escins Ib and IIb on serum Mg^{2+} in $MgSO_4$-loaded mice

Treatment	Dose (mg/kg, p.o.)	n	Serum Mg^{2+} (mg/dl)	Increase (%)
Normal (not $MgSO_4$-loaded)	–	8	2.9±0.1	–
0.5 M $MgSO_4$-loaded (10 ml/kg, p.o.)				
Control	–	8	3.3±0.1	–
Escin Ib	5	8	3.7±0.2	12.1
	12.5	8	3.9±0.1[a]	18.2
	25	8	4.1±0.2[b]	24.2
Escin IIb	5	8	3.4±0.1	3.0
	12.5	8	4.0±0.2[b]	21.2
	25	8	4.2±0.1[b]	27.3

Each value represents the mean±S.E.M.
[a]Significantly different from the relative control group, $P < 0.05$.
[b]Significantly different from the relative control group, $P < 0.01$.

levels were determined 90 min after administration of the samples.

2.7. Mg^{2+} absorption from the gastrointestinal tract in L-NAME-pretreated and $MgSO_4$-loaded mice

In order to investigate the role of endogenous NO in the effects of escins Ib and IIb on Mg^{2+} absorption, L-NAME (1–20 mg/kg, dissolved in 10 ml saline, i.p.) or saline (10 ml/kg, i.p.) was given 30 min before administration of the samples. The $MgSO_4$ solution was administered orally 30 min after the samples, and serum Mg^{2+} levels were determined 60 min after administration of the $MgSO_4$ solution as described above.

2.8. Mg^{2+} absorption from the gastrointestinal tract in D-NAME-, L-NAME + L-arginine-, or L-NAME + D-arginine-pretreated and $MgSO_4$-loaded mice

In order to confirm the role of L-NAME, D-NAME (10 mg/kg, dissolved in 10 ml saline), L-NAME (10 mg/kg) and L-arginine [600 mg/kg (Mascolo et al., 1993), dissolved in 10 ml saline], or L-NAME (10 mg/kg) and D-arginine [900 mg/kg (Mascolo et al., 1993), dissolved in 10 ml saline] was injected intraperitoneally to the 20-h fasted mice 30 min before administration of the samples. The $MgSO_4$ solution was administered orally 30 min after the samples. Serum Mg^{2+} levels were determined 60 min after administration of the $MgSO_4$ solution.

2.9. Mg^{2+} absorption from the gastrointestinal tract in dexamethasone-pretreated and $MgSO_4$-loaded mice

In order to investigate the involvement of inducible NO synthase in the effects of escins Ib and IIb on Mg^{2+} absorption, dexamethasone (0.05 and 0.5 mg/kg, dissolved in 10 ml saline, s.c.) was administered to the 20-h fasted mice 2 h before the samples. The $MgSO_4$ solution was administered orally 30 min after the samples. Serum Mg^{2+} was determined 60 min after administration of the $MgSO_4$ solution.

2.10. Statistics

Values are expressed as means ± S.E.M. Student's *t*-test or one-way analysis of variance (ANOVA) following Dunnett's test for parametric data was used for statistical analysis. Probability (P) values less than 0.05 were considered significant.

3. Results

3.1. Effects on Mg^{2+} absorption from the gastrointestinal tract in $MgSO_4$-loaded mice

As shown in Table 1, compared to those in normal mice, the serum Mg^{2+} levels in $MgSO_4$-loaded mice tended to be increased. Escins Ib and IIb (5–25 mg/kg, p.o., 30 min before administration of the $MgSO_4$ solution) dose dependently increased the serum Mg^{2+} levels 60 min

