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## Transient inhibition of CYP3A in rats by star fruit juice

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Running title: Star fruit altered carbamazepine pharmacokinetics in rats

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### **Abstract**

Star fruit juice is a potent *in vitro* inhibitor of CYP3A; however, few reports are available on the inhibition of CYP3A activities by star fruit juice *in vivo*. Therefore, in this study, we investigated the CYP3A-mediated star fruit-drug interaction *in vivo*. The effect of star fruit juice on carbamazepine pharmacokinetics was examined in rats. In comparison with water, the area under the concentration time curve (AUC) of carbamazepine was approximately 1.3-fold greater when star fruit juice (2 ml) was orally administered 1 h prior to the oral administration of carbamazepine (50 mg/kg). On the other hand, the elimination half-life of carbamazepine and the AUC ratio of carbamazepine 10,11-epoxide to carbamazepine were not altered by the administration of star fruit juice. These results suggest that star fruit juice impairs the function of enteric CYP3A, but not of hepatic CYP3A. In addition, we evaluated the time course of recovery of CYP3A activity that was reduced following the treatment with star fruit juice. The inhibition by star fruit juice was recovered within approximately 24 h. This data suggests that the effect of star fruit juice is mainly reversible and transient. Thus, we discovered that star fruit juice alters the carbamazepine pharmacokinetics in rats.

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## Introduction

It has been reported that the intake of grapefruit can alter the bioavailability of drugs. Previous studies have shown that coadministration of grapefruit juice with dihydropyridine calcium channel antagonists—felodipine and nifedipine—resulted in a large increase in the plasma concentration of these drugs, which can cause serious adverse reactions such as headaches, hypotension, facial flushing, and lightheadedness (Bailey et al., 1991; Lundahl et al., 1995). Grapefruit juice interacts with orally administered drugs that undergo substantial presystemic metabolism mediated by cytochrome P450 (CYP) 3A4 (Bailey et al., 1998). The mechanism of this interaction involves reversible or irreversible (mechanism-based) inhibition of CYP3A4 in the small intestine (Bailey et al., 2000). In addition, recent reports have indicated that various types of fruits, including those of the citrus species, have an inhibitory effect on CYP3A activities in the liver and gut wall and thereby alter the pharmacokinetics of certain drugs (Di Marco et al., 2002; Bailey et al., 2003; Egashira et al., 2004; von Moltke et al., 2004).

In a previous study, we attempted to identify fruits that had an inhibitory effect on CYP3A activity. Star fruit was found to be a potent *in vitro* inhibitor of CYP3A activity; it almost completely inhibits midazolam 1'-hydroxylase activity in human liver microsomes (Hidaka et al., 2004). Star fruit has been gaining increasing popularity in Japan, and higher star fruit consumption increases the possibility of star fruit-drug interactions. However, few

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reports are available on the inhibition of CYP3A activities by star fruit juice *in vivo*. Hence, it is important to evaluate the CYP3A-mediated drug interactions.

In the present study, we conducted an experiment to confirm the CYP3A-mediated star fruit-drug interaction *in vivo*. In our previous study, carbamazepine was found to be suitable for evaluating the inhibitory effects of fruit juice on enteric CYP3A activity in rats (Hidaka et al., 2005); therefore, carbamazepine was adopted as a substrate for CYP3A. The inhibition by star fruit juice of the predominant cytochrome P450 enzyme in the small intestine and/or liver could alter carbamazepine pharmacokinetics. Hence, we investigated the effect of star fruit juice on carbamazepine pharmacokinetics in rats.

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## Materials and Methods

**Chemicals.** Carbamazepine and carbamazepine 10,11-epoxide were purchased from Sigma-Aldrich (St. Louis, MO). All the chemicals and solvents were of the highest grade commercially available.

**Fruit Sample.** Star fruit (*Averrhoa carambola*) (Miyazaki, Japan) was obtained from local commercial sources. Star fruit juice was obtained by squeezing the edible portion of the fruit. All the samples were treated immediately after they were squeezed and filtered.

