ปีที่ ๑๙ เล่มที่ ๑ Vol. 19, No. 1
มกราคม-มิถุนายน ๒๕๓๐ January – June 1987

วารสารสำนักงานคณะกรรมการวิจัยแห่งชาติ
Journal of the National Research Council

THE HYPOGLYCEMIC ACTIVITY OF *MOMORDICA CHARANTIA* LINN. IN NORMAL AND ALLOXAN – INDUCED DIABETIC RABBITS

ฤทธิ์ของสารสกัดจากมะระในการลดน้ำตาลในเลือดของกระต่ายปกติ และกระต่ายที่เป็นเบาหวานจาก alloxan

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ABSTRACT

The acidified water-chloroform extract of unriped fruits of Momordica charantia *Linn. was tested for hypoglycemic activity at several dosage levels in normal and alloxan-induced diabetic rabbits. The blood glucose was measured at 2 h interval for 10 h period by O-toluidine method. In diabetic rabbits, a significant decrease in blood glucose level occurred at 10 and 20 mg/kg after intravenous injection whereas no hypoglycemic action was found in*

normal rabbits even at the high dosage of 20 mg/kg. This pattern of the acidified water-chloroform extract action contrasted with that of tolbutamide which produced the hypoglycemic action only in normal rabbits.

บทคัดย่อ

การทดสอบฤทธิ์ในการลดน้ำตาลในเลือดของยาที่สกัดด้วย acidified water-chloroform จากผลมะระ (*Momordica charantia* Linn.) ในกระต่ายปกติและกระต่ายที่ทำให้เป็นเบาหวานโดยการฉีด alloxan พบว่า การฉีดสารสกัดนี้ 10 และ 20 มก./กก. เข้าเส้นเลือดดำ ทำให้กระต่ายที่เป็นเบาหวานมีระดับน้ำตาลในเลือดลดลงอย่างมีนัยสำคัญทางสถิติ แต่ไม่มีผลในกระต่ายปกติ เมื่อเปรียบเทียบกับ tolbutamide พบว่าให้ผลแตกต่างกัน คือ tolbutamide มีฤทธิ์ลดน้ำตาลในเลือดเฉพาะในกระต่ายปกติ แต่ไม่มีผลกับกระต่ายที่ทำให้เป็นเบาหวาน แสดงว่ากลวิธานการออกฤทธิ์ของยาสกัดด้วย acidified water-chloroform จากผลมะระน่าจะแตกต่างออกไปจาก tolbutamide

INTRODUCTION

Momordica charantia Linn. (Family Cucurbitaceae), commonly known as bitter gourd, which was widely cultivated in Thailand and other tropical countries was usually used as vegetable.[16,17,22] In addition, various parts of this herb were useful in folkloric medicine, one of which was the antidiabetics which has long been used in Puerto Rico.[5] The scientific studies of this plant began in 1941 by Rivera[18,19] who isolated the hypoglycemic principles in the alcoholic extract of the fruits of this plant. Since then, there were many investigators[1,3,7-14,20,21,23,24] who were interested in the hypoglycemic action of this plant. Sharma et al.[20] administered the fruit juice to normal and alloxan-induced diabetic rabbits and reported a decrease in blood glucose of both groups. The hypoglycemic action of the fruit juice was confirmed in the pituitary extract induced diabetic rats which fed with glucose,[7] in non-diabetic rats and non-insulin dependent diabetic patients.[12] On the other hand, oral administration of the dried juice in normal rabbits showed no hypoglycemic effect either as an acute single dose or a chronic administration. However, it prolonged the action of tolbutamide.[10] Akhtar et al.[1] studied the hypoglycemic action of dried fruit powder in normal and alloxan-induced diabetic rabbits and found that a lower dosage was required for normal animals. They proposed that there might be more than one kind of active principles in the fruits of this plant. In fact, Lotlikar and Rao[13] had isolated a non-nitrogenous substances with a phytosterol like property named charantin, which brought about the hypoglycemic action[14] in normal rabbits by both oral and intravenous administration. The structure of charantin was identified by Sucrow[21] as ß-D-glucosides of ß-sitosterol and stigmasta-5, 25 diene-3ß-ol in proportion of 1:1. Moreover, an insulin like polypeptide was also isolated and identified from this plant.[3,9] Recently, Visarata