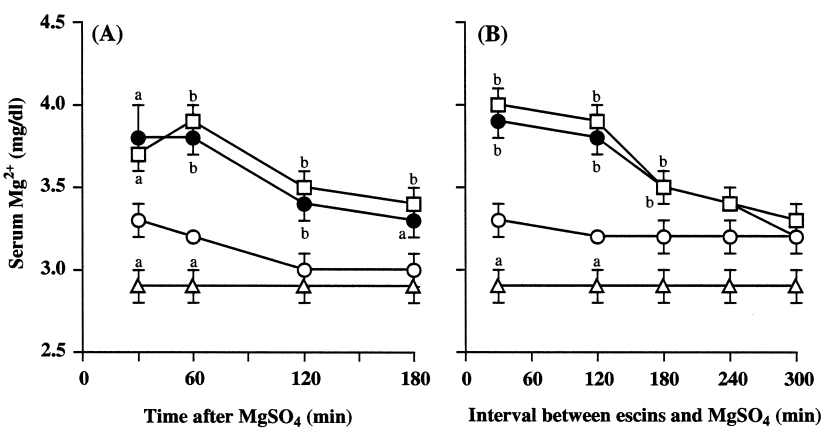

Fig. 2. Effects of escins Ib and IIb on Mg^{2+} absorption in $MgSO_4$-loaded mice at different times. (A) Escins were given orally 30 min before administration of $MgSO_4$. Blood samples were collected at different times (30–180 min) after administration of $MgSO_4$. (B) Escins were given orally at different times (30–300 min) before administration of $MgSO_4$. Blood samples were collected 60 min after administration of $MgSO_4$. △: normal (not $MgSO_4$-loaded), ○: control, ●: escin Ib (12.5 mg/kg, p.o.), □: escin IIb (12.5 mg/kg, p.o.). Each point represents the mean ± S.E.M. ($n = 8$). Significantly different from the control group, [a]$P < 0.05$, [b]$P < 0.01$.

Table 2
Effects of escins Ib and IIb on Mg^{2+} content of small intestinal fluid in $MgSO_4$-loaded mice

Treatment	Dose (mg/kg, p.o.)	n	Mg^{2+} (mg)	Decrease (%)
Control	–	10	1.19±0.05	–
Escin Ib	12.5	8	0.72±0.03[b]	39.5
Escin IIb	12.5	8	0.71±0.03[b]	40.3

Each value represents the mean±S.E.M.
[b]Significantly different from the control group, $P < 0.01$.

after the administration of the $MgSO_4$ solution. As shown in Fig. 2(A), the administration of escins Ib and IIb (12.5 mg/kg) 30 min before the $MgSO_4$ solution significantly increased the serum Mg^{2+} levels 30, 60, 120 and 180 min after the $MgSO_4$ solution. The peak effects of escins Ib and IIb, with 18.8% and 21.9% increases, were obtained 60 min after administration of the $MgSO_4$ solution. As shown in Fig. 2(B), the effects of escins Ib and IIb lasted for 180 min. The maximum effects of escins Ib and IIb, with 18.2% and 21.2% increases, were seen 30 min after administration of the samples, and the effects disappeared 240 min after administration of the samples.

3.2. Effects on Mg^{2+} levels of intraluminal fluid of the small intestine in $MgSO_4$-loaded mice

As shown in Table 2, escins Ib and IIb (12.5 mg/kg, p.o. 30 min before administration of the $MgSO_4$ solution) significantly decreased the Mg^{2+} contents of the washed intraluminal fluid of the small intestine, by 39.5% and 40.3%, respectively, 60 min after administration of the $MgSO_4$ solution.

3.3. Effects on serum Mg^{2+} in normal mice

As shown in Table 3, escins Ib and IIb (25 mg/kg, p.o., 90 min after their administration) did not significantly increase the serum Mg^{2+} levels.

3.4. Effects on Mg^{2+} absorption from the gastrointestinal tract in L-NAME-, D-NAME-, L-NAME + L-arginine-, L-NAME + D-arginine-, or dexamethasone-pretreated and $MgSO_4$-loaded mice

As shown in Table 4, the effects of escins Ib and IIb (12.5 mg/kg) on serum Mg^{2+} levels were attenuated in a dose-related manner by the pretreatment with L-NAME (3–20 mg/kg, i.p.). As shown in Table 5, differently from L-NAME, pretreatment with D-NAME (10 mg/kg, i.p.) or dexamethasone (0.05 and 0.5 mg/kg, s.c.) did not attenuate the effects of escins Ib and IIb (12.5 mg/kg) on the serum Mg^{2+} levels in $MgSO_4$-loaded mice. The attenuation by L-NAME (10 mg/kg) of the effects of escins Ib and IIb was abolished by the simultaneous administration of L-arginine (600 mg/kg, i.p.), but not by that of D-arginine (900 mg/kg, i.p.).

