EFFECT OF CAPSAICIN AND ETHANOL FEEDING ON GROWTH AND DISACCHARIDASE ACTIVITY IN RATS

การออกฤทธิ์ของแคพไซซินและเอทานอลต่อการเจริญเติบโตและเอนไซม์ไดแซ็กคาริเดสในหนูพุกขาว

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ABSTRACT

Long term feeding of the rats with capsaicin, and ethanol was studied for 3 and 6 months. During the first two weeks, the growth rate of the rats fed with both ethanol and 42 mg% capsaicin was quite less than that of other groups as well as the food consumption which was the direct effect of ethanol. Food consumption of this group was increased from 5.86 to 14.3 g/100 g BW in the first two weeks. After that, food consumption in all groups was quite similar to one another. It was indicated that the rats could adapt to higher level of capsaicin and ethanol. In physiological study of small intestine, 14 or 42 mg% capsaicin decreased maltase activity and maltose digestion but increased sucrase activity and sucrose digestion in the jejunum of the rats. However, both capsaicin and ethanol stimulated glucose absorption in the jejunal everted sacs of the rats so the amount of glucose administration was not changes. Stimulation of glucose absorption might be due to intestinal cell proliferation, after which intestinal cells were damaged by capsaicin and ethanol.

บทคัดย่อ

แคพไซซิน (สารเผ็ดในพริก) และเอทานอลมีผลต่อการเจริญเติบโต การดูดซึมของกลูโคส และการทำงานของเอนไซม์ในลำไส้เล็กของหนูพุกขาวเพศผู้เมื่อให้แคพไซซิน และเอทานอลควบคู่กันไปเป็นเวลา 3 และ 6 เดือน ในช่วง 2 สัปดาห์แรก หนูกลุ่มที่ให้เอทานอลและแคพไซซิน 42 มก.% ในช่วง 2 สัปดาห์แรก มีการเจริญเติบโตและการกินอาหารน้อยกว่าหนูกลุ่มอื่น ๆ ซึ่งอาจเป็นผลมาจากเอทานอล หลังจาก 2 สัปดาห์แรกแล้ว พบว่าการกินอาหารของหนูกลุ่มนี้จะเพิ่มขึ้นอย่างมากคือจาก 5.86 เป็น 14.3 ก./น้ำหนักหนู 100 ก. หลังจากนั้นการกินอาหารของหนูทุกกลุ่มจะเท่ากัน แสดงว่าหนูกลุ่มที่ให้แคพไซซินและเอทานอลสามารถทนต่อระดับที่เพิ่มมากขึ้นของแคพไซซินและเอทานอลได้ การศึกษาในลำไส้เล็กพบว่า แคพไซซินปริมาณ 14 หรือ 42 มก.% ในอาหารสามารถที่จะลดการทำงานของเอนไซม์มอลเตสมีผลทำให้การย่อยของน้ำตาลมอลโตสลดลง แต่กลับไปเพิ่มการทำงานของเอนไซม์ซูเครส ทำให้มีการย่อยของน้ำตาลซูโครสในลำไส้เล็กของหนูเพิ่มมากขึ้น เป็นผลให้ปริมาณน้ำตาลกลูโคสที่ถูกดูดซึมได้ไม่เปลี่ยนแปลง และการที่แคพไซซินและเอทานอลสามารถกระตุ้นการดูดซึมของน้ำตาลกลูโคสได้นั้น อาจเนื่องมาจากการเพิ่มมากขึ้นของจำนวนเซลล์ดูดซึมในลำไส้เล็ก หลังจากเซลล์ดูดซึมบางส่วนถูกทำลายโดยแคพไซซินและเอทานอล

INTRODUCTION

Capsaicin is the pungent material in capsicum fruits which is one of the most common food ingredient used daily in many tropical countries. The average capsaicin in diet consumed by Thai people is approximately 14 mg%. An experiment in rats fed with capsaicin in six different diets for 28 and 56 days, normal diet, high-protein and low-protein diets, with or without capsaicin, were similar in protein and carbohydrate content to those consumed by the people of the central, southern and northeastern regions of Thailand, respectively.[17] It showed that there was a reduction in absorptive ability of the epithelial absorptive cells influenced by capsaicin. The remained of absorptive cells from capsaicin injury could absorb added amount of protein in the diet which was adequate for the normal growth. The increase in the number of goblet cells in all capsaicin groups represented a protective mechanism by producing excess mucous. Feeding of red pepper (5%) and synthetic capsaicin (15 mg%) in rats for 12 months was investigated.[21] Red pepper (5%) increased in food intake, weight gain over the control. Synthetic capsaicin (15 mg%) lowered significantly the food intake, weight gain at 4 weeks. Long term feeding of red pepper (5%) and synthetic capsaicin (15 mg%) did not affect growth rate or cause mortality.

It was interesting to clarify whether capsaicin at the average level in diet of

Thai people (14 mg%) and higher level (42 mg%) which is 3 times as much as the average level diet can affect the gastrointestinal tract and induce the adaptation in the animals. Accumulating evidences suggested toxicity of ethanol on gastrointestinal tract. The additive effects of capsaicin and ethanol was another attractive field. Thai people concurrently acclimatize to consume pungent food and ethanol. It was of interest to investigate whether toxicity of capsaicin could be modified by ethanol during prolong administration.

MATERIALS AND METHODS

Synthetic capsaicin and absolute ethanol were obtained from E. Merck Co. (Germany). Glucose peroxidase-oxidase enzyme and other chemicals were purchased from Sigma Chemical Co. (USA).

