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''EFFACT BLACK PEPPER, RED PEPPER, AND GINGER ON THE INTAKE OF B-CAROTENE BY RAT INTESTINES''

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ABSTRACT

given the widespread deficiency of A in populations hooked in to plant foods, it's desirable to enhance the bioavailability of β-carotene. Specific dietary spices may alter the ultrastructure and permeability characteristics of the intestines. Few common spices were studied here for his or her possible influence on intestinal absorption of β-carotene by examining its uptake by the intestines from rats fed black pepper, red pepper, ginger, piperine, and capsaicin. Higher in vitro absorption of β-carotene within the intestines was evidenced altogether spice-fed animals. Dietary piperine and ginger increased the uptake of β-carotene by 147% and 98%, respectively. While the rise in absorption was 59% and 27% in black pepper and red pepper fed animals, respectively, dietary capsaicin increased the identical by 50%. Thus, significantly enhanced intestinal uptake of β-carotene as a results of consumption of pungent spices was evidenced, which could form a food-based strategy to possibly reduce anti ophthalmic factor deficiency.

KEYWORDS

Dietary pungent spicesβ-Carotene Intestinal uptake Micronutrient deficiency

1. INTRODUCTION

The deficiency of antiophthalmic factor may be a serious public ill health resulting in blindness among children in India (WHO, 1998). While animal foods (egg, milk, liver) are good sources of preformed axerophthol, the bulk of the Indian population is however obsessed with plant foods, which give carotenes, especially β-carotene. Several factors like diet composition (fat, fiber, protein) and methods employed for food processing affect the bioaccessibility of β-carotene from foods (Rodriguez & Irwin, 1972). Studies have shown that absorption of carotenoids from uncooked foods is low, which mild

cooking enhances their absorption (Ogulensi and Lee, 1979, Veda et al., 2006).

The presence of dietary factors like food acidulants and antioxidant spice ingredients influences retention and bio accessibility of β-carotene. In an earlier study, it absolutely was revealed that the inclusion of food acidulants (tamarind and citric acid) and antioxidant spices (turmeric and onion) during heat processing of vegetables generally improved the retention of β-carotene (Gayathri et al., 2004, Veda et al., 2008). Because a majority of the Indian population depends on plant foods to satisfy their requirement of antiophthalmic factor, it's desirable to evolve dietary strategies to enhance the bioavailability of β-carotene from these sources. Food acidulants – amchur and lime beneficially enhanced the bio accessibility of β-carotene from green leafy and yellow–orange vegetables (Veda et al., 2008). This improved bio accessibility was evident in both raw and heat-processed vegetables. The presence of the spice turmeric significantly enhanced the bio accessibility of β-carotene from these vegetables, especially when heat-processed, while the presence of onion also enhanced the bio accessibility of β-carotene from pressure-cooked carrot and amaranth leaf and open-plan boiled pumpkin and fenugreek leaves (Veda et al., 2008).

Spices are commonly employed in Indian culinary. Specific spices may alter the intestinal ultrastructure and permeability characteristics. Piperine, the most important alkaloid present in black pepper is thought to extend the bioavailability of medication and other phytochemicals, which can be attributed to increased absorption, resulting from alteration in membrane lipid dynamics and alter within the conformation of enzymes within the intestine (Srinivasan, 2007, Srinivasan, 2009). The lipophilic spice compounds – capsaicin (red pepper), and gingerol, and ginger (phytochemicals of ginger) share a substantial amount of structural homology with piperine. Whether such dietary spices that have the potential to change the ultrastructure and permeability of intestinal brush border beneficially influence the absorption of β-carotene has to be evidenced.

Spices are a gaggle of esoteric food adjuncts that are in use for thousands of years to

reinforce the sensory attributes of foods. the number and style of spices consumed in tropical countries are particularly extensive. These spice ingredients impart characteristic flavors and attractive colors to foods (Srinivasan, 2008). except these sensory qualities, a bunch of beneficial physiological influences also are attributed to spices. Among these, the flexibility to stimulate digestion, beneficial influence on lipid metabolism, efficacy as antidiabetic, antioxidant property, anti-inflammatory, and cancer preventive potential are extensively documented (Srinivasan, 2005).