Table 3
Effects of escins Ib and IIb on serum Mg^{2+} in normal mice

Treatment	Dose (mg/kg, p.o.)	n	Serum Mg^{2+} (mg/dl)	Increase (%)
Control	–	12	3.0±0.1	–
Escin Ib	25	8	3.1±0.1	3.3
Escin IIb	25	8	3.1±0.2	3.3

Each value represents the mean±S.E.M. No significant differences were observed with respect to the control group.

Table 4
Effects of escins Ib and IIb on serum Mg^{2+} in saline- or L-NAME-pretreated and $MgSO_4$-loaded mice

Treatment	Dose (mg/kg, p.o.)	n	Serum Mg^{2+} (mg/dl)	Increase (%)
Saline (10 ml / kg, i.p.)-pretreated				
Control	–	8	3.6±0.1	–
Escin Ib	12.5	8	4.4±0.2[b]	22.2
Escin IIb	12.5	8	4.5±0.1[b]	25
L-NAME (1 mg / kg, i.p.)-pretreated				
Control	–	8	3.7±0.2	–
Escin Ib	12.5	8	4.7±0.2[b]	27.0
Escin IIb	12.5	8	4.6±0.1[b]	24.3
L-NAME (3 mg / kg, i.p.)-pretreated				
Control	–	8	3.6±0.1	–
Escin Ib	12.5	8	4.1±0.1[b]	13.9
Escin IIb	12.5	8	4.2±0.1[b]	16.7
L-NAME (5 mg / kg, i.p.)-pretreated				
Control	–	8	3.6±0.1	–
Escin Ib	12.5	8	3.9±0.1	8.3
Escin IIb	12.5	8	3.8±0.1	5.6
L-NAME (10 mg / kg, i.p.)-pretreated				
Control	–	10	3.6±0.1	–
Escin Ib	12.5	10	3.8±0.1	5.6
Escin IIb	12.5	10	3.5±0.3	−2.8
L-NAME (20 mg / kg, i.p.)-pretreated				
Control	–	8	3.7±0.1	–
Escin Ib	12.5	8	3.9±0.1	5.4
Escin IIb	12.5	8	3.8±0.1	2.7

Each value represents the mean±S.E.M.
[b]Significantly different from the control group, $P < 0.01$.

4. Discussion

The present study demonstrated that escins Ib and IIb (12.5 and 25 mg/kg, p.o.) significantly increased the serum Mg^{2+} levels in $MgSO_4$-loaded mice, but did not interfere with the serum Mg^{2+} levels in normal mice. Furthermore, escins Ib and IIb (12.5 mg/kg) significantly decreased the Mg^{2+} levels in the fluid of the small intestine in $MgSO_4$-loaded mice. The results suggest that escins Ib and IIb enhance Mg^{2+} absorption from the gastrointestinal tract in mice.

Table 5
Effects of escins Ib and IIb on serum Mg^{2+} in D-NAME-, L-NAME + L-arginine-, L-NAME + D-arginine-, or dexamethasone-pretreated and $MgSO_4$-loaded mice