Male weanling Wistar rats (50-60 g) obtained from the National Animal Center, Mahidol University. All animals were housed individually in stainless steel hanging cages and were kept in a room with temperature of $28 \pm 2°C$ and humidity of 65%, and were provided with commercial rat chow and water *ad libitum.*

Experimental protocols

Rats were randomly selected and divided into 5 groups of 10 rats each. These groups of rats were given with the following diet. Group I, rats were fed with control diet. Group II, control diet supplemented with 14 mg% capsaicin. Group III, control diet supplemented with 42 mg% capsaicin. Group IV, control diet and 40% ethanol (4 g/kg BW) i.g., 3 times per week. Group V, control diet supplemented with 42 mg% capsaicin and 40% ethanol (4 g/kg BW) i.g., 3 times per week.

All animals were fed with the diet and water *ad libitum.* Body weight, food and capsaicin consumption of the rats were recorded 2 times per week for 6 months. At the end of 3 and 6 months, each rat from all groups was anesthetized with ether. The abdominal wall was opened. Whole length of small intestine was removed and washed with 0.9% NaCl. The small intestine was blotted, weighed and measured the total length. The everted sacs of the upper parts of small intestine were prepared by a method of Wilson and Wiseman for glucose absorption.[22] The lower part of small intestine was taken to determine nucleic acid, disaccharidase enzyme activity and protein.

Determination of disaccharide digestion

The lower part of small intestine (about 8 cm) was removed and everted. One sac was prepared for maltose and the other for sucrose digestion. The sacs were incubated in maltose or sucrose in Krebs-Henseleit-HCO_3^- buffer which was added only in mucosal side. In the serosal side, only Krebs-Henseleit-HCO_3^- buffer was added. At the end of incubation period, the mucosal and serosal contents were collected, recorded their volume and analysed for glucose concentration by oxidase method.[19] The remained mucosal maltose or sucrose was determined by carbazole method.[6]

Determination of glucose absorption

The upper part of small intestine (about 3 cm) was removed and everted. Glucose in Krebs-Henseleit-HCO_3^- buffer was incubated in both mucosal and serosal sides. At the end of incubation period, the mucosal and serosal fluid were collected, recorded their volume and analysed for glucose concentration by oxidase method.[19]

Biochemical composition of small intestine

Intestinal disaccharidase enzyme activity of mucosal homogenate was determined by the method of Dahlqvist.[4,5] DNA from mucosal content was determined by the method of Giles and Meyers.[7] RNA from mucosal content[8] was determined by the method of Colowick and Kaplan.[3] Protein from mucosal content was determined by the method of Lowry et al.[13]

RESULTS

Effect of capsaicin and ethanol on the growth rate of the rats body weight

Body weight of the rats fed with capsaicin and ethanol for 6 months is shown in Figure 1. In the first few days of the experiment, the body weight of the rats fed with control diet, 14 mg% capsaicin, 42 mg% capsaicin and only ethanol were not different from one another except the body weights of the rats fed with both ethanol and 42 mg% capsaicin was less than the others. At the third week, it was obvious that in rats fed with both ethanol and 42 mg% capsaicin, the body weight (138.86 ± 8.58 g) was less than the other (160-180 g). It remained in this manner until the fourth week. After that (in the sixth to the tenth week) the body weight of the rats fed with only ethanol and both ethanol and 42 mg% capsaicin was not significant different but they were significantly different from the other 3 groups ($P < 0.05$). In the eleventh week, the body weight of the rats fed with both ethanol and 42 mg% capsaicin (283.17±10.13 g) was less than that of only ethanol (331.99±14.09 g, $P < 0.001$). In the seventeenth week, the body weight of the rats fed with both ethanol and 42 mg% capsaicin (345.20±9.43 g) was less than that of only ethanol (373.66± 11.47 g, $P < 0.01$). It remained in this condition until 6 months. The body weight of the rats fed with control diet,14mg% capsaicin and 42 mg% capsaicin was not significantly different.

Food consumption.

Food consumption of the rats fed with capsaicin and ethanol for 6 months is shown in Figure 2. In the first few days of the experiment, the rats fed with both ethanol and 42 mg% capsaicin (5.86 g/100 g BW) consumed food less than the other 4 groups (12.5-15.0 g/100 g BW). In the third week, rats fed with both ethanol and 42 mg% capsaicin consumed food more than the other groups. It was found that food consumption in rats was gradually decreased whereas the body weight was gradually increased. After the fifth week, rats fed with both ethanol and 42 mg% capsaicin still consumed more food than the others but these values were not significantly different. It remained in this manner until the end of experiment.

Capsaicin consumption

Capsaicin consumption of the rats fed with capsaicin and ethanol for 6 months is shown in Figure 3. In the first few days of the experiment, capsaicin consumption of the rats fed with both ethanol and 42 mg% capsaicin (2.46 ± 0.92 mg/100 g BW) was less than that of 42 mg% capsaicin (6.29 ± 0.81 mg/100 g BW) because the former consumed food less than the latter. Until the second week, capsaicin consumption of the rats in both groups was about equal because they consumed similar amount of food. It remained in this manner until the end of the experiment. Capsaicin consumption of the rats fed with 14 mg% capsaicin was less than that of both ethanol and 42 mg% capsaicin and only 42 mg% capsaicin because the former group consumed quite similar amount of food to the latter 2 groups. In conclusion, the relative food consumption of all animals was gradually decreased as the body weight increased and so did capsaicin consumption.

Effect of capsaicin and ethanol on glucose absorption

Glucose absorption of the rats fed with capsaicin and ethanol for 3 months is summarized in Table 1. There was no significant difference in glucose absorption in all groups.

Table 2 summarizes glucose absorption of the rats fed with capsaicin and ethanol for 6 months. Glucose absorption of the rats fed with 42 mg% capsaicin (319.7 ± 22.5 μmol/g wet wt/60 min, $P < 0.01$) and only ethanol (236.0 ± 26.8 μmol/g wet wt/60 min, $P < 0.01$) was higher than that of control diet (146.2 ± 17.7 μmol/g wet wt/60 min). Glucose absorption of the rats fed with both ethanol and 42 mg% capsaicin (186.9 ± 26.6 μmol/g wet wt/60 min) was lower than that of the rats fed with only ethanol and 42 mg% capsaicin. Glucose absorption of rats fed with both ethanol and 42 mg% capsaicin was higher than that of control diet but again these values were not significantly different.