Evaluation of the effect of specific dietary spices on the absorption of β-carotene particularly by the everted sacs of intestinal segments isolated from experimental rats is that the objective of this study. Dietary black pepper, red pepper, ginger, and their active principles are examined specifically during this investigation for any influence on β-carotene absorption under alteration within the ultrastructure and fluidity of intestinal brush border. Such basic information on the bioavailability of β-carotene is important to optimize dietary approaches to boost the identical, and it also helps in rationalizing the recommended daily allowance for antiophthalmic factor (β-carotene).

2. Materials and methods

2.1. Materials

Fresh carrot (Daucus carota) was procured from an area market (Mysore, India), cleaned, and used as a source of β-carotene during this study. All chemicals used were of analytical grade and also the solvents were distilled before use. The spice bioactive compounds – piperine and capsaicin were procured from M/s Fluka Chemie, Buchs, Switzerland. Standard β-carotene, porcine pancreatic pepsin, and pancreatin and bile extract (porcine) were procured from Sigma Chemicals Co., St. Louis, MO, USA. Casein utilized in the animal diets was procured from M/s. Nimesh Corporation (Mumbai, India). Double-distilled water was employed throughout the whole study. All glassware used was acid washed.

2.2. Animal treatment

Animal experiments were dole out taking appropriate measures to reduce pain or discomfort following the rules laid down regarding the care and use of animals for experimental procedures and with due approval from the Institutional Animal ethics panel (Approval# IAEC-66/05). Young male Wistar rats (8 per group) weighing 80–85 g obtained from the Experimental Animal Production Facility of Central Food Technological Research Institute, Mysore were maintained on specific semi-synthetic diets for 8 weeks. The basal diet comprised of casein, 21%; cane sugar, 10%; corn starch, 54%; refined vegetable oil, 10%; salt mixture (Bernhart & Tommarelli, 1966), 4% and vitamin mixture (National Research Council, 1972), 1%. Spices – black pepper (0.5%), red pepper (3.0%), ginger (0.05%), spice bioactive compounds piperine (0.02%), and capsaicin (0.01%) were included during this basal diet to allow various experimental diets. The animals were housed in individual chrome steel cages and had free access to food and water. The diet consumption and therefore the gain in weight during the experimental regimen in allspice groups were such as controls.

2.3. Food source of β-carotene

The carrot was finely chopped and mashed. it had been subjected to simulated digestion at pH 2.0 within the presence of pepsin at 37 °C (16 g in 100 mL 0.1 M HCl), followed by simulated intestinal digestion within the presence of pancreatin-bile extract mixture (4 g porcine pancreatin and 25 g bile extract in 1000 mL of 0.1 M NaHCO3), pH 7.5 at 37 °C for two h (Veda et al., 2006). The resultant carrot digesta was used as a source of β-carotene within the study of its intestinal uptake.

2.4. In vitro intestinal absorption studies

The rats were stunned with a blow on the pinnacle and after laparotomy, the little intestine was quickly excised. After thoroughly washing both inside and outdoors with 0.9% saline, it absolutely was everted and take segments of uniformly 10 cm long. Uptake of β-carotene in vitro by these segments of intestine isolated from spice pre-treated animals was evaluated (Table 1).

Rat group	Recovery of β-carotene after 3 h of incubation (μg)			
	Mucosal fluid	Serosal fluid	Intestinal epithelium	Percent absorption
Control	22.09 ± 0.52	0.166 ± 0.022	0.256 ± 0.023	1.87
Black pepper	21.30 ± 0.51	0.261 ± 0.032	0.392 ± 0.046	2.97*
Red pepper	22.51 ± 0.47	0.169 ± 0.018	0.379 ± 0.052	2.37*
Ginger	22.03 ± 0.40	0.384 ± 0.043	0.465 ± 0.060	3.71*
Piperine	21.27 ± 0.36	0.332 ± 0.047	0.695 ± 0.097	4.61*
Capsaicin	22.36 ± 0.52	0.332 ± 0.036	0.315 ± 0.032	2.81*

Values are mean ± SEM of eight independent determinations.

