RESEARCH ARTICLE

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DEVELOPMENT OF *ZINGIBER CASSUMUNAR* FOR ASTHMATIC TREATMENT : PHARMACOKINETICS IN ANIMALS

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ABSTRACT

Pharmacokinetic studies of Plai preparation prepared from an ethanolic extract of Zingiber cassumunar *rhizome were performed in rats and monkeys. The apparent first order transfer rate constant, using* in situ *intestinal loop technique, was found to be 0.0731 min^{-1}.* In vivo *study by oral administration of drug to rats and determination of the concentration of bronchodilating active component of this plant in plasma also showed rapid absorption with a rate constant of 0.0385 min^{-1}. Maximum plasma level of 0.75 μg/ml was observed at 1.04 hours after drug administration.*

Elimination half-life in monkeys (2.31 hours) was found to be not statistically different from rats (2.00 hours). Prolonged half-life after multiple dosing was observed in monkeys. An accumulation ratio of 3.13 was found after long term drug administration.

The pharmacokinetic parameters obtained from this study provides valuable information for future use in clinical evaluation of Zingiber cassumunar *for the treatment of asthmatic patients. The study is also of benefit as a model for pharmacokinetic studies of other medicinal plants.*

INTRODUCTION

Zingiber cassumunar Roxb., commonly known as Plai, is a plant in the Zingiberaceae family. The rhizome of this plant has been widely used in Thai traditional drug preparations to relieve inflammation and muscle pain.[1] In combination with other medicinal plants it was found to be effective in relieving asthmatic symptoms in children and adults.[2, 3] Investigators have performed pharmacological testings of this

plant and found that its hexane extract had antispasmodic action on isolated tracheal chain preparation of guinea-pig.[4] In an attempt to isolate active components, a large number of aromatic compounds was isolated from the hexane extract of Plai rhizome and their structures have been elucidated.[5,6] Among these compounds, Compound D, (E-4- (3′, 4′ - dimethoxyphenyl) but-3-en-l-ol) was found to have an antispasmodic effect on the guinea-pig ileum and tracheal smooth muscle.[7]

In view of these results, it would be of great interest and benefit to develop this plant for the treatment of asthma. Prior to clinical evaluation, pharmacokinetic studies in animals, following the concentration of Compound D in the blood, have to be performed. The information obtained from this study e.g. absorption and eliminaion rate constants, maximum plasma concentration, and accumulation of drug, would be useful for adjusting dosage regimen and for the design of a proper form of Plai drug preparation.

MATERIALS AND METHODS

Drugs and chemicals

Pulverized Plai rhizome was exhaustively extracted with absolute ethanol using the soxhlet apparatus. Plai preparation was prepared from the obtained extract in the form of an emulsion using 2% tween 80 and 4% span 80 as the emulsifying agents.

Synthetic Compound D (reference standard) and Compound E, (8-(3′, 4′ - dimethoxyphenyl) - 2-methoxynaphtho-1,4-quinone), (internal standard) were obtained from the Synthesis Section, Division of Medical Research, Department of Medical Sciences.

Solvents for High Performance Liquid Chromatography (HPLC) were of HPLC grade and chemicals used for extraction and purification of samples for analysis were of analytical reagent (AR) grade.

Equipment for analysis

High Performance Liquid Chromatograph[a] used for the analysis of Compound D comprised of Pumps[b], an Automated Gradient Controller[c], an Injector[d], a Programmable Multiwavelength Detector[e] and a Data Module[f].

Animals

Two species of animals, male Wistar rats weighing 200-250 g and adult male cynomolgus monkeys *(Macaca fascicularis)* weighing 6.0-8.1 kg (age 9-16 yrs), were used.

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a) Waters Associates, Milford, Massachusetts, USA. b) Pump, model 510 and 590. c) Automated Gradient Controller, model 680. d) Injector, model U6K. e) Programmable Multiwavelength UV Detector, model 490. f) Data Module, model 730.

Transfer of drug from rat intestine

Rats were fasted but allowed access to water for about 18 hours before the experiment. Each animal was anesthetized with urethane,1.25 g/kg, intraperitoneally.

