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Bioavailability

Enhanced intestinal uptake of iron, zinc and calcium in rats fed pungent spice principles – Piperine, capsaicin and ginger (*Zingiber officinale*)

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ABSTRACT

In view of the wide-spread deficiency of iron and zinc in populations dependent on plant foods, it is desirable to improve the bioavailability of the same. Specific dietary spices may alter the ultrastructure and permeability characteristics of intestines. Groups of Wistar rats were fed piperine, capsaicin and ginger containing diets for 8 weeks in order to examine their possible influence on intestinal absorption of iron, zinc and calcium. Everted segments of duodenum, jejunum and ileum portions of small intestines isolated from these rats were examined for *ex vivo* uptake of iron, zinc and calcium from incubations containing digesta of finger millet. Higher uptake of iron, zinc and calcium by the intestinal segments from spice-fed animals was observed. The increase in the mineral uptake was the highest for calcium with >100% in some cases. The positive influence of dietary capsaicin was more pronounced on zinc uptake as compared to that of iron. Uptake of the glutamic acid standard was 87% and 62% higher in the case of jejunal segments of rats fed piperine and ginger. The higher intestinal uptake of iron and zinc as a result of consumption of pungent spices could encourage a strategy to reduce deficiency of these trace elements prevalent in population dependent on plant based foods.

Introduction

Spices are commonly used in Indian culinary. Specific spices may alter the intestinal ultrastructure and permeability characteristics. Piperine, the active principle of black and long pepper is known to increase bioavailability of drugs and other phytochemicals [1,2]. Potential of piperine to increase the bioavailability of drugs in humans is of great clinical significance. The available data suggest that piperine increases bioavailability of drugs either by promoting rapid absorption from the gastrointestinal tract, or by retarding the drug from being metabolized in the liver or by a combination of these two mechanisms [1].

The effect of piperine, the pungent principle of black pepper on the absorptive function of the intestine has been studied in *in vitro* experiments which showed that piperine (25–100 μM) significantly stimulated γ-glutamyl transpeptidase (γ-GT) activity, enhanced the uptake of radiolabeled amino acids and increased lipid peroxidation in freshly isolated epithelial cells of rat jejunum [3]. It is suggested that piperine may interact with the lipid environment to produce effects leading to increased permeability of the intestinal cells. It is hypothesized that piperine's bioavailability-enhancing property may be particularly attributed to increased absorption, which may be due to alteration in membrane lipid dynamics and change in the conformation of enzymes in the intestine [4]. Results of membrane fluidity studies showed an increase in intestinal brush border membrane fluidity [4]. Piperine modulates the membrane dynamics due to its apolar nature by interacting with surrounding lipids and hydrophobic portions in the protein vicinity, which may decrease the tendency of membrane lipids to act as steric constraints to enzyme proteins and thus modify enzyme conformation. Ultra structural studies with piperine showed an increase in microvilli length with a prominent increase in free ribosomes and ribosomes on the endoplasmic reticulum in enterocytes, suggesting that synthesis or turnover of cytoskeletal components or membrane proteins may be involved in the observed effect [2].

In our previous study, dietary spices – black pepper, red pepper and ginger were evidenced to induce alterations in brush border membrane fluidity and passive permeability property, associated with the induction of an increased microvilli length, resulting in an increased absorptive surface of the small intestine [5]. Also, these dietary spices were shown to stimulate the activities of brush border membrane enzymes – glycyl-glycine dipeptidase, leucine aminopeptidase and γ-glutamyl transpeptidase in the jejunal mucosa, suggesting a modulation in membrane dynamics leading to increased absorption through small intestine.

Minerals are needed by the body as structural components and regulators of chemical reactions and body processes. Among the

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micro minerals, iron and zinc are gaining attention in view of the widespread deficiency of these minerals among the population dependent on plant foods. Iron absorption is influenced by both endogenous factors including the physiological characteristics of the organism and the exogenous factors such as dosage and the ingested form of iron and the dietary matrix [6]. Iron absorption is responsive to mentioned dietary properties, iron stores, erythropoiesis, possible hypoxia, and in the females by pregnancy and lactation. Absorption takes place in the upper small intestine and is controlled by the mucosal cells, mediated by specific receptors on the intestinal mucosal surface [6]. Zinc is absorbed in the small intestine with jejunum being the site for maximum absorption [7]. Zinc absorption is a carrier-mediated transport process which is not saturated under normal physiological conditions. The mechanism of zinc absorption involves two pathways: (1) a saturable carrier mechanism operating most efficiently when luminal zinc concentrations are low, and (2) a passive mechanism involving paracellular movement when zinc intakes and luminal concentrations are high [8]. Approximately 5–10% of dietary iron and 20% of dietary zinc are absorbed. During digestion, free iron and free zinc are complexed with ligands such as amino acids, phosphates and organic acids [9]. Phytates and oxalates may form insoluble complexes that inhibit absorption [10].