Table 1. Uptake of β-carotene from carrot homogenate by everted intestinal segments from rats fed spices.

Rat group Recovery of β-carotene after 3 h of incubation (μg)
Mucosal fluid Serosal fluid Intestinal epithelium Percent absorption
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Black pepper 21.30 ± 0.51 0.261 ± 0.032 0.392 ± 0.046 2.97□
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Ginger 22.03 ± 0.40 0.384 ± 0.043 0.465 ± 0.060 3.71□
Piperine 21.27 ± 0.36 0.332 ± 0.047 0.695 ± 0.097 4.61□
Capsaicin 22.36 ± 0.52 0.332 ± 0.036 0.315 ± 0.032 2.81□
Values are mean ± SEM of eight independent determinations.

Denotes significantly higher compared to the Control group ($p < 0.05$).

Each intestinal everted sac was stuffed with Krebs–Ringer phosphate buffer containing 10 mM glucose. Absorption of β-carotene was examined by incubating aerobically, the

everted rat intestinal segments within the same Krebs–Ringer phosphate buffer – 10 mM glucose medium (10 mL) placed in 25 mL conical flask containing a known amount of β-carotene source (carrot digesta containing 24 µg β-carotene) that was subjected to simulated gastrointestinal digestion employing pepsin, pancreatin, and bile salts in step with the same procedure described above. The flasks were aerated with 95% oxygen and 5% carbonic acid gas mixture and incubated at 37 °C in a very Julabo shaking water bath for 3 h (110 strokes/min). At the top of incubation, the sacs were removed, the mucosal surface was washed and serosal contents were collected (Suresh & Srinivasan, 2007). The mucosal medium, serosal fluid, and intestinal tissue were extracted for β-carotene employing an appropriate procedure (described below) and therefore the extracts were analyzed for β-carotene by an appropriate HPLC procedure (described below). the quantity of β-carotene absorbed was computed by the values of the β-carotene present within the serosal and mucosal side and therefore the intestinal epithelium.

2.5. ANALYSIS OF B-CAROTENE

β-Carotene within the aqueous serosal and mucosal medium samples after incubation was extracted initially with a combination of acetone/ethanol (1:1, v/v) and subsequently with petroleum ether (Hedren, Mulkozi, & Svanberg, 2002). the method was repeated several times to make sure complete extraction of β-carotene. The extracts were pooled and therefore the solvent was evaporated to dryness in an exceedingly rotary evaporator. The residue was re-dissolved in petroleum ether and stored within the cold pending analysis. Before analysis, petroleum ether was evaporated under nitrogen and therefore the residue was re-dissolved within the mobile phase used for HPLC determination. β-Carotene within the intestinal tissue samples was extracted in step with the tactic of Mercado, Holmgren, Fox, and Russel (1989). The intestinal tissue was homogenized in 10 mL chloroform/methanol (2:1, v/v) in a very tissue homogenizer fitted with a Teflon pestle. The homogenate was mixed with 2 mL 0.9% saline so vortexed. The blend was allowed to settle and separate into two layers and centrifuged at 2500g for 10 min. the underside chloroform layer was separated and evaporated to dryness under a

stream of nitrogen gas. The residue was re-dissolved within the mobile phase used for the HPLC determination of β-carotene.

Determination of β-carotene was allotted by reverse-phase HPLC (Model: Shimadzu LC 10 AVP; Shimadzu Corporation, Kyoto, Japan), equipped with a photodiode array (PDA) detector. β-Carotene was separated on a C18 column (SS Exil, Dandenong, Australia). The mobile phase consisted of a combination (v/v) of 65% acetonitrile, 15% chloride, and 20% methanol containing 1.3 mmol/L ammonium acetate. β-Carotene was monitored at a wavelength of 450 nm using β-apo-8′-carotenal as an enclosed standard. the height identities and λmax were confirmed by their retention time (14 min 30 s) and characteristic spectra of ordinary chromatograms.

During the steps of incubation, and extraction of β-carotene, precautions were taken to attenuate the exposure of samples to light and air and thus prevent the oxidative breakdown of β-carotene. Air was replaced by nitrogen before stoppering the flask in the slightest degree stages of incubation and storage. All operations were disbursed under yellow lighting and glassware was covered with black cloth to forestall exposure to light.