Effect of capsaicin and ethanol on disaccharide digestion

Disaccharide digestion of the rats fed with capsaicin and ethanol for 3 months is summarized in Table 3. There was no significant difference in disaccharide digestion in all groups.

Table 4 summarizes disaccharide digestion of the rats fed with capsaicin and ethanol for 6 months. Maltose digestion of the rats fed with 14 mg% capsaicin (35.6 ± 2.2 μmol/g wet wt/60 min, $P < 0.001$) or 42 mg% capsaicin (45.6 ± 3.9 μmol/g wet wt/60 min, $P < 0.01$) or ethanol (50.2 ± 7.5 μmol/g wet wt/60 min, $P < 0.01$) was lower than that of control diet (70.4 ± 4.0 μmol/g wet wt/60 min). In rats fed with 42 mg% capsaicin, maltose digestion was lower than that of both ethanol and 42 mg% capsaicin (63.7 ± 5.3 μmol/g wet wt/60 min, $P < 0.01$) but higher than that of 14 mg% capsaicin ($P < 0.05$).

Sucrose digestion of the rats fed with 14 mg% capsaicin (91.8 ± 11.9 μmol/g wet wt/60 min, $P < 0.01$) or 42 mg% capsaicin (74.6 ± 11.3 μmol/g wet wt/60 min, $P < 0.05$) was higher than that of control diet (51.2 ± 3.0 μmol/g wet wt/60 min). In rats fed with ethanol (34.8 ± 4.4 μmol/g wet wt/60 min, $P < 0.01$) and both ethanol and 42 mg% capsaicin (44.3 ± 0.8 μmol/g wet wt/60 min, $P < 0.01$) was lower than that of control diet. Sucrose digestion of the rats fed with both ethanol and 42 mg% capsaicin was higher than that of ethanol ($P < 0.05$) but lower than that of 42 mg% capsaicin ($P < 0.01$).

Final mucosal maltose concentration of the rats fed with 14 mg% capsaicin (261.8 ± 0.7 mg%, $P < 0.001$) or 42 mg% capsaicin (214.8 ± 2.6 mg%, $P < 0.01$) or ethanol (208.4 ± 8.2 mg%, $P < 0.01$) was higher than that of control diet (181.8 ± 11.4 mg%). However, in rats fed with 42 mg% capsaicin, final mucosal maltose concentration was less than that of 14 mg% capsaicin ($P < 0.001$) but higher than that of both ethanol and 42 mg% capsaicin (199.7 ± 3.9 mg%, $P < 0.01$).

In case of sucrose, final mucosal sucrose concentration of the rats fed with 14 mg% capsaicin (162.8 ± 4.6 mg%, $P < 0.01$) or 42 mg% capsaicin (158.5 ± 23.8 mg%, $P < 0.05$) was less than that of control diet (209.5 ± 2.7 mg%). In rats fed with ethanol, final mucosal sucrose concentration (238.7 ± 6.3 mg%, $P < 0.01$) or both ethanol and 42 mg% capsaicin (220.7 ± 3.8 mg%, $P < 0.01$) was higher than that of control diet. However, final mucosal sucrose concentration of the rats fed with both ethanol and 42 mg% capsaicin was less than that of ethanol ($P < 0.01$) but higher than that of 42 mg% capsaicin ($P < 0.01$).

Effect of capsaicin and ethanol on biochemical compositions of small intestine

Biochemical compositions of small intestine in the rat fed with capsaicin and ethanol for 3 months are shown in Table 5. Small intestinal length of the rats fed with both ethanol and 42 mg% capsaicin (98.4 ± 1.8 cm) was less than that of control diet (107.8 ± 1.8 cm, $P < 0.01$), ethanol (104.6 ± 1.6 cm, $P < 0.01$) and 42 mg% capsaicin (108.6 ± 2.2 cm, $P < 0.01$). There was no statistically difference in maltase and sucrase activities in all groups. The amount of intestinal DNA of the rats fed with both ethanol and 42 mg% capsaicin (12.6 ± 0.9 mg/g mucosa) was higher than that of 42 mg% capsaicin (10.3 ± 0.2 mg/g mucosa, $P < 0.01$). There was no significant difference in the amount of RNA and protein in all groups.

After 6 months (Table 6), there was no significant difference in small intestinal length of the rats in all groups. Maltase activity of the rats fed with 14 mg% capsaicin

(149.3 ± 9.7 unit/g protein, $P < 0.001$) or 42 mg% capsaicin (173.8 ± 8.3 unit/g protein, $P < 0.01$) or ethanol (182.0 ± 8.1 unit/g protein, $P < 0.01$) was lower than that of control diet (206.6 ± 4.1 unit/g protein). In rats fed with 42 mg% capsaicin, maltase activity was higher than 14 mg% capsaicin ($P < 0.05$) whereas lower than that of both ethanol and 42 mg% capsaicin (195.3 ± 8.0 unit/g protein, $P < 0.05$). Sucrase activity of the rats fed with 14 mg% capsaicin (72.1 ± 3.9 unit/g protein, $P < 0.01$) and 42 mg% capsaicin (67.5 ± 2.5 unit/g protein, $P < 0.05$) was higher than that of control diet (59.1 ± 3.8 unit/g protein). In rats fed with both ethanol and 42 mg% capsaicin, sucrase activity (51.1 ± 1.5 unit/g protein) was lower than that of control diet ($P < 0.05$) and 42 mg% capsaicin ($P < 0.001$). However, sucrase activity of the rats fed with ethanol (46.9 ± 2.7 unit/g protein) was lower than that of control diet ($P < 0.01$) and both ethanol and 42 mg% capsaicin ($P < 0.05$).