**Pharmacokinetic Experiments.** Male Wistar rats (Kiwa Animal Lab Service Co., Ltd., Wakayama, Japan), weighing 280 to 300 g and maintained at the Department of Bio-resources, Division of Biotechnology, Frontier Science Research Center, Miyazaki University, were used in the study. The experimental protocol was approved by the Ethics Review Committee for Animal Research of Miyazaki University. The effects of star fruit juice on carbamazepine pharmacokinetics in rats were evaluated by our previously reported method (Hidaka et al., 2005) with minor modifications. Briefly, 2 ml of star fruit juice or water was orally administered to rats (N = 6). Carbamazepine at a dose of 50 mg/kg was orally administered through gastric intubation at 1, 3, 12, and 24 h after the pretreatment. Blood samples (approximately 0.2 ml) were collected through the carotid artery at 15 and 30 min and 1, 2, 4, 6, 8, 10, 12, and 24 h after the oral administration of carbamazepine. Analysis of carbamazepine and carbamazepine 10,11-epoxide and their pharmacokinetic analysis were

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performed as described in our previous study (Hidaka et al., 2005).

**In Situ Closed Loop Method.** The in situ closed loop method used was a modification of the procedure of Toyobuku et al. (2003). The experiments were performed 1 h after a single administration of star fruit juice or water (2 ml each p.o.), and five rats were used in each experiment. In brief, an abdominal incision was carefully done in order to expose the intestines. The intestines were divided along the four segments with 4-0 silk suture, i.e., duodenum, jejunum, ileum, and cecum (8 cm in length). The intestinal contents were discarded with prewarmed (37°C) phosphate buffered saline from the respective segments. Then, 1 ml of carbamazepine (2.5 mg/ml) dissolved in lactated Ringer's solution with 10% ethanol was introduced into the divided segments with a syringe, and both ends of the segment were carefully ligated. The intestinal segment was placed in the body for 15 or 30 min along with a heating lamp to maintain the body temperature at 37°C. Following this, the luminal solution in the loop was collected, and the loop was rinsed with saline, resulting in a total solution volume of 50 ml. Carbamazepine absorption was measured by subtracting the remaining amount in the intestinal lumen from the administered amount, and it was expressed as the percentage of dose absorbed.

**Data Analysis.** Data from the experiments are expressed as mean  $\pm$  S.E.M.. Unpaired Student's *t* test and one-way analysis of variance, followed by least-significant difference analysis, were used to test for significant difference in mean values. The significant level was

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set at  $p < 0.05$ .



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## Results and Discussion

Since star fruit is a potent inhibitor of the CYP3A-mediated metabolism of carbamazepine, we considered the possibility that coadministration of star fruit juice may alter carbamazepine pharmacokinetics. In order to verify this hypothesis, we examined the effect of star fruit juice on carbamazepine pharmacokinetics in rats. As shown in Fig. 1, the plasma concentrations of carbamazepine and carbamazepine 10,11-epoxide were significantly higher in rats treated with star fruit juice than in rats treated with water. The pharmacokinetic parameters are summarized in Table 1. The mean area under the concentration-time curves (AUCs) of carbamazepine and carbamazepine 10,11-epoxide observed in rats treated with star fruit juice were significantly greater than the values obtained in rats treated with water. On the other hand, the  $t_{1/2}$  values of carbamazepine and carbamazepine 10,11-epoxide and the AUC ratios (carbamazepine 10,11-epoxide/carbamazepine) were not significantly different between the two groups. If the component(s) that inhibits CYP3A is absorbed into the systemic circulation, it would decrease the elimination of carbamazepine and the formation of carbamazepine 10,11-epoxide by inhibiting hepatic CYP3A. These results suggest that absorption of the component(s) of star fruit juice into the systemic circulation was not sufficient to inhibit hepatic CYP3A.

Furthermore, it has been recently reported that grapefruit juice influences intestinal uptake and efflux transporters, as well as CYP3A (Dresser and Bailey, 2003; Lilja et al., 2003;

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Honda et al., 2004). Hence, we considered that the increase in the AUC of carbamazepine in rats that were administered star fruit juice was caused by the enhancement of carbamazepine absorption as well as by the inhibition of carbamazepine metabolites, even though carbamazepine is not a substrate of P-glycoprotein (Sethia and Squillante, 2004). Therefore, to confirm this hypothesis, the effect of star fruit juice on the absorption of carbamazepine was investigated. The absorption of carbamazepine by various parts of the rat intestine was examined in the presence or absence of star fruit juice by using the in situ closed loop technique. The total amounts of carbamazepine absorbed through the rat intestine during a 30-min period were not significantly different among the two groups in each part of the rat intestine (Fig. 2); similar results were obtained in a 15-min period (data not shown). The results indicate that star fruit juice increases the AUC without affecting the absorption of carbamazepine.