and Ungsurungsie[23] using a modified extraction method of Rivera[18] have detected the steroidal compounds in acidified water-chloroform extract which exhibited antibacterial activity *in vitro*.[24] Whether this extract could decrease blood glucose has not been reported. Therefore, the present investigation was undertaken to study the hypoglycemic effect of the acidified water-chloroform extract in normal and alloxan-induced diabetic rabbits in comparison with the standard hypoglycemic drug, tolbutamide.

MATERIALS AND METHODS

Large variety of *M. charantia,* fresh green fruits, (Figure 1) was obtained from vegetable markets in Bangkok area. After the seeds were removed, the fruits were cut into small pieces and dried at 60°C. They were powdered and extracted according to the method described by Visarata and Ungsurungsie[23] as shown in Figure 2. The acidified water-chloroform extract was evaporated until dry and collected for pharmacological testing. The extract was dissolved in normal saline solution before the injection was made intravenously into the rabbits.

Male adult healthy albino rabbits of local strain weighing 1.5-2.5 kg were used in this experiment. The rabbits were kept in the animal room of the Department of Pharmacology and fed with commercial food and tap water *ad libitum*. They were divided into 2 groups composed of normal and diabetic rabbits and were induced diabetes by injection 80 mg/kg of alloxan monohydrate intravenously.[15] The blood glucose levels were determined daily for 7 days after the injection. The blood glucose of more than 150 mg/100 ml was considered diabetes. Any rabbits which were not diabetics were repeatedly injected with the same dose of alloxan until the blood glucose level was in the range of diabetes.

Both normal and diabetic rabbits were randomly divided into 5 groups of 6 animals each. Group 1 was served as a control. The rabbits were received 4 ml of normal saline intravenously. Group 2, 3 and 4 were intravenously administered with a solution of acidified water-chloroform extract in normal saline at 5, 10 and 20 mg/kg respectively. The last group was treated with tolbutamide 100 mg/kg orally.

Prior to testing, all rabbits were fasted for 14 h and 1 ml of blood samples were drawn from an ear vein into NaF-coated test tubes at 0, 2, 4, 6, 8 and 10 h. The blood samples were centrifuged and the plasma were kept for the glucose determination by O-toluidine method.[4] The blood glucose levels were measured at 2 h interval for a period of 10 h.

The blood glucose was expressed in mg/100 ml (Mean $\pm$ SEM). Changes in blood glucose levels of normal and diabetic rabbits at various time intervals (2, 4, 6, 8 and 10 h) from initial value before drug treatment (0 h) were analysed by using paired Student's t test. $P < 0.01$ was considered statistically significant.

RESULTS

Effect of *Momordica charantia* Linn. and tolbutamide on blood glucose level in diabetic rabbits

Intravenous administration of 5 mg/kg acidified water-chloroform extract of *M. charantia* had no significant effect on blood glucose. However, the hypoglycemic action was observed at higher doses. At a dose of 10 mg/kg, the extract produces a significant decrease in blood glucose in the alloxan-induced diabetic rabbits at 8 and 10 h after the treatment. At 20 mg/kg, the significant decrease was seen earlier at 4 h and maintained for at least 10 h (Table 1). On the contrary, oral administration of tolbutamide at 100 mg/kg produced only a slight but not significant decrease in blood glucose as in the control group (Table 1).

Effect of *Momordica charantia* Linn. and tolbutamide in the normal rabbits

In contrast to the diabetic rabbits, acidified water-chloroform extract at the same dosage produced no hypoglycemic action in the normal rabbits. As shown in Figure 3, the highest dosage at 20 mg/kg which significantly decreased the blood glucose level in diabetic rabbits with a maximum effect of about 30%, had no significant effect on the blood glucose level in normal rabbits. On the other hand, oral administration of tolbutamide at 100 mg/kg decreased the blood glucose level significantly about 40% (Table 2) whereas there was no effect in the diabetic rabbits (Figure 4).