2.6. STATISTICAL ANALYSIS

All determinations were made in eight replicates and also the results were expressed as mean ± standard error of the mean. Data were analyzed statistically and comparisons between groups were made using an unpaired Student's t-test (Snedecor & Cochran, 1976). Differences were considered significant at $p < 0.05$

3. RESULTS AND DISCUSSION

The amount of carrot digesta to be included within the incubation medium as a source of β-carotene was optimized in an exceedingly trial study using the identical length of intestinal segments and ranging the concentration of the source of β-carotene. An amount of digesta such as 0.08 g dry carrot present within the incubation medium provided maximum intestinal uptake of β-carotene under the experimental conditions of duration of incubation and therefore the length of rat intestinal segment (Fig. 1). The die-

tary levels of piperine and capsaicin employed in this study roughly correspond to the dietary level of their respective parent spices – black pepper and red pepper, respectively employed in this animal study.

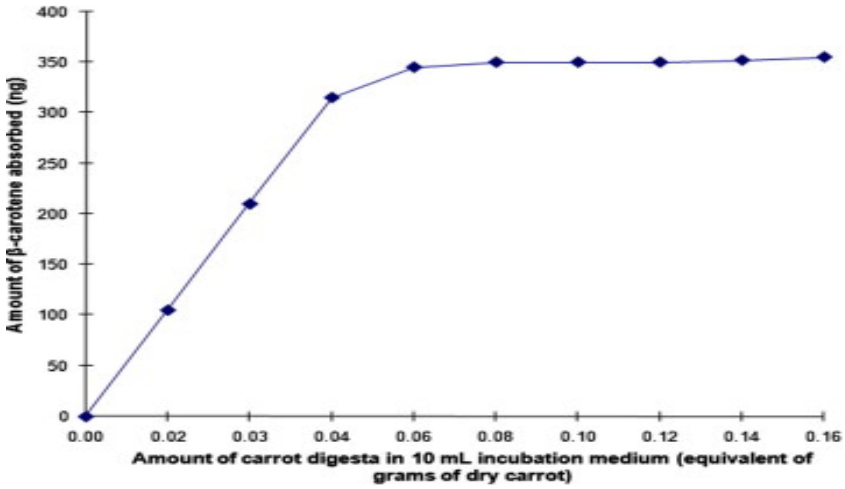

Fig. 1. Standardization of absorption of β-carotene by everted intestinal sac from the carrot digesta.

A significantly increased uptake of β-carotene from carrot homogenate by intestinal segments from the spice-fed animals was generally evidenced (Table 1). Among the test spices, dietary piperine produced the best increase in β-carotene absorption and was 247% of the control value. Whereas dietary ginger increased the intestinal uptake of β-carotene by 98%, dietary black pepper, capsaicin, and red pepper brought approximately a rise of 59%, 50%, and 27%, respectively. Thus, both black pepper and its bioactive constituent – piperine are evidenced here to push β-carotene absorption within the intestine (Fig. 2). the opposite pungent spice – red pepper and its pungent constituent – capsaicin were also effective in promoting intestinal β-carotene absorption, but to a relatively lesser degree. Ginger enhanced β-carotene uptake by the intestines, quite either black pepper or red pepper (Fig. 2).