Since our previous data suggested that star fruit juice contained few mechanism-based inhibitor(s) of CYP3A (Hidaka et al., 2004), we assumed that the inhibition of CYP3A by star fruit juice is mainly reversible, and the pharmacokinetics could recover rapidly. Therefore, we evaluated the time course of recovery of CYP3A activity in rats after treatment with star fruit juice. The AUCs of carbamazepine obtained after exposure to the juice were normalized by the respective control values in the same manner as reported by Greenblatt et al. (2003). As shown in Fig. 3, the AUC progressively returned to the control value as time elapsed. The

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ratios of mean AUC values at 1, 3, 12, and 24 h after the exposure to star fruit juice were 1.31, 1.14, 1.04, and 1.02, respectively. A plot of the time after exposure to star fruit juice versus the ratio of mean AUC values yielded a half-life of recovery that was estimated to be 6 h. These results suggest that CYP3A activity in rats treated with star fruit juice could recover within 24 h and star fruit contains few mechanism-based inhibitor(s).

In conclusion, we demonstrated that a component(s) of star fruit modifies the oral pharmacokinetics of carbamazepine in rats. Since the major metabolic pathways of carbamazepine in rats are similar to those in humans (Lertratanangkoon and Horning, 1982), we are tempted to propose that star fruit may influence the pharmacokinetics of CYP3A-mediated drugs in humans. However, since the biotransformation rate of carbamazepine in rats differs from that in humans (Faigle and Feldman, 1995), it is difficult to quantitatively extrapolate our results to humans. In addition, the relative role of hepatic and enteric extraction of CYP3A substrates in rats differs from that in humans, and thus, the interactions with CYP3A inhibitors in rats will be quite different from those in humans (Kotegawa et al., 2002). Therefore, further investigations in humans are necessary to develop our findings.

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### Legends for figures

**Fig. 1.** Plasma concentration-time profiles of rats treated with 50 mg/kg carbamazepine at 1 h after a single exposure to star fruit juice or water (2 ml p.o., each). A, carbamazepine; B, carbamazepine 10,11-epoxide; ●, control; ○, star fruit juice. Each point and bar represents the mean and S.E.M. of six rats. \*  $p < 0.05$ , \*\*  $p < 0.01$  versus control values.

**Fig. 2.** Effect of star fruit juice on the carbamazepine absorption by various regions of the rat intestine evaluated by the in situ closed loop method.

In the in situ closed loop method, carbamazepine absorption from the intestinal segments was determined. The experiments were performed at 1 h after a single administration of star fruit juice or water (2 ml each p.o.). Each column and bar represents the mean and SEM of five experiments.

**Fig. 3.** Relationship between the time after single exposure to star fruit juice and the fractional increase of area under the plasma concentration-time curve (AUC) of carbamazepine relative to the control value. The recovery half-life was estimated as 6 h ( $r^2 = 0.94$ ). Each point represents the mean value obtained from six rats.

**Table 1*****Pharmacokinetic parameters of carbamazepine 10,11-epoxide after administration Of carbamazepine in 1 h after a single exposure to star fruit juice or water in rats***Data are presented as mean  $\pm$  S.E.M. of six rats.