Drug absorption was investigated by the *in situ* intestinal loop technique of Doluisio *et al*[8] with some modifications.[9] Preparation included midline lapalotomy and cannulation of small intestine, at the beginning of the duodenum and ending of ileum, using glass cannulas. The bile duct was ligated to prevent the secretion of bile into the intestinal lumen. Seven milliliters of Plai preparation in the form of emulsion, containing 12.0 mg percent of Compound D, was instilled into the lumen of the cannulated segment of the small intestine. Samples of 0.2 ml from the intestinal loop were removed at 0, 2, 5, 10, 20 and 30 minutes for analysis of Compound D. The volume of drug solution in the intestine was kept constant by the addition of 0.9% sodium chloride before the removal of each sample. The percent of drug remaining in the lumen of the intestinal segment was determined as a function of time for a period of 30 minutes.

***In vivo* study in rats**

A group of 9 rats, 1 control and 8 drug-treated, was used to obtain 1 set of data. The emulsion of Plai preparation was administered to rats orally through an intubation tube at a dose equivalent to 2.0 g of pulverized Plai rhizome/kg body weight (equivalent to a Compound D dose of 15.0 mg/kg). The animals were anesthetized with urethane, 1.25 g/kg intraperitoneally before blood sampling. Blood was drawn from the control rat prior to dosing, and from drug-treated rats at 15, 30, 45, 60, 120, 240, 360 and 480 minutes post-administration, one rat at each time interval, by heart puncture. The plasma was separated and stored in a freezer pending assay. Fifteen groups of rats were used in this study, hence 15 sets of data were obtained.

Multiple dosing of Plai preparation in monkeys

Five male monkeys were fasted overnight but allowed access to water. An emulsion of Plai preparation filled in capsule was administered to monkeys orally at a dose equivalent to 1.5 g of pulverized Plai rhizome/kg body weight (equivalent to a Compound D dose of 11.25 mg/kg). The drug was administered once every day, 2 hours before morning feed, for 28 days. Blood samples were drawn from each monkey through the femoral vein at intervals of 1, 2, 4, 6 and 8 hours post-administration on the first day and the twenty-eighth day after drug administration. Plasma was separated and analyzed for Compound D concentration. Prior to drug administration and blood sampling, each monkey was sedated with Ketalar Parke-Davis, containing Ketamine HCI equivalent to Ketamine base 50 mg/ml), 5 mg/kg intramuscularly.

Assay

Compound D concentrations in the intestinal fluid and in the plasma were determined by High Performance Liquid Chromatography (HPLC).[10] Briefly, one milliliter of plasma sample containing 0.1 ml of internal standard solution (Compound

E) was acidified with 0.2 ml of 0.1 N sulfuric acid. Subsequently, the sample was extracted with 20.0 ml of 10% isopropanol in chloroform for 10 minutes before centrifugation at 4,000 rpm for 10 minutes. The organic phase was then transferred to separate tubes and dried under reduced pressure. Finally, the residue was redissolved in 50 μl of mobile phase and 10 μl was injected into the HPLC. Analysis was carried out on a silica column (Radial-Pak Silica Column of Waters Associates, 10 μm, 5 mm diameter and 10 cm long) with a mobile phase consisting of *n*-hexane : ethyl acetate : isopropanol (75:25:2). Compound D was detected by UV monitoring at 260 nm and was quantitated according to standard curves (generated by least-square regression analysis).

Pharmacokinetic analysis

The data were analyzed using a simple linear one-compartment open model[11] to determine the pharmacokinetic parameters, comprising of the absorption rate constant, elimination rate constant and half-life, peak plasma concentration, time taken to reach peak concentration, and accumulation of drug.

Statistical analysis

Regression analysis was used in drawing a line to obtain pharmacokinetic parameters. All results were expressed as mean ± SEM. The statistical significance between the rat and monkey groups was determined by the student's unpaired t-test whereas the student's paired t-test was used to determine the significance between single and multiple dose studies in monkeys. The level of significance of $p < 0.05$ was used.

RESULTS

The transfer rate of Plai preparation instilled into a cannulated segment of small intestine of anesthetized rats is shown in Figure 1 as a plot of percent of Compound D remaining in the intestine as a function of time. The apparent first-order transfer rate constant for Compound D was determined for individual rats from slope of the line (fitted by the method of least squares) of semilogarithmic plots of percent of Compound D remaining in the intestine versus time. The mean transfer rate constant obtained from 15 rats was 0.0731 min^{-1} corresponding to a half-life of 9.58 mins. The rate constant was corrected for drug removed in the samples and converted to clearance per centimeter of intestine. The mean value of this parameter was calculated to be 5.60 $\mu l. min^{-1}. cm^{-1}$. Table 1 shows the mean values and standard errors of the means of these parameters.