It would be most relevant to examine if piperine or its parent spice – black pepper or even other pungent spices such as red pepper (or capsaicin) and ginger have any influence on micronutrient absorption by virtue of alteration in the ultra structure and fluidity of intestinal brush border. Hence in the present study, everted segments of small intestines isolated from rats fed capsaicin, piperine and ginger were examined for the uptake of iron, zinc and calcium present in the incubation medium. Uptake of radio-labeled amino acid was also evaluated in everted intestinal sac suspended in solution containing this radio-labeled amino acid as a 'standard compound' in this *ex vivo* system.

Materials and methods

Spice principles – capsaicin and piperine were obtained from M/s Fluka Chemie (Buchs, Switzerland). Ginger rhizomes (*Zingiber officinale*) were procured from local market, freeze-dried and milled to a fine powder. Finger millet (*Eluisine coracana*) was purchased from local supermarket; and powdered in a grinder with stainless steel blade assembly. Food grade casein was purchased from M/s Nimesh Corporation (Mumbai, India). Corn starch, sugar powder and refined groundnut oil were purchased from local market. Salt mixture (Bernhardt–Tommarelli modified) was purchased from SISCO Research Laboratories (Mumbai, India). L-Glutamic acid U-^{14}C (specific activity: 107.5 mCi/mmol) was obtained from Bhabha Atomic Research Centre (Mumbai, India). Triethanolamine, vitamin E acetate, vitamin A acetate, cholecalciferol, and EDTA were obtained from Himedia Laboratories (Mumbai, India). All other chemicals used were of analytical grade and solvents were distilled before use. Porcine pepsin–pancreatic preparation and bile extract were procured from Sigma Chemicals Co. (St. Louis, MO, USA). Milli Q water (Millipore, Bedford, USA) was employed throughout the study. All glassware used were acid washed by soaking overnight in 10% nitric acid, rinsed five times with distilled water and dried before use. Reagent blanks were taken through the procedure and the iron content measured.

Animal treatment

Animal study was carried out taking appropriate measures to minimize pain/discomfort in accordance with standard guidelines for care and use of experimental animals and with due approval from the Institute's Animal Ethics Committee. Male Wistar rats 6-week old, weighing 65–70 g (12 per group) from Experimental Animal Production Facility of this Institute were housed in individual stainless steel cages and maintained on various experimental diets *ad libitum* for 8 weeks. The animals had free access to water. The basal diet consisted of (%): casein, 21; cane sugar, 10; cornstarch, 54; NRC vitamin mixture, 1; Bernhart-Tommarelli modified NRC salt mixture, 4, fat soluble vitamins at the recommended levels and refined peanut oil, 10. The test spices/spice principles were incorporated into this diet, replacing an equivalent amount of cornstarch to give the various experimental diets containing: piperine (0.02 g%), capsaicin (0.015 g%), and ginger (0.05 g%).

In vitro intestinal absorption studies

At the end of the experimental duration, overnight fasted animals were sacrificed under light ether anesthesia. The small intestine was quickly excised. After thoroughly washing both inside and outside with 0.9% saline, it was everted and cut into segments of uniform length (10 cm). *Ex vivo* uptake of micronutrients – Fe, Zn, Ca, and glutamic acid by these segments of intestine isolated from spice pre-treated animals was evaluated with appropriate incubations described hereunder. After a period of incubation at 37 °C, the amounts of each micronutrient mineral present in the serosal side and in the intestinal tissue were quantitated by following an appropriate extraction and clean-up procedure. Uptake of radio-labeled amino acids was also evaluated in a similar *ex vivo* system consisting of everted intestinal sac suspended in Krebs–Ringers solution containing the radio-labeled amino acid.

Preparation of finger millet digesta [11]

The finger millet powder (10 g) or finger millet powder fortified with iron and zinc salts (ferric stearate and zinc sulfate) (to provide iron or zinc at 5 mg/100 g flour) was mixed with 80 g water in separate 250 mL Erlenmeyer flasks. The pH was adjusted to 2.0 by adding 6 M HCl. The pH was checked after 15 min and readjusted to 2.0. Freshly prepared pepsin solution (3 g) was added and the sample was made up to 100 g with water. (A pepsin digestion mixture was prepared by suspending 16 g pepsin in 100 mL 0.1 M HCl.) After mixing, the sample was incubated at 37 °C in a shaking water bath for 2 h. The water bath settings were 100–120 strokes/min and an arm movement of 2 cm. The pH of this homogenized gastric digest was adjusted to 5.0 by adding sodium hydroxide, 25 g of the pancreatin–bile extract mixture was added and incubated in a shaking water bath for 2 h at 37 °C. (The pancreatin–bile extract mixture contained 4 g pancreatin and 25 g bile extract dissolved in 1 L of 0.1 M sodium bicarbonate.) At the end of the incubation period the pH was measured and set to 7.0. The Erlenmeyer flasks were closed with parafilm in order to reduce CO_2 losses. Portions (20 g) of this digesta were taken as intestinal incubation medium for *in vitro* intestinal uptake studies.