<b>Parameters</b>	<b>Control</b>		<b>Star fruit</b>	
	<b>Average</b>	<b>S.E.M.</b>	<b>Average</b>	<b>S.E.M.</b>
<b>Carbamazepine</b>				
Cmax ( $\mu\text{mol/ml}$ )	55.2	$\pm$ 4.0	70.7	$\pm$ 4.7 *
AUC ( $\mu\text{mol}\cdot\text{h/ml}$ )	398.1	$\pm$ 22.9	520.9	$\pm$ 22.4*
t1/2 (h)	9.5	$\pm$ 1.1	10.2	$\pm$ 1.0
<b>Carbamazepine 10,11-epoxide</b>				
Cmax ( $\mu\text{mol/ml}$ )	17.6	$\pm$ 2.9	22.8	$\pm$ 2.1
AUC ( $\mu\text{mol}\cdot\text{h/ml}$ )	269.2	$\pm$ 32.2	345.9	$\pm$ 14.1 *
t1/2 (h)	12.5	$\pm$ 2.0	13.5	$\pm$ 2.3
<b>Carbamazepine 10,11-epoxide/carbamazepine</b>				
AUC ratio <sup>a</sup>	0.70	$\pm$ 0.09	0.67	$\pm$ 0.04

\*  $p < 0.05$  vs. control values.<sup>a</sup> AUC of carbamazepine 10,11-epoxide/AUC of carbamazepine