The amount of intestinal DNA of the rats fed with 42 mg% capsaicin (13.3 ± 1.1 mg/g mucosa, $P < 0.01$) or ethanol (12.7 ± 1.1 mg/g mucosa, $P < 0.01$) or both ethanol and 42 mg% capsaicin (13.4 ± 0.9 mg/g mucosa, $P < 0.01$) was higher than that of control diet (9.4 ± 0.8 mg/g mucosa) whereas the amount of DNA of the rats fed with 42 mg% capsaicin was also higher than that of 14 mg% capsaicin (9.7 ± 0.7 mg/g mucosa, $P < 0.01$). Intestinal RNA content of the rats fed with 14 mg% capsaicin (8.3 ± 0.3 mg/g mucosa, $P < 0.01$) or 42 mg% capsaicin (8.5 ± 0.2 mg/g mucosa, $P < 0.01$) was less than that of control diet (10.5 ± 0.7 mg/g mucosa). In rats fed with both ethanol and 42 mg% capsaicin, RNA content (9.3 ± 0.4 mg/g mucosa) was higher than that of 42 mg% capsaicin ($P < 0.05$). Intestinal protein content in rats fed with ethanol (146.5 ± 13.1 mg/g mucosa) was higher than that of control diet (113.6 ± 0.1 mg/g mucosa, $P < 0.01$) and both ethanol and 42 mg% capsaicin (108.8 ± 2.4 mg/g mucosa, $P < 0.01$). In rats fed with 14 mg% capsaicin, intestinal protein content (128.6 ± 4.6 mg/g mucosa) was higher than that of control diet ($P < 0.01$) whereas in rats fed with both ethanol and 42 mg% capsaicin was lower than that of control diet ($P < 0.05$).

DISCUSSION

Long term effects of capsaicin was previously reported in the rats by Nopanitaya.[17] It was found that the growth rate of male Sprague-Dawley rats fed with 14 mg% capsaicin was slightly increased than that fed with control diet during the first 2 weeks. However, their growth rate was decreased in a longer period of capsaicin feeding. It was obvious that the rats fed with 14 mg% capsaicin diet was not significantly different in case of body weight from that of control diet within 56 days. In the experiment, the growth rate of male Wistar rats fed with 14 mg% capsaicin diet was similar to those of control diet after feeding for 3 and 6 months. Even higher level of capsaicin (42 mg%) in diet could not significantly influence the growth rate of the rats when compared to those fed with 14 mg% capsaicin and control diet. Recently, Srinivasan[21] reported the effect of 5.0%

red pepper and 15 mg% capsaicin on the growth rate and food consumption in male Wistar rat fed with these diets for 1 year. It was observed that red pepper diet caused an increase in food intake and weight gain that would reverse to normal later, whereas 15 mg% capsaicin diet could slightly reduce food intake and weight gain at 4 weeks after feeding and persist only 8 weeks. It suggested that long term feeding of red pepper and synthetic capsaicin (14 or 15 mg%) should not affect growth rate or mortality. This suggestion in supported by the results showing that 42 mg% capsaicin did not significantly reduce the growth rate or induce any lethality during 6 months of feeding. In addition, the growth rate and mortality of rabbits fed with red pepper 5.0 g/kg BW daily) for 12 months were not affected even there was some histopathologic changes in several organs including liver.[11,12]

In the ethanol experiment, the results indicated that the body weight gain of the rats given 4 g/kg BW ethanol (i.g.) was less than that in the control diet, 14 mg% capsaicin and 42 mg% capsaicin. Ethanol exerted inhibitory action on the growth rate of these rats. This finding was similar to those reported by Khanna et al.[10] in that chronic ethanol administration to male Wistar rats in a liquid diet for 4 weeks with the daily intake of ethanol range from 10-12 g/kg BW throughout the experimental period. Ariyoshi[1] gave 2.0% ethanol solution as drinking water in the rats for 20 days. It was found that the body weight gain of the ethanol-treated rats was inhibited by ethanol in this period. It seems that feeding pattern of ethanol to the rats may influence the body weight gain.

Food consumption of the rats fed with ethanol and 42 mg% capsaicin was markedly decreased when compared to those of control diet, 14 mg% capsaicin, 42 mg% capsaicin, and ethanol during the first week of the experiment. In the second week, however, food consumption was gradually increased from 6 g/100 g BW to about 14.5 g/100 g BW. With the reduction of food consumption in the first week would initiate the lower body weight of the rats in this group. Although, the rats could consume a larger amount of food in the following weeks. After the sixth week, food consumption in all groups was quite similar to one another. These findings suggest that 42 mg% capsaicin and both ethanol and 42 mg% capsaicin have no influence on food consumption and body weight gain of the rats during the later period of treatment, except both ethanol and 42 mg% capsaicin decreased food intake during the first 2 weeks of experiment. It is therefore established that the rats in the present study were accustomed to consume high level of capsaicin (42 mg%) with ethanol.

As the result in reduction of food intake during the first few days of the experiment, capsaicin consumption of the rats fed with ethanol and 42 mg% capsaicin was ultimately lower than the others. The reduction of food intake and capsaicin consumption of the rats in this group is rather due to direct action of ethanol since the rats fed with 42 mg% capsaicin can normally take this diet even at the first day of serving. It is different from experience in the children that could take only a very limited amount of red pepper in the food because of its pungent and pain sensation. However, they were accustomed to consume an increase amount of capsaicin with time.