The cumulative percent of absorption as a function of time was calculated from the percent of Compound D remaining in the intestinal lumen to be absorbed. It was found that 92.68% of drug was absorbed in 30 minutes and the plot is shown in Figure 2.

Pharmacokinetic parameters after oral administration of Plai preparation to rats at a dose equivalent to 15.0 mg of Compound D/kg body weight were determined. The semilogarithmic plots of concentrations of Compound D in plasma as a function of time are shown in Figure 3. The data points are the average of 15 groups of rats. By analyzing individual sets of data, using a one compartment open model, pharmacokinetic parameters were determined. Mean values of absorption rate constant, elimination rate constant and elimination half-life, were found to be 0.0385 min^{-1}, 0.3648 h^{-1} and 2.00 h respectively. At 1.04 hours after drug administration the maximum plasma level of 0.75 μg/ml was observed. The area under the curve was calculated to be 3.00 $\mu g.h^{-1}. ml^{-1}$. All these parameters are summarized in Table 2.

Drug accumulation after multiple dosing of Plai preparation for 28 days was studied in 5 monkeys. The mean plasma levels of Compound D as a function of time on the first day, and on the twenty-eighth day after drug administration were plotted as shown in Figure 4. The plasma data of individual monkey were fitted to a one compartment open model system for the determination of pharmacokinetic parameters. The values obtained after drug administration for a period of 1 day and 28 days were compared as in Table 3. The results showed that after repeated drug administration, the maximum plasma level of Compound D was significantly increased from 0.38 μg/ml to 1.19 μg/ml, while the time taken to reach peak concentration was not statistically different. The elimination half-life was found to be noticeably increased from 2.31 hours to 3.61 hours. The accumulation ratio after long term drug administration was calculated to be 3.13.

DISCUSSIONS

The results of this investigation show a rapid absorption of Plai preparation in rats *(in situ* study) with a transfer rate constant from the intestinal lumen of 0.0731 min^{-1} (Table 1). It was also found that 92.68% of the drug was absorbed from the intestinal segment in 30 minutes as shown in Figure 2. An *in vivo* study, by oral administration of Plai preparation to rats followed by analysis of Compound D concentrations in the plasma, also revealed rapid absorption with a rate constant of 0.0385 min^{-1} and a maximum plasma level of 0.75 μg/ml being reached in 1.04 hours. The fast absorption rates obtained agreed well with the physicochemical nature of the active component of Plai, Compound D, which is non-polar.[12] However, several studies have reported that emulsifiers can effect membrane permeability.[13, 14] Thus, the rapid absorption of drug observed in this study may be partially due to the use of emulsifier in the drug preparation. Rapid elimination of drug was also observed in the rats with a mean elimination rate constant of 0.3648 h^{-1} corresponding to a half-life of 2.00 hours (Table 2).

The pharmacokinetics of Plai preparation were further investigated in monkeys. Due to limitation of the amount of blood that can be drawn from a monkey, blood sampling could only be performed at 5 consecutive time intervals per day. Since

only a few data points were obtained in the absorptive phase, it was not possible to determine the absorption rate constant. The absorption rate was therefore evaluated from the time measured from after drug administration until maximum plasma concentration was reached (T_{max}), because the absorption rate is inversely related to the time required to reach maximum concentration. The peak level of Compound D in the plasma of monkeys was reached at 3.25 hours after a single dose of drug administration (Table 3). The difference in species may also be another factor responsible for this variation.

The peak level of 0.38 μg/ml in monkeys was lower than that in rats (0.75 μg/ml) as shown in Table 2 and 3. This discrepancy was due to the difference in the dose of Plai preparation used. It is well established that peak level is absorption rate dependent and therefore the slow rate of absorption in monkeys may also account for the lower peak level obtained.

The elimination half-life of Compound D in monkeys in the single dose study was in good agreement with the result from rats (Table 2 and 3). No statistical difference between the values of elimination half-life obtained from rats and monkeys was observed.