Evaluation of mineral absorption by everted gut sacs

Segments of 10 cm length were cut from each region of the intestine. Duodenum segments were taken from the first 12 cm posterior to the common bile duct, jejunal segments were taken from 15 cm beyond 10 cm of pyloric end and ileal segments from the region immediately anterior to ileo-cecal junction. The intestinal segment was everted over a thin glass rod. Each everted intestinal sac was filled with Krebs–Ringer phosphate buffer of pH 7.4 containing 10 mM glucose. Absorption of the specific mineral was examined by incubating aerobically, the everted rat intestinal segments in the same Krebs–Ringer phosphate buffer – 10 mM glucose medium placed in 50 mL conical flask containing a known amount (10 mL)

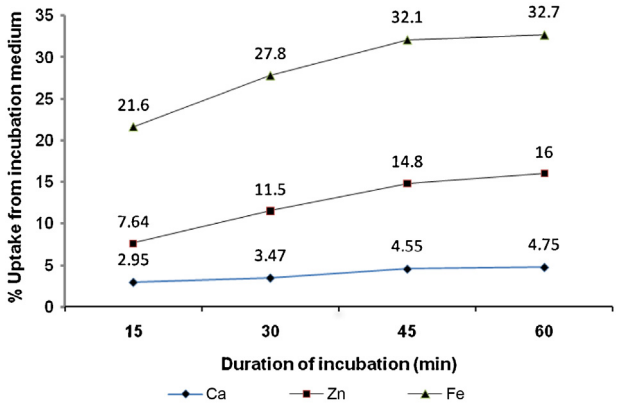

Fig. 1. Uptake of Ca, Zn and Fe from the incubation medium containing digesta of finger millet by the intestinal ileum segments as a function of duration of incubation. Values are mean of 4 replicates containing identical length of intestinal segment.

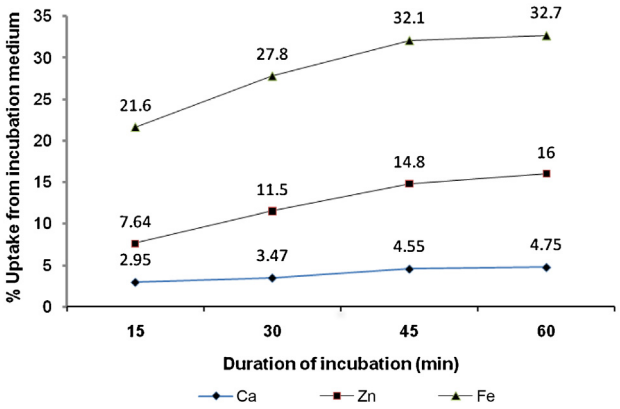

Fig. 2. Uptake of Ca, Zn and Fe from the incubation medium containing digesta of fortified finger millet by the intestinal ileum segments as a function of duration of incubation. Values are mean of 4 replicates containing identical length of intestinal segment.

of mineral source, *viz.*, finger millet digesta (finger millet subjected to simulated gastrointestinal digestion employing pepsin, pancreatin and bile salts according to a standardized procedure described above. The flasks were aerated with 95% oxygen and 5% carbon dioxide mixture and incubated at 37 °C in a shaking water bath (Julabo, Siskin Instruments Co., Bangalore, India) for exactly 30 min (110 strokes/min). At the end of incubation, the sacs were removed, the fluid adhering to the mucosal surface was washed into the medium; the mucosal medium, serosal fluid and the intestinal tissue were collected separately [12]. The intestinal sacs were dried at 65 °C in an oven to a constant weight. Dried sacs were wet ashed for 16 h at 600 °C in a mixture of concentrated nitric, sulfuric and perchloric acids (3:3.1:1, v/v). The mucosal and serosal fluid samples were acidified to 5% nitric acid.