DISCUSSION AND CONCLUSION

The present experiment reveals that acidified water-chloroform extract of *M. charantia* produces a significant decrease of blood glucose level in alloxan-induced diabetic rabbits. However, higher dosages should be further tested in normal group. The hypoglycemic effect of this extract is obviously different from that of tolbutamide which produced a significant effect only in the normal rabbits. The major acute action of tolbutamide is able to stimulate a secretion of insulin from the pancreatic ß-cell[11] but is ineffective in completely pancreectomized or alloxan-induced diabetic animals.[6,11] Therefore, the hypoglycemic mechanism of the acidified water-chloroform extract of this plant is different from tolbutamide and may be extra-pancreatic, a direct action on glucose absorption and metabolism. It is also noted that this acidified water-chloroform extract has a slow onset, even through the intravenous route it needs as much as 4 h to be effective. This is in contrast to a rapid action of the orally administered tolbutamide. However, both drugs have long duration of action, which last for more than 10 h. Moreover, the pattern of blood glucose changes by acidified water-chloroform extract is different from that of charantin, a sterol glycoside which is isolated and identified by Lotlikar and Rao.[13,14]

The intravenous (15 mg/kg) and oral (25 mg/kg) administration of charantin in normal fasting rabbits produced the same hypoglycemic effect whereas a variable response was observed in alloxan-diabetic rabbits.[14] Moreover, the hypoglycemic effect of charantin will only last for 4 h after which the blood sugar is tended to reach its same level before treatment.[14]

Besides charantin, an isolation of other active principles of a polypeptide class which has an insulin-like activity after subcutaneous and intramuscular injection in diabetic patients have been reported.[3,9] Although this polypeptide had been thought to be hydrolysed if the fruit juice was taken orally, many literatures reported its effectiveness.[7,12,20] Therefore, this polypeptide may be acid resistant which can be experimentally proof. There may be some other active principles in the fruits of this plant beyond p-insulin like substances and charantin. One of which may be the steroidal substances which has been identified in the acidified water-chloroform extract.[23] In addition to the hypoglycemic action, this extract has an advantageous antibacterial activity[24] for the diabetic patients. An isolation and purification of these substances from acidified water-chloroform extract for pharmacological and toxicological studies are therefore worthwhile in order to elucidate the active principles in this extract. Since the fruits of *Momordica charantia* Linn. contain many active hypoglycemic components of different mechanisms, this plant may be very useful for the diabetic patients and further studies on this plant should be performed.

ACKNOWLEDGEMENT

The authors wish to thank the National Research Council of Thailand for a partial support of this work.

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Table 1. The blood glucose level (Mean ± SEM) in diabetic rabbits at various time intervals after intravenous administration of acidified water-chloroform extract from *Momordica charantia* L., NSS and oral administration of 100 mg/kg tolbutamide.

Treatment	Blood glucose level (mg/100 ml)					
	0 h	2 h	4 h	6 h	8 h	10 h
Acidified water-chloroform extract						
5 mg/kg (n = 5)	284±52	296±54	276±47	298±42	294±43	275±42
10 mg/kg (n = 5)	268±48	272±48	247±51	235±56	221±51*	201±50*
20 mg/kg (n = 6)	355±75	339±70	300±35*	274±36*	250±31*	241±24*
NSS (n = 6)	306±30	315±29	312±32	296±31	292±29	280±28
Tolbutamide (n = 6)	332±40	349±40	338±40	301±42	305±46	295±45

* $P < 0.01$

Table 2. The blood glucose level (Mean ± SEM) in normal rabbits at various time intervals after intravenous administration of 20 mg/kg acidified water-chloroform extract and oral administration of 100 mg/kg tolbutamide.