Some drugs e.g. analgesics, hypnotics, and antiemetics, may be effectively used as a single dose treatment. Other drugs have to be given on a continuous basis in long-term treatment. For drugs administered in multiple dosing, the peak plasma level following the second and succeeding doses of a drug is higher than that of the peak level after the first dose, consequently the drug accumulates in the body relative to the initial dose. Plai preparation is intended to be used in patients to relieve asthmatic symptoms which require long-term therapy. In the present study, the monkey was chosen as the animal model for the study of drug accumulation. It was evident that, after chronic administration of the drug to monkeys, the peak plasma level was significantly increased from 0.38 μg/ml to 1.19 μg/ml (Table 3), hence an accumulation ratio of 3.13 was obtained. It was also found that the mean elimination half-life of 2.31 hours which was obtained on the first day of the experiment had increased to 3.61 hours on the twenty-eighth day (Table 3). The resulting prolonged half-life upon repeated drug administration may be due to the inhibition of drug on the metabolizing enzyme. The observed slower rate of drug elimination after long term administration correspond very well with the increase in peak plasma level.

The pharmacokinetic parameters obtained from this study is useful for adjusting dosage regimen in clinical evaluation of this plant for asthmatic treatment. Strong emphasis should be made that, in using the Plai preparation for long-term treatment, accumulation problem must be considered. Also drug level monitoring should be performed, if possible, to assure that plasma drug concentrations are within the therapeutic range.

Very few studies concerning pharmacokinetics of medicinal plants have been performed for the following reasons. Firstly, analysis of pharmacological active components in the blood tends to be rather chaotic due to interferences from impurities such as a large number of inactive components in the plant as well as their metabolites which will also appear in the blood. Secondly, there is a lack of knowledge in pharmacokinetics required for the design of proper research protocol. Therefore, this study should be of benefit as a model leading to pharmacokinetic studies of other medicinal plants.

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บทคัดย่อ

ศึกษาทางเภสัชจลนศาสตร์ของสารสำคัญ (สาร D) ในยาไพลซึ่งเตรียมจากสารสกัดเหง้าไพลด้วยแอลกอฮอล์ในหนูขาวและลิงแสม พบว่าค่าคงที่การลำเลียงสารสำคัญของยาไพลจากส่วนของลำไส้ของหนูขาวที่ทำให้เป็น loop (*In situ* intestinal loop) เท่ากับ 0.0731 ต่อนาที เพื่อศึกษาค่าคงที่ทางเภสัชจลนศาสตร์ในหนูขาว โดยการตรวจวิเคราะห์ความเข้มข้นของสาร D ในเลือดที่เวลาต่าง ๆ หลังจากป้อนยาไพลทางปาก พบว่าสาร D ดูดซึมได้เร็วด้วยค่าคงที่ของการดูดซึมยาเท่ากับ 0.0385 ต่อนาที โดยมีความเข้มข้นของยาสูงสุดในเลือดเท่ากับ 0.75 ไมโครกรัม/มล. ที่เวลา 1.04 ชม. หลังการป้อนยา ผลที่ได้จากการศึกษานี้มีความสอดคล้องกับคุณสมบัติทางเคมีและฟิสิกส์ของสารออกฤทธิ์ในไพล การศึกษาในลิงพบว่าค่าครึ่งชีวิตของการขจัดยาออกจากร่างกาย เมื่อให้ยาวันแรกมีค่าเท่ากับ 2.31 ชม. ซึ่งไม่แตกต่างจากในหนูขาว (2.00 ชม.) แต่เมื่อป้อนยาให้ลิงทุกวันติดต่อกันนาน 28 วันพบว่าค่าครึ่งชีวิตของการขจัดยาออกจากร่างกายนานขึ้น และมีอัตราส่วนการสะสมยาในร่างกายเท่ากับ 3.13 ค่าทางเภสัชจลนศาสตร์ที่ได้รับจากการศึกษานี้จะเป็นประโยชน์ในการศึกษาทางคลีนิกของยาไพลในผู้ป่วยโรคหืด และจะเป็นแบบอย่างสำหรับการศึกษาทางเภสัชจลนศาสตร์ของสมุนไพรตัวอื่น ๆ ต่อไป

TABLE 1. Transfer rate constant, half-life, and volume clearance of Compound D from intestinal loop of anesthetized rats after emulsion of *Zingiber cassumunar* extract was instilled into a cannulated segment of small intestine.