Iron, zinc and calcium contents in the mucosal medium, serosal fluid and intestinal tissue samples were determined by Atomic Absorption Spectrometry (Shimadzu AAF-6701, Kyoto, Japan). Calibration of the mineral measurements was performed using iron and zinc standards and appropriate acid blanks. Iron standards containing 0.1, 0.2, 0.3, 0.6, 0.9, 1.2 and 1.5 pg Fe(II) mL^{-1} in 10 g/L HCl, Zinc standards containing 0.1, 0.2, 0.3, 0.6, 0.9, 1.2 and 1.5 ppm Zn in 15 g/L HNO_3 and calcium standards containing, 0.5, 1.0, 1.5 and 2.0 ppm Ca in 0.25% lanthanum chloride were used for establishing a calibration curve. All measurements were carried out with standard flame operating conditions as recommended by the manufacturer. Amount of mineral absorbed was computed by the values of mineral present in the serosal fluid and the intestinal epithelial tissue.

A pilot study was made for optimization of necessary incubation duration in these determinations. In this pilot study, the incubation of the intestinal segments was carried out for varying time intervals, *viz.*, 15, 30, 45 and 60 min. Based on the results of this pilot study (Figs. 1 and 2), an incubation period of 30 min was fixed in all subsequent experiments. Incubation period for amino acid absorption was also set for 30 min based on a pilot study (Fig. 3).

Evaluation of amino acid absorption by everted gut sacs

The everted segments (10 cm) from jejunum and ileum regions of small intestine were filled with Krebs–Ringer phosphate buffer of pH 7.4 containing 10 mM glucose. Absorption of ^{14}C-glutamic acid was examined by incubating aerobically, the everted rat intestinal segments in the same Krebs–Ringer phosphate buffer containing 10 mM glucose and 10 mM glutamic acid (including ^{14}C-glutamic acid) placed in 50 mL conical flask. The flasks were aerated with 95% oxygen and 5% carbon dioxide mixture and incubated at 37 °C in a shaking water bath (Julabo) for exactly 30 min (110 strokes/min).

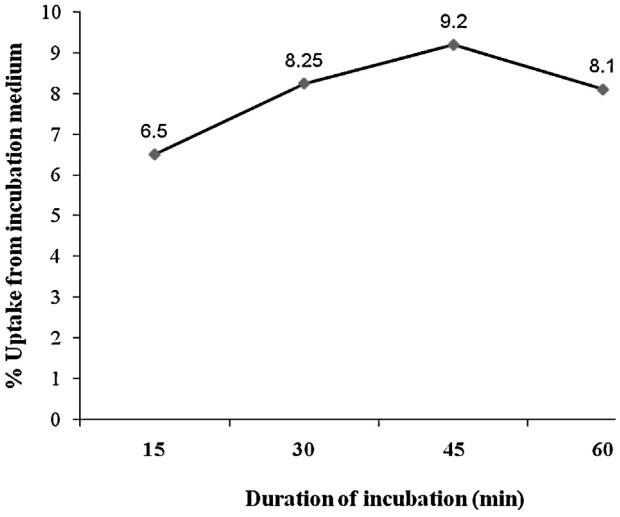

Fig. 3. Uptake of ^{14}C-glutamic acid from the incubation medium by the intestinal ileum segments as a function of duration of incubation. Values are mean of 4 replicates containing identical length of intestinal segment.

At the end of incubation, the sacs were removed, the fluid adhering to the outer mucosal surface was washed into the leftover incubation medium (mucosal fluid); the serosal fluid contained in the sac was carefully collected and the intestinal tissue was also separately collected [9]. Radioactivity in aliquots of the mucosal and serosal fluids were counted in a liquid scintillation counter (Perkin Elmer Tri-carb 2900 TR) using Bray's solution. The intestinal tissue was solubilized in 1 mL of 1N NaOH and radioactivity in aliquots of the same was counted similarly.

Statistical analysis

Results are expressed as mean ± SEM, and at first one-way ANOVA was computed [13]. Comparisons among different groups were made applying Dunnette test. Differences were considered significant when $p < 0.05$.

Results

Finger millet was used as the source of Ca, Zn and Fe for studying the uptake of these minerals by the intestinal segments. The concentrations of these minerals inherently present in this grain (mg/100 g) were: Ca: 325 ± 9.23, Zn: 1.73 ± 0.04, and Fe:

Table 1
Uptake of iron from the digesta of finger millet by everted intestinal segments from rats fed spices as compared with control (without spices' addition).