Treatment	Blood glucose level (mg/100 ml)					
	0 h	2 h	4 h	6 h	8 h	10 h
Acidified water-chloroform extract (n = 6)	98±4	104±6	106±2	108±3	109±4	102±5
Tolbutamide (n = 6)	104±5	88±7*	71±4*	68±4*	62±5*	61±2*

* $P < 0.01$

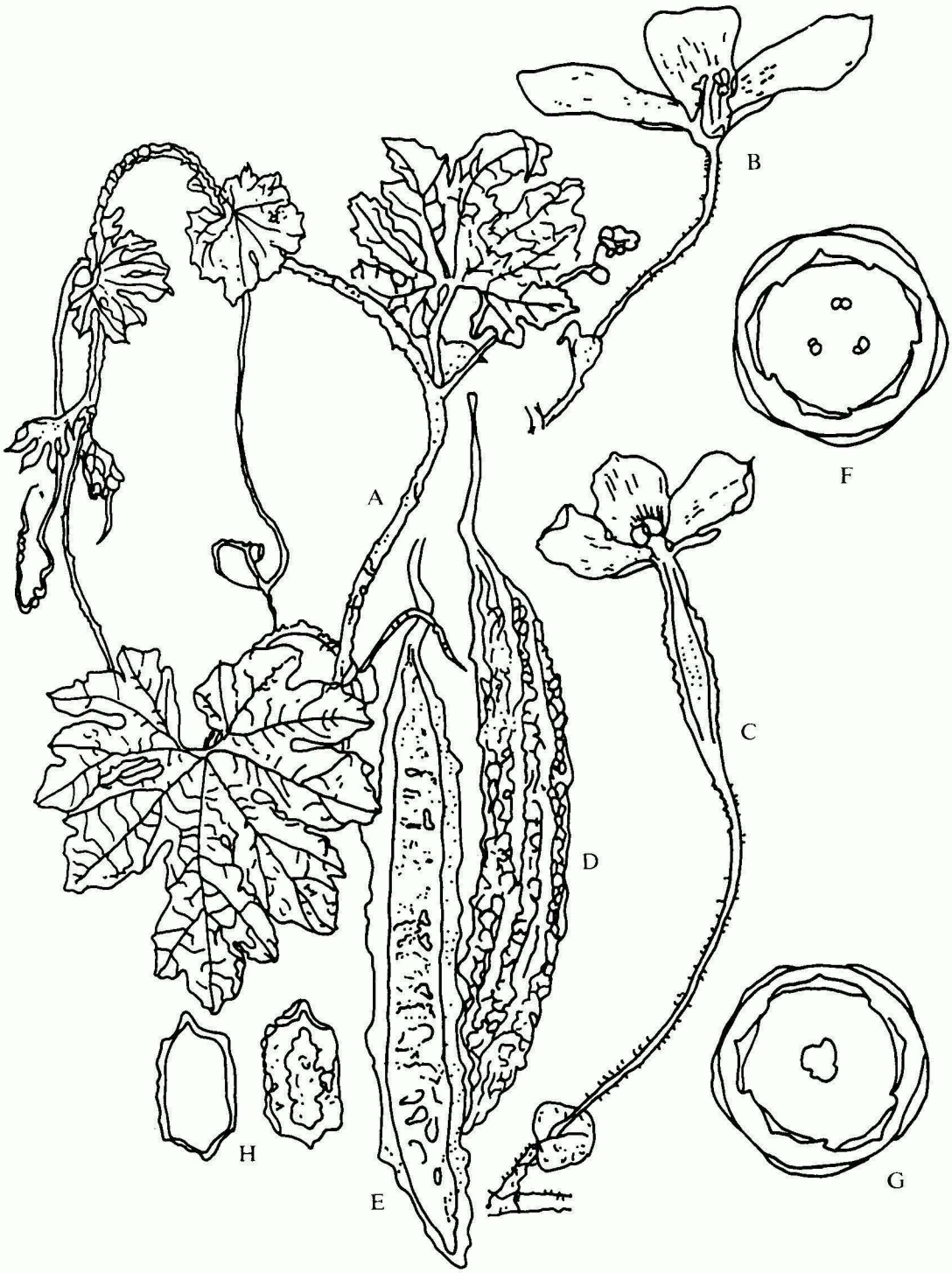

Fig. 1 *Momordica charantia* **Linn. (Mara-chean), showing leaves and tendrils, fruits, male and female flowers.**