Pharmacokinetic Parameters	**mean★ ± SEM**
Transfer Rate Constant (min^{-1})	0.0731 ± 0.002
Half-life of Transfer (min)	9.58 ± 0.28
Volume Clearance ($\mu l.min^{-1}. cm^{-1}$)	5.60 ± 0.16

★ n = 15

TABLE 2. Summary of pharmacokinetic parameters of Compound D in rats after emulsion of *Zingiber cassumunar* extract was given orally (equivalent to Compound D 15.0 mg/kg)

Pharmacokinetic Parameters	**mean★ ± SEM**
Absorption Rate Constant (Ka), min^{-1}	0.0385 ± 0.0049
Half-life of Absorption ($T_{1/2\ a}$), min	21.80 ± 2.34
Elimination Rate Constant (Ke), h^{-1}	0.3648 ± 0.0219
Elimination Half-life ($T_{1/2\ e}$), h	2.00 ± 0.13
Time to Maximum Concentration (T_{max}), h	1.04 ± 0.07
Maximum Concentration (C_{max}),$\mu g/ml$	0.75 ± 0.18
Area Under Curve (AUC), $\mu g.h.ml^{-1}$	3.00 ± 0.65

★ n = 15

TABLE 3. Pharmacokinetic parameters of Compound D in monkeys after emulsion of *Zingiber cassumunar* extract was given orally (equivalent to Compound D 11.25 mg/kg)

Pharmacokinetic Parameters	Period of Drug Administration 1 day	28 days	Statistical Analysis
Elimination Rate Constant (Ke), h^{-1}	0.3022 ± 0.0119	0.2094 ± 0.0333	$p < .05$
Elimination Half-life ($T_{1/2\ e}$), h	2.31 ± 0.10	3.61 ± 0.50	$p < .05$
Time to Maximum Concentration (T_{max}), h	3.25 ± 0.30	3.90 ± 0.10	NS
Maximum Concentration (C_{max}), μg/ml	0.38 ± 0.05	1.19 ± 0.07	$p < .0005$

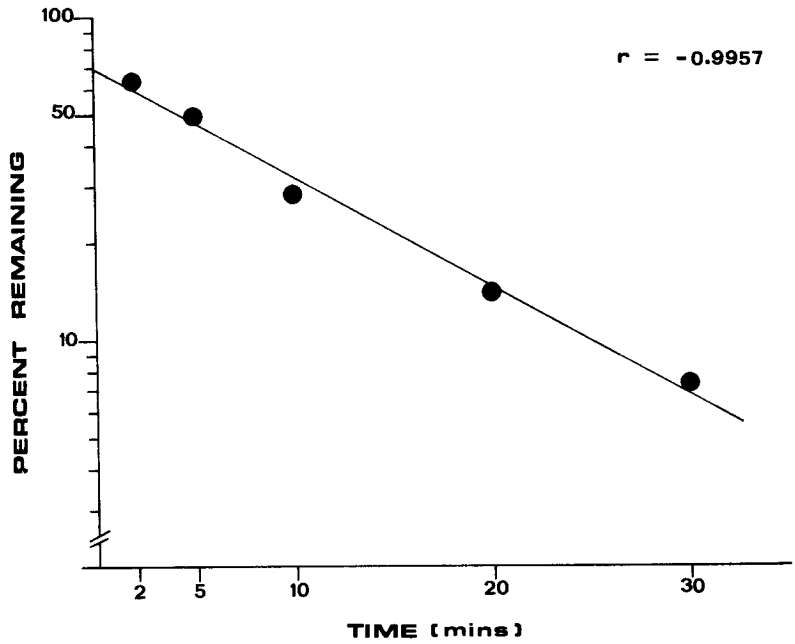

Fig. 1 Percent of Compound D remaining in the intestine as a function of time after emulsion of *Zingiber cassumunar* extract was instilled into a cannulated segment of rats' small intestine (average of 15 rats).