Diet group	Intestinal uptake of iron			
	μg/g tissue/30 min		Per cent of iron uptake from the incubation medium	
	Jejunum	Ileum	Jejunum	Ileum
Control	27.7 ± 1.14	25.6 ± 1.36	30.9 ± 1.17	31.2 ± 0.73
Capsaicin	28.6 ± 1.61	28.5 ± 0.42*	32.1 ± 0.59	35.3 ± 1.20*
Piperine	31.9 ± 1.70*	32.0 ± 2.83*	34.0 ± 1.22*	34.4 ± 0.56*
Ginger	36.1 ± 1.69*	35.7 ± 3.73*	34.9 ± 0.72*	36.9 ± 1.69*

Values are mean ± SEM of 8 animals per group.
Amount of mineral absorbed was computed by the values of mineral present in the serosal fluid and the intestinal epithelial tissue at the end of incubation, while the amount that remained in the mucosal fluid was the unabsorbed portion.
* Significantly different from control group ($p < 0.05$).

Table 2
Uptake of iron from the digesta of fortified finger millet[a] by everted intestinal segments from rats fed spices as compared with control (without spices' addition).

Diet group	Intestinal uptake of iron					
	μg/g tissue/30 min			Per cent of iron uptake from the incubation medium		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Control	27.4 ± 1.21	25.1 ± 0.84	25.9 ± 1.23	17.8 ± 0.50	18.3 ± 0.39	19.1 ± 0.68
Capsaicin	29.7 ± 1.16	28.1 ± 2.22	28.2 ± 1.16	21.6 ± 0.88*	22.6 ± 1.40*	23.8 ± 0.79*
Piperine	32.7 ± 1.83*	30.1 ± 2.17*	28.2 ± 2.28	24.7 ± 1.97*	26.3 ± 1.30*	25.0 ± 1.14*
Ginger	32.4 ± 1.31*	32.7 ± 2.25*	32.7 ± 2.03*	25.7 ± 2.04*	28.5 ± 2.09*	27.3 ± 2.25*

Values are mean ± SEM of 8 animals per group.
Amount of mineral absorbed was computed by the values of mineral present in the serosal fluid and the intestinal epithelial tissue at the end of incubation, while the amount that remained in the mucosal fluid was the unabsorbed portion.
[a] Finger millet powder fortified with iron and zinc salts to provide iron or zinc at 5 mg/100 g flour.
* Significantly different from control group ($p < 0.05$).

2.13 ± 0.06. In a preliminary trial of incubation conditions for the evaluation of *in vitro* uptake, optimum duration of incubation and ingredients of incubation medium were standardized by using an identical length of intestinal segments and varying concentrations of the source of Fe, Zn and Ca in the incubation medium. Incubation of the everted intestinal segments was carried out in a medium containing finger millet with a known amount of exogenous iron and zinc. Calcium was not exogenously added to this system as finger millet as such is very rich in this mineral. The simulated gastrointestinal procedure of Miller et al. [6] was adopted where a digesta of finger millet (10 g) or finger millet (10 g) fortified with iron and zinc (5 mg/100 g flour) was used in the incubation medium.

Effect of dietary capsaicin, piperine and ginger on the uptake of iron by segments of duodenum, jejunum and ileum portions of small intestine when incubated with digesta of finger millet flour or finger millet flour fortified with iron (5 mg/100 g) is presented in Tables 1 and 2, respectively. Iron uptake from the digesta of either native finger millet or iron fortified finger millet was significantly higher in all the three spice groups. The increase in the uptake of iron by the segments of jejunum where digesta of finger millet was used as source of iron was highest in the case of ginger group, followed by piperine (Table 1). Similarly, the increase in iron uptake by segments of ileum was also highest in ginger group, followed by piperine and capsaicin. The increase in percent absorption of iron in segments of jejunum was also highest in the case of ginger group followed by piperine, whereas for the ileum, ginger group showed highest increase in iron uptake, followed by capsaicin and piperine.

In comparison with the control (without spice addition) the uptake of iron by the duodenal segments from the incubation medium containing digesta of finger millet fortified with iron was similarly higher for piperine, followed by ginger and capsaicin (Table 2). Dietary ginger produced highest increase in iron uptake, followed by piperine and capsaicin in the case of jejunum. Increase in iron uptake by ileum was highest in ginger fed group, while piperine and capsaicin also produced marginal increases. The increase in percent absorption of iron was highest in ginger group (44.4%, 56%, and 43.2% increases, respectively) in duodenum, jejunum and ileum sections followed by piperine and capsaicin. Thus, all the three dietary pungent spices effectively increased the uptake of iron from jejunal, ileal and duodenal segments of small intestine.

There was a significant increase in the uptake of zinc from the incubation medium containing the digesta of finger millet (Table 3) or of finger millet fortified with zinc salt (Table 4) by the small

Table 3
Uptake of zinc from the digesta of finger millet by everted intestinal segments from rats fed spices as compared with control (without spices' addition).