Fig. 1

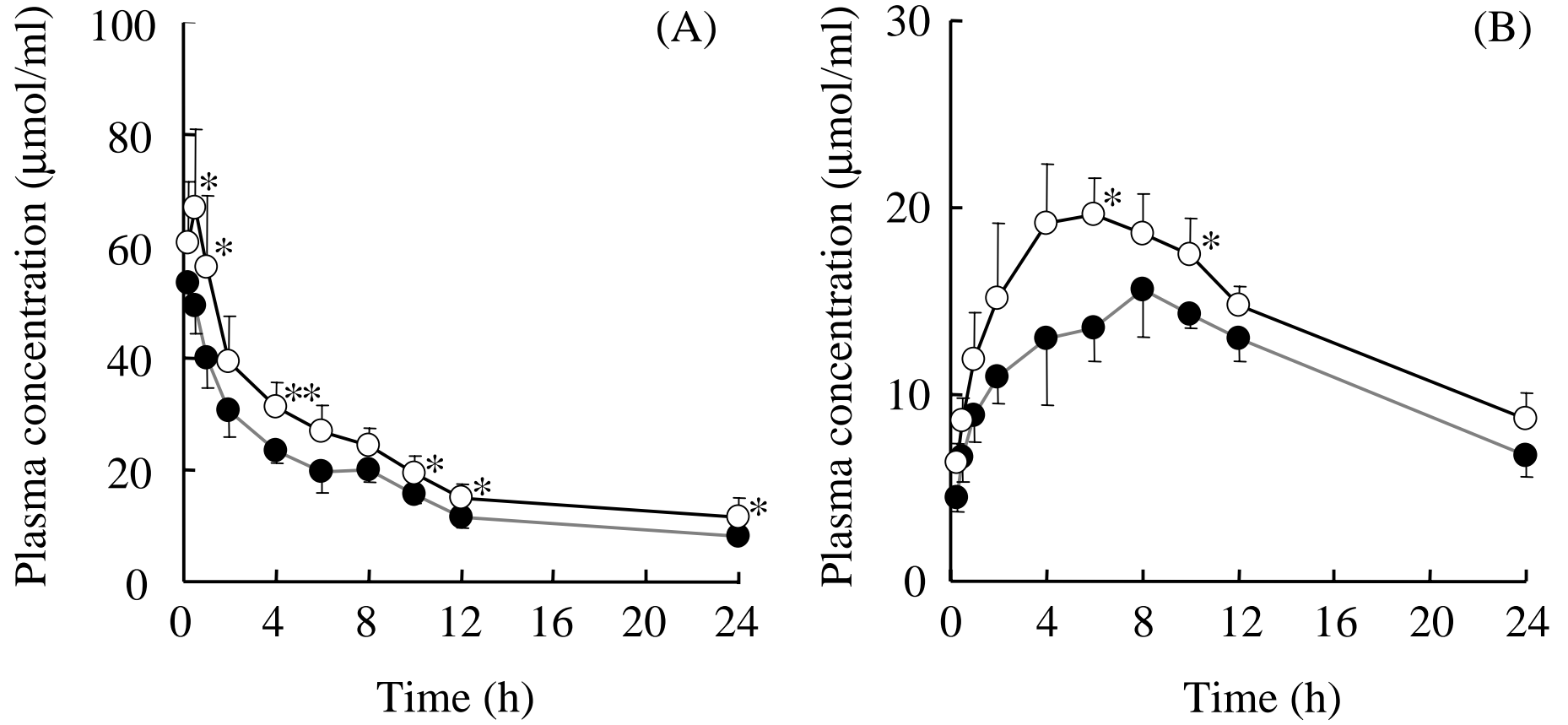


Fig. 2

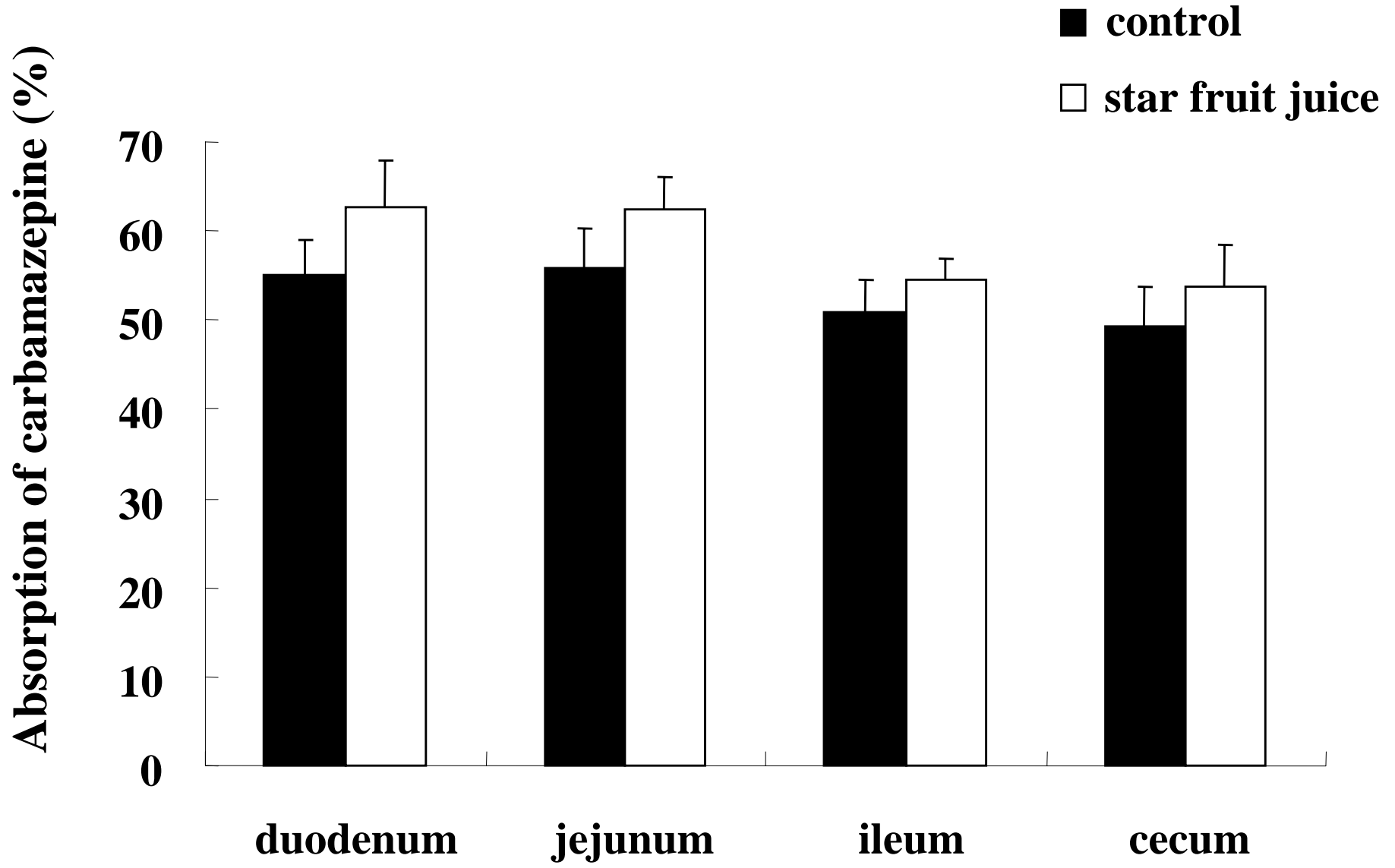


Fig. 3