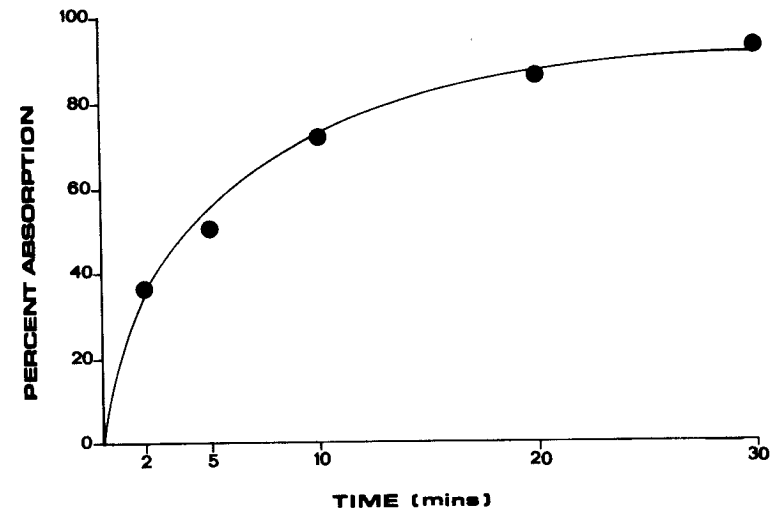

Fig. 2 Cumulative percent of Compound D absorption from a cannulated segment of rats' small intestine as a function of time after instilled with emulsion of *Zingiber cassumunar* extract (average of 15 rats).

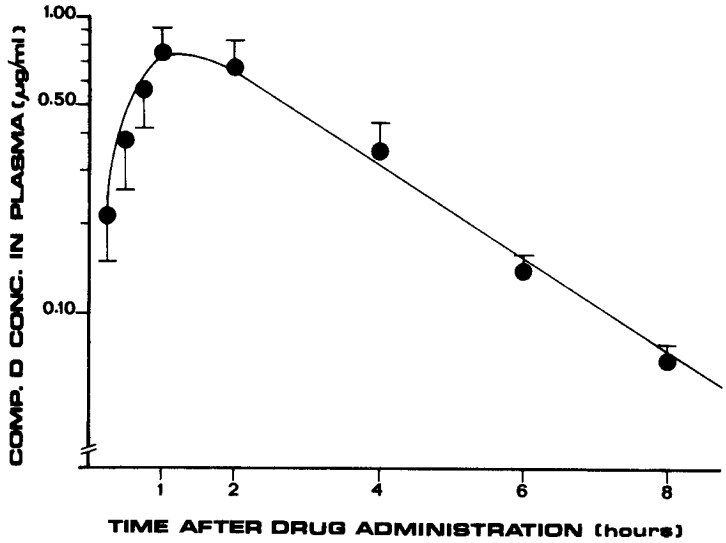

Fig. 3 Semilogarithmic plot of average plasma concentration of Compound D in rats versus time after oral administration of emulsion of *Zingiber cassumunar* extract (equivalent to Compound D 15.0 mg/kg).

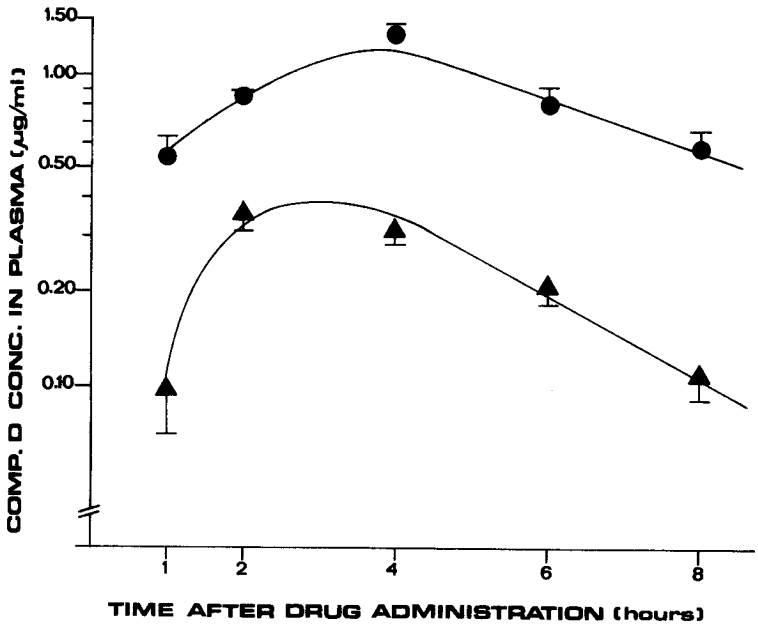

Fig. 4 Average plasma concentration of Compound D in monkeys versus time after single dose (1 day) and multiple dose (28 days) oral administration of emulsion of *Zingiber cassumunar* extract (equivalent to Compound D 11.25 mg/kg).

▲ represent data of single dose

● represent data of multiple dose