In addition to the reduction of food intake that initiated the decrease in body weight of rats fed with ethanol or both ethanol and 42 mg% capsaicin, inhibition of digestion and/or absorption of the nutrients such as starch, sugar and glucose may also potentiate the decrease in the growth rate of the rats. In this study, it was shown that capsaicin (14 and 42 mg%) and also ethanol caused a decrease in maltose digestion as resulted from a reduction of maltase activity in the small intestine of the rats fed with those diets for 6 months. Capsaicin was recently reported that no changes in disaccharidase activity in the rats administered i.g. for 7 days.[9] However, absorption of the digestion product of maltose, glucose was stimulated. From these results, it is probably that no net change in glucose supply to the rats from dietary source. Furthermore, it is of interest to observe that ethanol has a modified effect on restoration of maltose digestion, while decrease in glucose absorption when fed with 42 mg% capsaicin. Capsaicin (14 and 42 mg%) and ethanol caused an increase in sucrose digestion as resulted from an increase in sucrase activity which was contrast to those observed by Rodgers and O'Brian[20] in which chronic feeding caused no changes in lactase and sucrase activity of rat jejunal mucosa. It seems that glucose supply from the source of sucrose to the rats should be greater than those of maltose. But this may not be the case as sucrose composition of our regular mouse pellet is much less than maltose. It may be possible to conclude that reciprocal effects of capsaicin and ethanol between maltose and sucrose digestion, and maltase and sucrase activity should not further decrease the growth rate of the rats.

With regard to the stimulating effect of capsaicin on glucose absorption, this finding was found to be opposite to the *in vitro* studies using everted sacs incubated in 14 mg% capsaicin in Krebs-Henseleit HCO_3^- buffer.[14] In that case, 14 mg% capsaicin was persisted through out the experiment of 60 min which was equivalent to acute exposure within shorter time. This acute effect would inhibit glucose absorption by decrease ATP production,[2,15] inhibition of $Na^+ - K^+ -$ATPase[16] and disorganization of microvillar membrane.[18] In the present study, however a smaller quantity of capsaicin was slowly ingested and also diluted by intestinal content which should not suddenly reach 14 mg% in a very short time of exposure. With low concentration of capsaicin, it might stimulate absorption of glucose as previously observed.[14] At present, the mechanism of biphasis response of intestinal absorption to capsaicin on glucose absorption is not yet known.

An increase in glucose absorption may be partly explained by an increase in total number of the intestinal absorptive cells as indicated by an increased in DNA content in the jejunal mucosa. An increase in number of intestinal absorptive cells may possibly be a result from cell injury by capsaicin and ethanol to certain cells, but not all cells of the jejunum[17] with a consequent of regeneration. This is a preposition that is difficult to get a support from microscopic examination in which no significantly histopathologic alterations was observed in the cells and length of the villi, with the exception of an increase in the total number of goblet cells. Therefore, the increased number of goblet cells may be partly attributed to an increase in DNA and protein (mucoprotein) content in the jejunal mucosa.

The results showed that the reduction of growth rate of the rats fed with ethanol alone and ethanol with high level of capsaicin resulted from a lower food consumption as unfamiliar with the pungent taste and pain sensation of capsaicin and toxicity of ethanol.

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Table 1. Glucose absorption in everted sacs of rat jejunum fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW, i.g.) for 3 months incubated in Krebs-Henseleit-HCO_3^- buffer (pH 7.4) containing 7.5 mM glucose at 37°C for 60 min.[a]

Treatment	Final Glucose conc. (mg%)			Glucose absorption (μ mol/g wet wt/60 min)
	Mucosa	Serosa	S/M ratio	
Control	93.3 ± 4.5	106.3 ± 17.4	1.14	195.7 ± 14.8
Capsaicin (14 mg%)	106.9 ± 1.8	128.3 ± 6.3	1.20	217.8 ± 21.7
Capsaicin (42 mg%)	107.1 ± 3.4	115.6 ± 15.8	1.08	247.7 ± 24.1
Ethanol (4 g/kg BW)	96.6 ± 4.5	122.8 ± 13.8	1.27	230.3 ± 27.0
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	109.6 ± 3.1	112.6 ± 31.5	1.03	222.6 ± 19.5

[a] Values are mean ± S.E.M. in 8-10 animals per group

Table 2. Glucose absorption in everted sacs of rat jejunum fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW, i.g.) for 6 months incubated in Krebs-Henseleit-HCO_3^- buffer (pH 7.4) containing 7.5 mM glucose at 37°C for 60 min.[a]

Treatment	Final glucose conc. (mg%)			Glucose absorption
	Mucosa	Serosa	S/M ratio	(μ mol/g wet wt/60 min)
Control	116.9 ± 2.2	132.5 ± 20.5	1.13	146.2 ± 17.7
Capsaicin (14 mg%)	109.3 ± 4.2	207.1 ± 20.3	1.89	167.1 ± 34.8
Capsaicin (42 mg%)	101.8 ± 3.1	246.0 ± 33.0	2.42	319.7 ± 22.5**
Ethanol (4 g/kg BW)	102.3 ± 3.2	234.9 ± 17.3	2.30	236.0 ± 26.8**
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	99.7 ± 5.3	223.6 ± 65.3	2.24	186.9 ± 26.6

[a] Values are mean ± S.E.M. in 8-10 animals

* Significant values (Student's t-test) are derived from comparison of the capsaicin (14 mg%), capsaicin (42 mg%), ethanol (4 g/kg BW) or capsaicin (42 mg%) and ethanol (4 g/kg BW) and control groups:

** $P < 0.01$

Table 3. Maltose and sucrose digestion by everted sacs of rat jejunum fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW, i.g.) for 3 months incubated in 7.5 mM maltose or sucrose in Krebs – Henseleit – HCO_3^- buffer (pH 7.4) at 37° C for 60 min.[a]