Treatment	Dose (mg/kg, p.o.)	*n*	Serum Mg^{2+} (mg/dl)	Increase (%)
D-NAME (10 mg / kg, i.p.)-pretreated				
Control	–	10	3.7 ± 0.1	–
Escin Ib	12.5	10	4.4 ± 0.2^{b}	18.9
Escin IIb	12.5	10	4.4 ± 0.1^{b}	18.9
L-NAME (10 mg / kg, i.p.) + L-Arginine (600 mg / kg, i.p.)-pretreated				
Control	–	10	3.7 ± 0.1	–
Escin Ib	12.5	10	4.3 ± 0.1^{b}	16.2
Escin IIb	12.5	10	4.4 ± 0.2^{b}	18.9
L-NAME (10 mg / kg, i.p.) + D-Arginine (900 mg / kg, i.p.)-pretreated				
Control	–	10	3.8 ± 0.1	–
Escin Ib	12.5	10	4.0 ± 0.1	5.3
Escin IIb	12.5	10	4.1 ± 0.1^{a}	7.9
Dexamethasone (0.05 mg / kg, s.c.)-pretreated				
Control	–	8	3.2 ± 0.1	–
Escin Ib	12.5	8	3.8 ± 0.2^{b}	18.8
Escin IIb	12.5	8	3.7 ± 0.1^{b}	15.6
Dexamethasone (0.5 mg / kg, s.c.)-pretreated				
Control	–	8	3.1 ± 0.1	–
Escin Ib	12.5	8	4.5 ± 0.2^{b}	45.2
Escin IIb	12.5	8	4.4 ± 0.1^{b}	41.9

Each value represents the mean ± S.E.M.
[a]Significantly different from the control group, $P < 0.05$.
[b]Significantly different from the control group, $P < 0.01$.

There are at least three isoforms of NO synthase. One constitutive form resides in the endothelial cells and releases NO over short periods in response to receptor-mediated increases in cellular Ca^{2+} (Lamas et al., 1992). A second constitutive form is responsible for the Ca^{2+}-dependent release from neurons. Many neural tissues, including nerves in the enteric nervous system, can generate NO (Bredt et al., 1990; Brookes, 1993). In some respects, NO has many characteristics of a neurotransmitter in that it is released from nerves and affects functions of effector cells. However, unlike other neurotransmitters, NO is not preformed or stored in presynaptic nerves, and does not produce its effect by acting on postsynaptic membrane receptors, but instead penetrates into the cell, acting directly on guanylate cyclase. The third form of NO synthase (Ca^{2+}-independent) is induced both by bacterial enterotoxins or following intestinal injury (Whittle, 1994). This NO synthase requires a lag period of 2–3 h and, once expressed, synthesizes NO in large amounts and for long periods of time. This latter form is significant in the pathophysiology of diarrhea associated with inflamed mucosa (Whittle, 1994; Miller and Gaginella, 1995). Recently, the inducible NO synthase has been also localized in the ileum of normal mice, suggesting a role for this enzyme in maintaining intestinal homeostasis (Hoffman et al., 1997).

It was reported previously (Matsuda et al., 1999c) that the accelerative effects of escins Ib and IIb on gastrointestinal transit in mice were attenuated markedly by L-NAME, a reversible inhibitor of constitutive and inducible NO synthase (Rees et al., 1990; Izzo et al., 1994), but not by dexamethasone, a glucocorticoid that inhibits the inducible form of NO synthase (Radomski et al., 1990; Moncada et al., 1991). Similarly, in this study, the enhancing effects of escins Ib and IIb on the Mg^{2+} absorption were attenuated by the pretreatment with L-NAME in a dose-related fashion, but with neither D-NAME (the enantiomer of L-NAME) nor dexamethasone. The effect of L-NAME was reversed by L-arginine (a substrate of NO synthase), but not by D-arginine (the enantiomer of L-arginine). These results suggest that the mechanism of NO release, probably through stimulation of the constitutive form of NO synthase, is involved in the effects of escins Ib and IIb on the Mg^{2+} absorption. Furthermore, the accelerative effects of the saponin mixture containing escins Ib and IIb on gastrointestinal transit took place within 30 min, disappeared 5 h after administration of the samples (Matsuda et al., 1999b) and their enhancing effects on Mg^{2+} absorption took place within 90 min, having lasted for only 3 h (in the present study) after administration. These results also support the possibility that constitutive, but not inducible, NO synthase is involved.

In conclusion, the present study demonstrated that escins Ib and IIb enhanced Mg^{2+} absorption from the gastrointestinal tract in mice, in which the constitutive, but not the inducible, NO synthase probably played an important role.

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