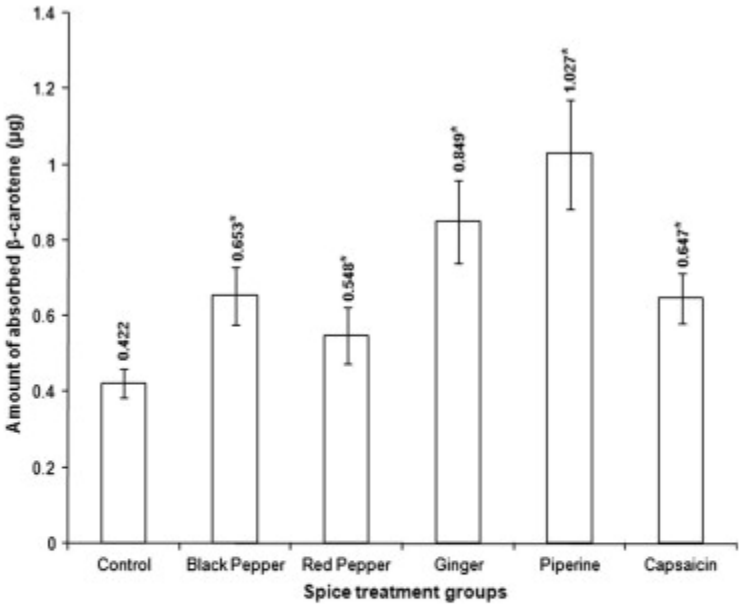

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Fig. 2. *In vitro* absorption of β-carotene by intestinal segments of spice fed rats. Values are mean ± SEM of eight independent determinations. *Significantly higher compared to Control group.

Piperine, the bioactive constituent of black pepper is established as a bioavailability enhancer of assorted structurally and therapeutically diverse drugs and other substances (Srinivasan, 2007). The potential of piperine or its parent spice – black pepper to extend the bioavailability of medicine in humans is of great pharmacological value. These constituents of the indigenous Ayurvedic system of medication are understood to extend the bioavailability of medicine either by promoting rapid absorption from the alimentary tract, or by protecting the drug from being metabolized in its first passage through the liver after being absorbed, or by a mixture of those two mechanisms (Atal, Zutshi, & Rao, 1981).

In vitro studies on the effect of piperine on the absorptive function of the intestine using freshly isolated epithelial cells of rat jejunum showed that piperine (25–100 µM) significantly stimulated γ-glutamyl transpeptidase activity and also the uptake of amino acids

(Johri, Thusu, Khajuria, & Zutshi, 1992). it's hypothesized that piperine's bioavailability-enhancing property is also attributed to increased absorption, which can result to alteration in membrane lipid dynamics and alter within the conformation of enzymes within the intestine (Khajuria, Thusu, & Zutshi, 2002). Results of membrane fluidity studies using an apolar fluorescent probe, pyrene (which measures the fluid properties of hydrocarbon core), showed a rise in intestinal brush border membrane fluidity. Ultrastructural studies with piperine showed a rise 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 is also involved within the observed effect (Khajuria et al., 2002). Thus, it's suggested that piperine may induce alterations in membrane dynamics and permeation characteristics, together with induction of the synthesis of proteins related to cytoskeletal function, increasing the tiny intestine absorptive surface, thus assisting efficient permeation through the epithelial barrier.

Dietary spices – black pepper, red pepper, ginger, and spice bioactive compounds – piperine and capsaicin which were evaluated in rats for his or her influence on the membrane fluidity in intestinal brush border membrane (BBM), the activity of intestinal enzymes passionate about the interaction with the lipid microenvironment of membrane and ultrastructural alterations within the intestinal epithelium revealed a rise in BBM fluidity in spice-fed animals (unpublished data). These dietary spices were also shown to stimulate the activities of glycyl-glycine dipeptidase, leucine aminopeptidase, and γ-glutamyl transpeptidase in jejunal mucosa, which suggest that these pungent spices modulate the membrane dynamics to switch enzyme conformation. Scanning microscopy observation of the intestinal villi from these spice/spice principles fed animals revealed alteration within the ultrastructure, especially a rise in microvilli length which might mean a beneficial increase within the absorptive surface of the little intestine, providing for increased bioavailability of micronutrients (unpublished data).

Thus, this study on the uptake of β-carotene by the intestinal segments isolated from

rats fed black pepper, red pepper, ginger, piperine, and capsaicin indicated higher absorption of β-carotene within the intestines of those spice-fed animals. This effect was highest within the case of dietary piperine followed by ginger and capsaicin. These pungent spices alter permeation characteristics presumably by increasing the absorptive surface and thereby enhance intestinal absorption of β-carotene, which could form a technique to cut back axerophthol deficiency. Such an influence of dietary pungent spices needs further in-depth investigation, concerning in vivo absorption of micronutrients. Such promising basic information is probably going to assist evolve diet-based strategies to combat fat-soluble vitamin deficiency diseases.

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