Treatment	Mucosal disaccharide	
	Final concentration (mg%)	Digestion (μ mol/g wet wt/60 min)
Maltose		
Control	250.0 ± 9.6	52.9 ± 8.4
Capsaicin (14 mg%)	245.4 ± 11.4	46.5 ± 6.0
Capsaicin (42 mg%)	255.4 ± 5.9	47.0 ± 10.9
Ethanol (4 g/kg BW)	240.3 ± 20.1	50.4 ± 4.3
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	227.6 ± 14.5	48.1 ± 14.7
Sucrose		
Control	195.9 ± 10.7	30.4 ± 1.9
Capsaicin (14 mg%)	184.9 ± 5.2	33.6 ± 2.1
Capsaicin (42 mg%)	190.8 ± 6.6	28.8 ± 4.3
Ethanol (4 g/kg BW)	200.2 ± 8.1	31.4 ± 4.5
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	201.0 ± 9.2	32.0 ± 2.9

[a] Values are mean ± S.E.M. in 8-10 animals

Table 4. Maltose and sucrose digestion by everted sacs of rat jejunum fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW, i.g.) for 6 months incubated in 7.5 mM maltose or sucrose in Krebs-Henseleit-HCO_3^- buffer (pH 7.4) at 37°C for 60 min.[a]

Treatment	Mucosal disaccharide	
	Final concentration (mg%)	Digestion (μ mol/g wet wt/60 min)
Maltose		
Control	181.8 ± 11.4	70.4 ± 4.0
Capsaicin (14 mg%)	261.8 ± 0.7***	35.6 ± 2.2***
Capsaicin (42 mg%)	214.8 ± 2.6***●●●	45.6 ± 3.9**●
Ethanol (4 g/kg BW)	208.4 ± 8.2**	50.2 ± 7.5**
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	199.7 ± 3.9$^{\pi\pi}$	63.7 ± 5.3$^{\pi\pi}$
Sucrose		
Control	209.5 ± 2.7	51.2 ± 3.0
Capsaicin (14 mg%)	162.8 ± 4.6***	91.8 ± 11.9**
Capsaicin (42 mg%)	158.5 ± 23.8*	74.6 ± 11.3*
Ethanol (4 g/kg WB)	238.7 ± 6.3	34.8 ± 4.4
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	220.7 ± 3.8**$^{\pi\pi}$‡	44.3 ± 0.8**$^{\pi\pi}$†

[a] Values are mean ± S.E.M. in 8-10 animals

* Significant values (Student's t-test) are derived from comparison of the capsaicin (14 mg%), capsaicin (42 mg%), ethanol (4 g/kg BW) or capsaicin (42 mg%) and ethanol (4 g/kg BW), and control groups: *P < 0.05; **P < 0.01; ***P < 0.001

† Significant values (Student's t-test) are derived from comparison of the capsaicin (42 mg%) and ethanol (4 g/kg BW), and ethanol (4 g/kg BW) groups : † P < 0.05; ‡ P < 0.01

● Significant values (Student's t-test) are derived from comparison of the capsaicin (14 mg%) and capsaicin (42 mg%) groups : ● P < 0.05; ●●● P < 0.001

$^{\pi}$ Significant values (Student's t-test) are derived from comparison of the capsaicin (42 mg%), and capsaicin (42 mg%) and ethanol (4 g/kg BW) groups: $^{\pi\pi}$ P < 0.01

Table 5. Protein and nucleic acid content, and disaccharidase activity of the small intestine in male rats fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW, i.g.) for 3 months.[a]

Treatment	Small intestine weight (g/100 g BW)	Small intestine length (cm)	Biochemical content of small intestine			Disaccharidase activity	
			Protein (mg/g mucosa)	DNA (mg/g mucosa)	RNA (mg/g mucosa)	Maltase (unit/g protein)	Sucrase (unit/g protein)
Control	2.21 ± 0.05	107.75 ± 1.79	126.27 ± 9.41	12.01 ± 1.39	7.44 ± 0.46	159.42 ± 13.26	67.28 ± 4.69
Capsaicin (14 mg%)	2.23 ± 0.05	110.17 ± 1.71	129.24 ± 2.94	10.72 ± 0.80	7.92 ± 0.16	139.20 ± 7.89	77.31 ± 4.11
Capsaicin (42 mg%)	2.21 ± 0.04	108.56 ± 2.24	133.3 ± 5.08	10.27 ± 0.23	8.04 ± 0.67	135.61 ± 5.64	75.38 ± 3.47
Ethanol (4 g/kg BW)	2.58 ± 0.21	104.57 ± 1.62	135.26 ± 6.84	11.95 ± 0.91	8.30 ± 0.50	135.28 ± 3.53	63.02 ± 4.84
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	2.16 ± 0.10†	98.42 ± 1.76$^{**\ddagger\pi\pi}$	142.04 ± 7.07	12.60 ± 0.91$^{\pi\pi}$	7.35 ± 0.45	136.47 ± 10.67	71.83 ± 4.56

[a] Values are mean ± S.E.M. in 8-10 animals

† Significant values (Student's t-test) are derived from comparison of the capsaicin (42 mg%) and ethanol (4 g/kg BW), and ethanol (4 g/kg BW) group : † P < 0.05; ‡ P < 0.01

π Significant values (Student's t-test) are derived from comparison of the capsaicin (42 mg %), and capsaicin (42 mg%) and ethanol (4 g/kg BW) groups : $\pi\pi$ P < 0.01

* Significant values (Student's t-test) are derived from comparison of the capsaicin (14 mg%), capsaicin (42 mg%), ethanol (4 g/kg BW) or capsaicin (42 mg%) and ethanol (4 g/kg BW) and control groups : ** P < 0.01

Table 6. Protein and nucleic acid content, and disaccharidase activity of the small intestine in male rats fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW, i.g.) for 6 months.[a]