Diet group	Intestinal uptake of zinc			
	μg/g tissue/30 min		Per cent of zinc uptake from the incubation medium	
	Jejunum	Ileum	Jejunum	Ileum
Control	2.35 ± 0.44	2.39 ± 0.26	7.08 ± 1.23	7.16 ± 0.66
Capsaicin	4.81 ± 0.57*	4.36 ± 0.46*	12.3 ± 1.6*	12.5 ± 1.19*
Piperine	3.37 ± 0.42*	2.72 ± 0.13	10.2 ± 1.9*	7.73 ± 0.46
Ginger	3.09 ± 0.20*	3.76 ± 0.32*	9.23 ± 0.77*	10.17 ± 0.76*

Values are mean ± SEM of 8 animals per group.
Amount of mineral absorbed was computed by the values of mineral present in the serosal fluid and the intestinal epithelial tissue at the end of incubation.
* Significantly different from control group ($p < 0.05$).

Table 4
Uptake of zinc from the digesta of fortified finger millet[a] by everted intestinal segments from rats fed spices as compared with control (without spices' addition).

Diet group	Intestinal uptake of zinc					
	μg/g tissue/30 min			Per cent of zinc uptake from the incubation medium		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Control	7.42 ± 0.34	6.91 ± 0.38	7.68 ± 0.22	10.7 ± 0.35	10.3 ± 0.51	10.6 ± 0.46
Capsaicin	8.77 ± 0.67	9.37 ± 0.88*	8.15 ± 0.60	12.9 ± 1.17	13.4 ± 1.03*	11.7 ± 0.64
Piperine	9.21 ± 0.37*	7.85 ± 0.75	9.11 ± 1.28*	12.4 ± 0.50*	11.5 ± 0.96	12.7 ± 1.09*
Ginger	9.12 ± 0.85*	7.19 ± 0.52	7.55 ± 0.64	13.1 ± 1.10*	11.4 ± 0.48	12.5 ± 1.27

Values are mean ± SEM of 8 animals per group.
Amount of mineral absorbed was computed by the values of mineral present in the serosal fluid and the intestinal epithelial tissue at the end of incubation, while the amount that remained in the mucosal fluid was the unabsorbed portion.
[a] Finger millet powder fortified with iron and zinc salts to provide iron or zinc at 5 mg/100 g flour.
* Significantly different from control group ($p < 0.05$).

Table 5
Uptake of calcium from the digesta of finger millet by everted intestinal segments from rats fed spices as compared with control (without spices' addition).

Diet group	Intestinal uptake of calcium			
	μg/g tissue/30 min		Per cent of calcium uptake from the incubation medium	
	Jejunum	Ileum	Jejunum	Ileum
Control	22.1 ± 0.36	22.5 ± 0.29	8.07 ± 0.17	8.36 ± 0.34
Capsaicin	31.6 ± 1.22*	30.6 ± 0.53*	9.72 ± 0.23*	10.2 ± 0.80*
Piperine	46.4 ± 2.35*	42.6 ± 1.43*	16.1 ± 1.15*	14.2 ± 0.73*
Ginger	43.9 ± 1.80*	40.1 ± 6.33*	14.3 ± 1.47*	13.3 ± 1.03*

Values are mean ± SEM of 8 animals per group.
Amount of mineral absorbed was computed by the values of mineral present in the serosal fluid and the intestinal epithelial tissue at the end of incubation, while the amount that remained in the mucosal fluid was the unabsorbed portion.
* Significantly different from control group ($p < 0.05$).

intestinal segments in all the three spice groups. The increase in the uptake of zinc by jejunum and ileum from digesta of finger millet was highest for the capsaicin group (Table 3). The positive influence of dietary capsaicin was more pronounced on zinc uptake as compared to that of iron. The increase in the uptake of zinc by duodenal and ileal segments from the digesta of finger millet fortified with zinc was highest through piperine, while capsaicin led to the highest zinc uptake by jejunal segments (Table 4).

Effect of dietary spices – capsaicin, piperine and ginger – on the uptake of calcium by segments of small intestine when incubated with finger millet or finger millet fortified with minerals are presented in Tables 5 and 6, respectively. There was a significant increase in the uptake of calcium in all the three spice groups. The increase in uptake of calcium by jejunal segments from the digesta of finger millet in the incubation medium was highest in piperine group, followed by ginger and capsaicin when compared to control, without added spices (Table 5). A similar trend was observed in calcium uptake by ileal segments where piperine produced the highest increase, followed by ginger and capsaicin. The uptake of calcium from the digesta of finger millet fortified with minerals by duodenum and jejunum segments was highest in piperine and ginger groups (Table 6). The uptake by the ileum was higher in ginger group followed by piperine and capsaicin. It was also evident that the positive influence of dietary pungent spices on mineral uptake by intestinal segments was much higher for calcium when compared to those of iron and zinc.