A = shoot
B = staminate flower
C = pistillate flower
D = fruit
E = long section of fruit
F = cross section of fruit
G = external feature of seed
H = diagrammatic layer of seed

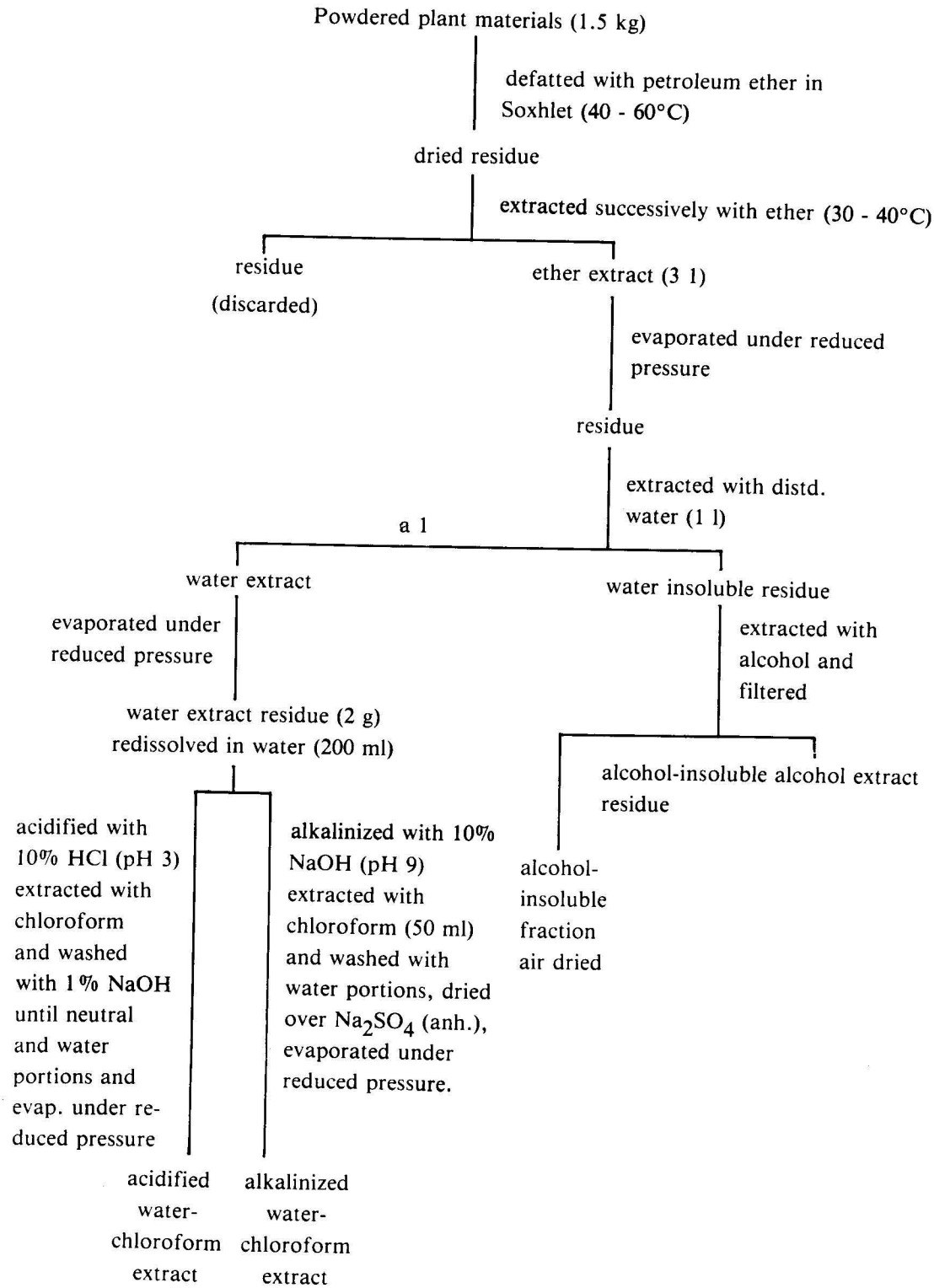

Fig. 2 Diagram showing the method of extraction of the fruits of *Momordica charantia* Linn.