Treatment	Small intestine weight (g/100 g BW)	Small intestine length (cm)	Biochemical content of small intestine			Disaccharidase activity	
			Protein (mg/g mucosa)	DNA (mg/g mucosa)	RNA (mg/g mucosa)	Maltase (unit/g protein)	Sucrase (unit/g protein)
Control	1.74 ± 0.05	102.00 ± 2.58	113.59 ± 0.06	9.43 ± 0.84	10.51 ± 0.72	206.61 ± 4.05	59.11 ± 3.75
Capsaicin (14 mg%)	1.78 ± 0.06	103.00 ± 2.12	128.57 ± 4.61**	9.70 ± 0.66	8.32 ± 0.31**	149.27 ± 9.71**	72.11 ± 3.97**
Capsaicin (42 mg%)	1.80 ± 0.11	105.29 ± 2.82	123.95 ± 8.87	13.30 ± 1.05••**	8.48 ± 0.21**	173.76 ± 8.31•**	67.54 ± 2.53*
Ethanol (4 g/kg BW)	1.84 ± 0.05	101.86 ± 1.77	146.48 ± 13.14**	12.71 ± 1.11**	8.88 ± 0.56	182.00 ± 8.09**	46.92 ± 2.66**
Capsaicin (42 mg%) + Ethanol (4 g/kg BW)	1.96 ± 0.04†**	100.71 ± 1.29	108.75 ± 2.36*‡	13.41 ± 0.90**	9.33 ± 0.41$^{\pi}$	195.31 ± 8.03$^{\pi}$	51.09 ± 1.49*†$\pi\pi\pi$

[a] Value are mean ± S.E.M. in 8-10 animals

† Significant values (Student's t-test) are derived from comparison of the capsaicin (42 mg%) and ethanol (4 g/kg BW), and ethanol (4 g/kg BW) group : †$P < 0.05$; ‡ $P < 0.01$.

• Significant values (Student's t-test) are derived from comparison of the capsaicin (14 mg%) and capsaicin (42 mg%) groups : • $P < 0.05$; •• $P < 0.01$

* Significant values (Student's t-test) are derived from comparison of the capsaicin (14 mg%), capsaicin (42 mg%), ethanol (4 g/kg BW) or capsaicin (42 mg%) and ethanol (4 g/kg BW), and control groups : *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

π Significant values (Student's t-test) are derived from comparison of the capsaicin (42 mg%), and capsaicin (42 mg%) and ethanol (4 g/kg BW) groups : $^{\pi}$ $P < 0.05$; $\pi\pi\pi$ $P < 0.001$

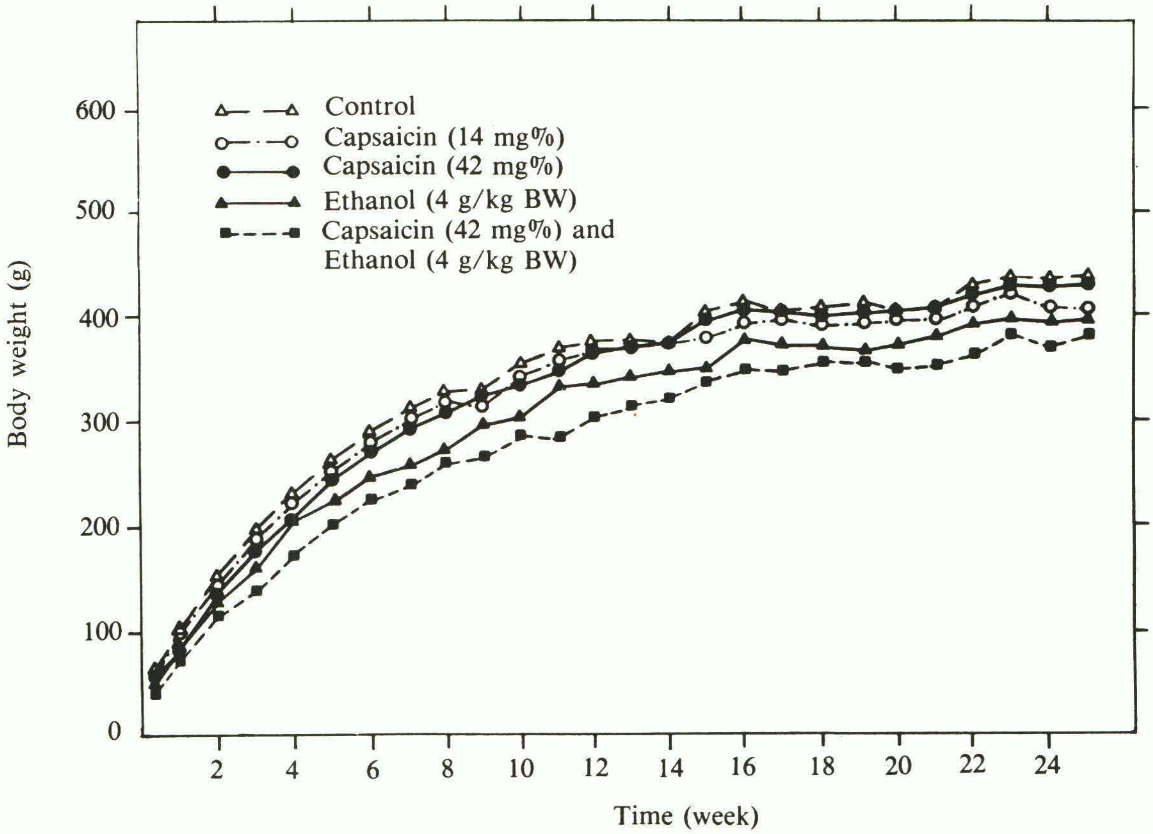

Fig. 1 Growth rate of male rats fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW) for 6 months.