Uptake of radio labeled glutamic acid by jejunal segment was significantly higher in rats treated with dietary piperine and ginger as compared with control, without spice addition (Table 7). The positive effect of dietary piperine and ginger on the uptake of this amino acid was to an extent of 87% and 62%, respectively. On the other hand, dietary capsaicin did not influence the uptake of this indicator amino acid by jejunal segments of the intestine. The uptake of the amino acid from ileal segments from spice treated rats was similar to that of control animals.

Dietary spices – capsaicin, piperine and ginger significantly increased the length of small intestine (Fig. 4). Dietary capsaicin decreased the body weight by 18%, followed by piperine (13%) and ginger (11.7%). When expressed on the basis of body weights, the increase in small intestinal length was highest in capsaicin fed

Table 6
Uptake of calcium from the digesta of fortified finger millet[a] by everted intestinal segments from rats fed spices as compared with control (without spices' addition).

Diet group	Intestinal uptake of calcium					
	μg/g tissue/30 min			Per cent of calcium uptake from the incubation medium		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Control	13.3 ± 1.65	13.1 ± 0.28	15.6 ± 0.73	5.42 ± 0.79	5.10 ± 0.65	5.69 ± 0.43
Capsaicin	29.7 ± 1.73*	22.5 ± 1.00*	24.7 ± 1.76*	8.59 ± 0.56*	6.93 ± 0.26*	7.34 ± 0.22*
Piperine	31.6 ± 1.57*	30.3 ± 1.99*	27.4 ± 1.63*	10.7 ± 1.21*	9.26 ± 0.88*	14.0 ± 0.73*
Ginger	31.7 ± 2.90*	30.3 ± 0.82*	30.8 ± 1.25*	11.9 ± 1.06*	14.1 ± 1.33*	12.1 ± 0.82*

Values are mean ± SEM of 8 animals per group.
Amount of mineral absorbed was computed by the values of mineral present in the serosal fluid and the intestinal epithelial tissue at the end of incubation, while the amount that remained in the mucosal fluid was the unabsorbed portion.
[a] Finger millet powder fortified with iron and zinc salts to provide iron or zinc at 5 mg/100 g flour.
* Significantly different from control group ($p < 0.05$).

Table 7
Intestinal uptake of glutamic acid by everted intestinal segments from rats fed spices as compared with control (without spices' addition).

Diet group	Intestinal uptake of glutamic acid			
	mmol/g tissue/30 min		Per cent of glutamic acid uptake from the incubation medium	
	Jejunum	Ileum	Jejunum	Ileum
Control	0.93 ± 0.05	1.50 ± 0.23	14.4 ± 0.94	20.4 ± 3.30
Capsaicin	1.00 ± 0.08	1.33 ± 0.14	15.6 ± 1.02	17.9 ± 0.88
Piperine	1.74 ± 0.15*	1.54 ± 0.24	22.6 ± 1.69*	20.3 ± 2.40
Ginger	1.51 ± 0.17*	1.18 ± 0.21	20.9 ± 1.36*	17.0 ± 1.95

Values are mean ± SEM of 8 animals per group.
Amount of amino acid absorbed was computed by the values of amino acid present in the serosal fluid and the intestinal epithelial tissue at the end of incubation, while the amount that remained in the mucosal fluid was the unabsorbed portion.
* Significantly different from control group ($p < 0.05$).

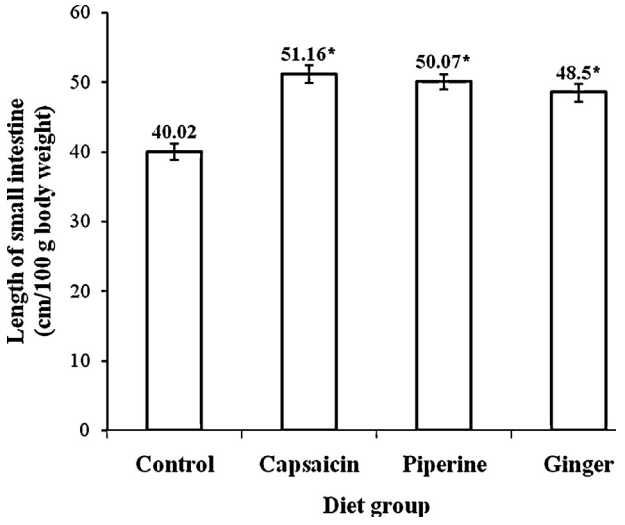

Fig. 4. Length of small intestine in rats fed spices. Values are mean ± SEM of 8 animals per group. *Significantly different from control group ($p < 0.05$).

animals (27.8% higher than the control value) followed by piperine (25.1% increase) and ginger (21.2% increase).