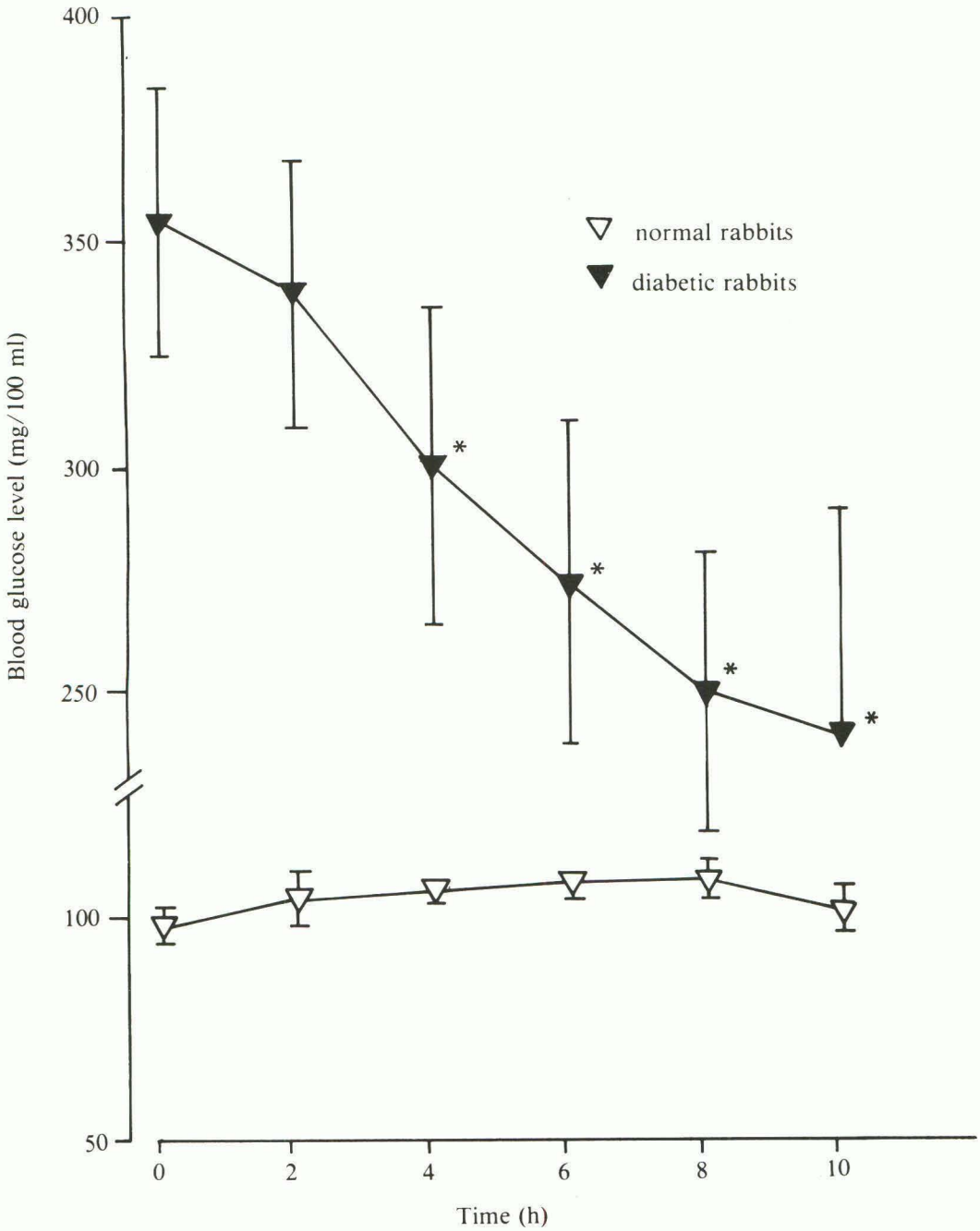

Fig. 3 Effect of intravenous administration of acidified water-chloroform extract at 20 mg/kg in normal and diabetic rabbits. (* $P < 0.01$)