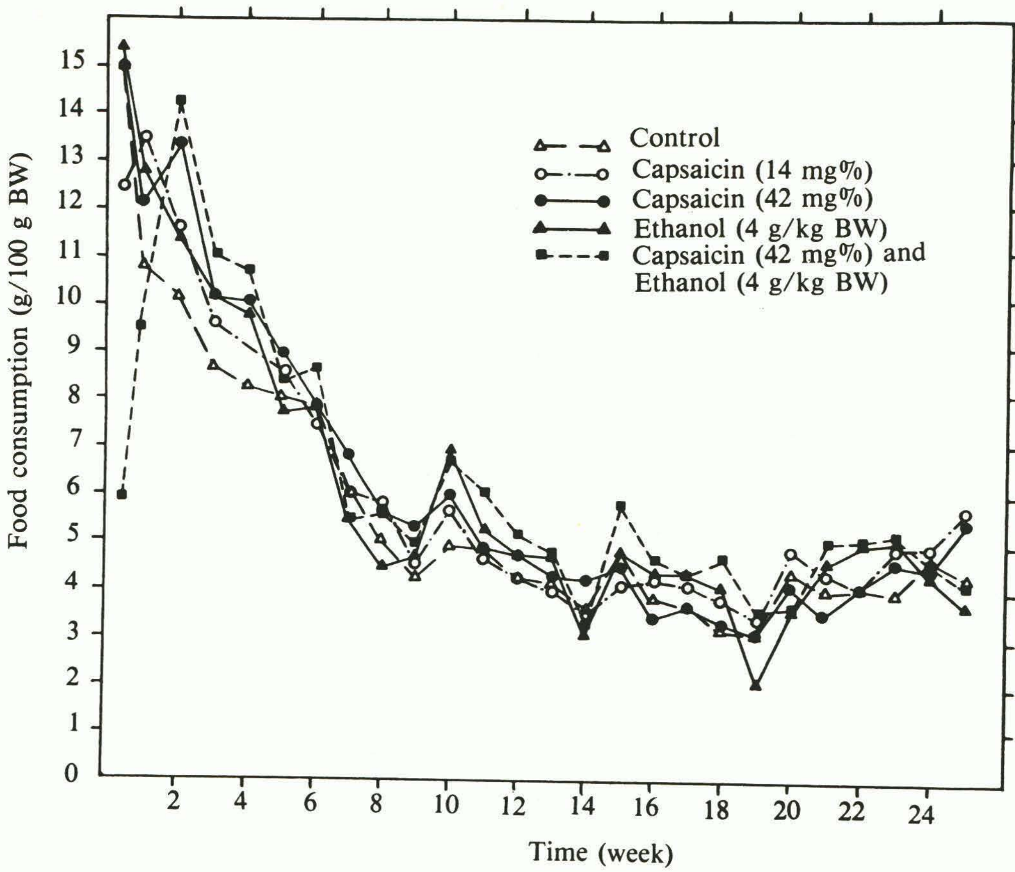

Fig. 2 Food consumption of male rats fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW) for 6 months.

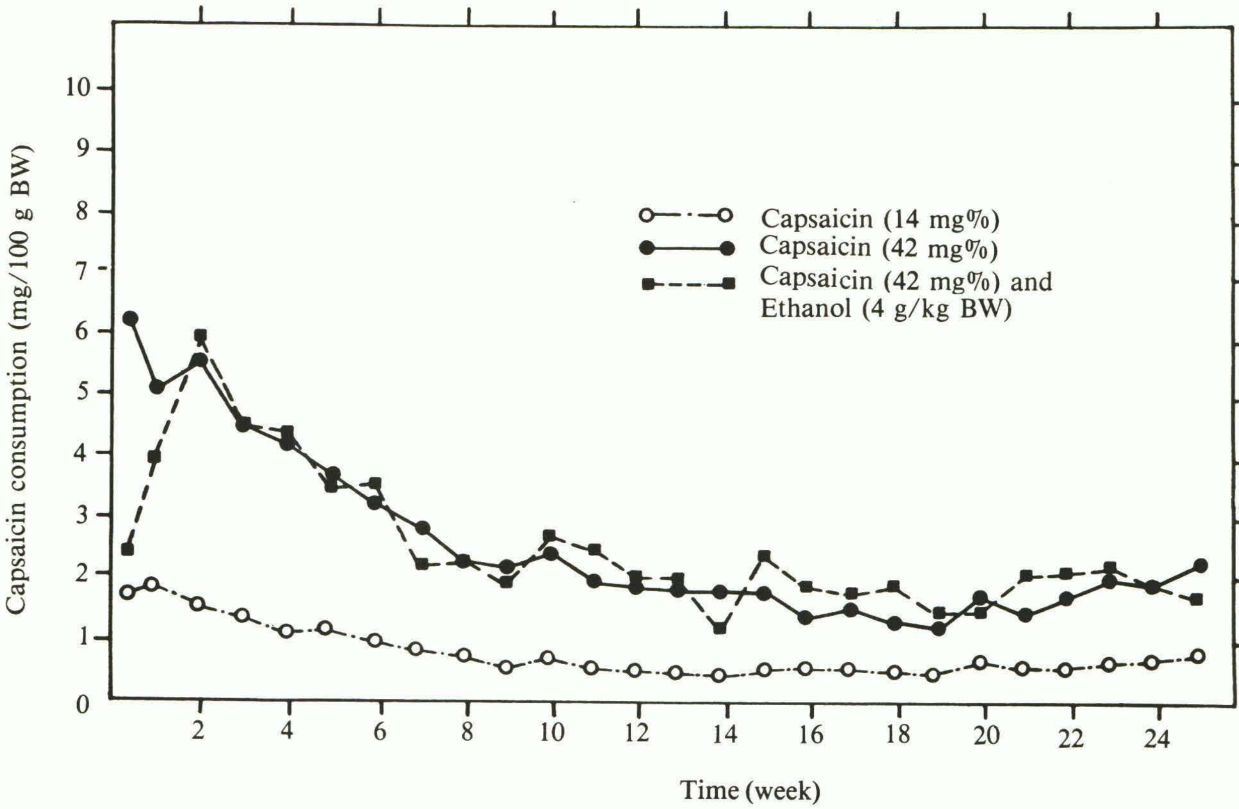

Fig. 3 Capsaicin consumption of male rats fed with capsaicin (14 or 42 mg%) with and without ethanol (4 g/kg BW) for 6 months.