Discussion

Piperine, the active principle of black and long pepper is now established as a bioavailability enhancer of various structurally and therapeutically diverse drugs and other phytochemicals [2]. Potential of piperine to increase the bioavailability of drugs in humans is of great clinical significance. The available data indicate that piperine increases bioavailability of drugs either by promoting rapid absorption from the gastrointestinal tract, or by retarding the drug from being metabolized in the liver or by a combination of these two mechanisms [14].

Dietary spices – black pepper, red pepper, ginger and the spice bioactive compounds piperine and capsaicin which were evaluated in rats for their influence on the membrane fluidity in intestinal brush border membrane (BBM), activity of intestinal enzymes and ultra structural alterations in the intestinal epithelium revealed an increase in BBM fluidity [5] and a stimulation in the activities of transpeptidases in jejunal mucosa. Therefore these pungent spices modulate the membrane dynamics by modifying enzyme conformation. Scanning electron microscopy of the intestinal villi from these spice/spice-agents' fed animals revealed an increased microvilli length which would mean an increased absorptive surface of the small intestine, providing for an increased bioavailability of micronutrients [5]. The current study has additionally evidenced increased length of small intestine in piperine, ginger and capsaicin fed animals. This would mean additional surface area for absorption of nutrients.

Few common spices have been studied earlier for their possible influence on intestinal absorption of β-carotene, wherein its *ex vivo* absorption by the intestinal segments isolated from rats fed black pepper, red pepper, ginger, piperine and capsaicin was examined [12]. Higher uptake of β-carotene in the intestines was evidenced in all these spice-fed animals. Such enhanced intestinal uptake of β-carotene as a result of consumption of pungent spices has been suggested to evolve a food based strategy to possibly reduce vitamin-A deficiency in developing countries.

In the case of calcium, the mineral concentration in intestinal segments was generally higher with incubations containing the finger millet digesta compared to the digesta of finger millet fortified with iron and zinc. This suggests the possibility of competition of the fortified minerals with calcium for the simultaneous uptake. The extent of uptake of iron by the intestinal segments was generally similar with the digesta of finger millet or of finger millet fotified with the same mineral. This suggests that the uptake of iron had reached its capacity even with the amount present inherently in the finger millet. Only in the case of zinc, a higher mineral content in the intestinal segments was observed when there was more mineral in the medium containing digesta of finger millet fortified with zinc.

The uptakes of iron by intestinal segments from the incubation medium containing the digesta of finger millet in this everted gut sac model are generally higher than the bioavailability figures for iron from plant based foods reported in *in vivo* studies [15–17]. Similarly, the uptakes of calcium by intestinal segments from the incubation medium containing the digesta of finger millet are often very low when compared to reported values of calcium bioavailability from plant foods [18,19]. This deviation could be due to the lack of subtle physiological factors controlling the absorption of minerals in this everted gut sac model, unlike the *in situ* situation. Nevertheless this everted gut sac model suffices to make meaningful comparisons between treatments such as the one in this study and the observed beneficial influence of dietary spices on the intestinal uptake of Fe, Zn and Ca is to be considered valid. This statement will also be not restricted by the absence of any effect of dietary capsaicin on the intestinal uptake of glutamic acid which conforms to a similar observation reported by Sambaiah et al. [20] and Finch and Hird [21].

Incidentally, a few common dietary spices including the three pungent spices studied in the present investigation are now understood to have significant beneficial influences on the gastrointestinal system with respect to stimulation of digestive capacity through a positive influence on bile secretion and digestive enzymes contributed by pancreatic juice [22] and improving the antioxidant status of the gastrointestinal tract [23]. The present investigation has added to the understanding of a newer dimension of the nutraceutical potential of these dietary spices, *viz.*, beneficial

influence of these spices on the intestinal absorption of micronutrient minerals.

Thus, the present study on the uptake of micronutrients by the intestinal segments isolated from rats fed piperine, capsaicin and ginger indicated a higher absorption of minerals by the intestines. The positive influence of dietary spices on the mineral uptake by the intestinal segments was highest for calcium. The influence of capsaicin was more pronounced on zinc uptake. The enhanced intestinal uptake of minerals by these spices was also complemented by increased small intestinal length suggesting that these pungent spices alter permeation characteristics presumably by increasing the absorptive surface. Another possibility could be through affecting the intestinal expression level of mineral transporters, which however remains to be verified. Thus, inclusion of spices in the diet could form a strategy to reduce deficiency of these trace elements prevalent in population dependent on plant based foods.

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