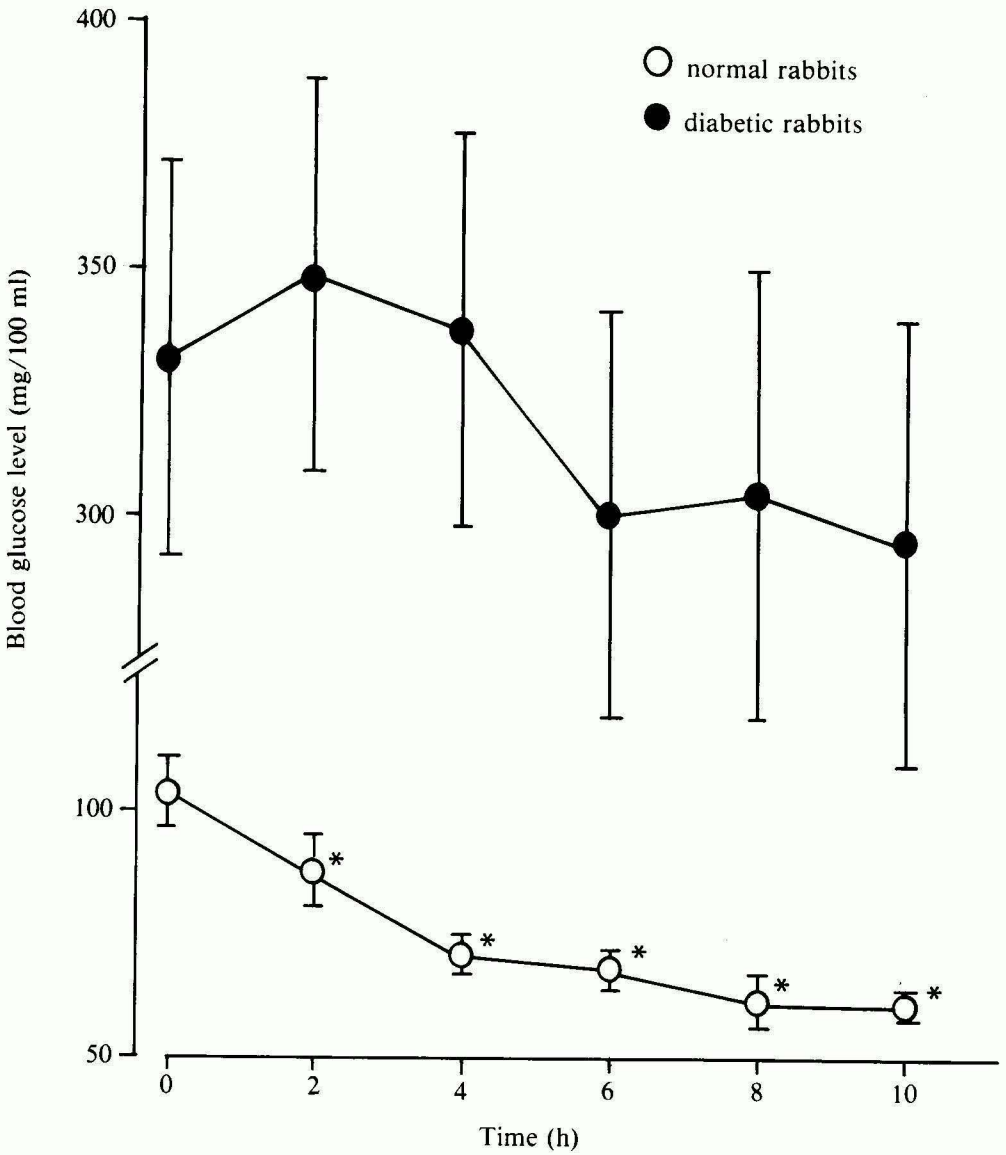

Fig. 4 Effect of oral administration of 100 mg/kg tolbutamide in normal and diabetic rabbits. (* $P < 0.01$)