Extra-pancreatic effects contributing to the hypoglycaemic activity of *Artocarpus heterophyllus*

M. R. Fernando[1]* and M. I. Thabrew[2]

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Abstract

Investigations were carried out to detemine whether the aqueous leaf extract of *Artocarpus heterophyllus* has extra-pancresatic effects which may contribute towards the observed hypoglycaemic activity of the leaf extract in rats. Administration of the leaf extract prior to glucose loading resulted in a significantly increased glycogen content in the liver (by 70%) and muscle (by 100%) and the triacylglycerol content in the adipose tissue (by 30%) in comparison with rats treated only with the glucose load. The leaf extract was also able to markedly inhibit the activity of insulinase. Pretreatment of fasted rats with the leaf extract of *Artocarpus heterophyllus* had no significant effect on the gluconeogenic capacity of kidney slices. Similar results were obtained with kidney slices pre-incubated with aqueous leaf extract of *Artocarpus heterophyllus*. The leaf extract had no effect on the intestinal glucose absorption.

Keywords: *Artocarpus heterophyllus*; medicinal plants; rats; mechanism of action; gluconeogenesis; triacylglycerol synthesis.

Introduction

Diabetes mellitus is the most common endocrine disorder and billions of people around the world are affected by it. This disease is characterized by hyperglycaemia due to the deficiency or diminished effectiveness of insulin. Ayurvedic physicians in Sri Lanka use aqueous extracts of several plant species to control the blood glucose concentrations in diabetic patients (1,2). Approximately 40 plants available in Sri Lanka are reputed to have oral hypoglycaemic activity. *Artocarpus heterophyllus* (L) Sinhala: Kos; Tamil: Pala; of the family Moraceae is a medical plant recommended by Ayurvedic physicians in Sri Lanka for the treatment of diabetes mellitus (1, 2). Previous studies carried out by Fernando *et al* (3) with laboratory animals, healthy human volunteers and diabetic patients provided evidence for the existence of oral hypoglycaemic activity in aqueous leaf extracts of *Artocarpus heterophyllus*. There are three main mechanisms by which oral hypoglycaemic drugs lower blood glucose concentrations; (1) release of insulin from β cells, (2) reduction of serum glucagon concentration and (3) extra-pancreatic effects. The present studies were undertaken to investigate the possible extra-pancreatic actions of *Artocarpus heterophyllus* which may contribute towards leaf extract mediated hypoglycaemic action. Investigations have therefore been carried out to determine the effects of the aqueous leaf extracts of *Artocarpus heterophyllus* on insulinase activity, intestinal glucose absorption, glycogen synthesis in liver and muscle tissue, triacylglycerol synthesis in adipose tissue, and gluconeogensis.

Material and Methods

Experimental animals

In all experiments, male Sprague-Dawley rats of body weight ranging from 125 to 175g were used and maintained on a standard laboratory diet and water *ad libitum*. The animals used for glycogen and triacylglycerol synthesis studies were fasted overnight before commencement of experiments. For studies on gluconeogenesis, the animals were fasted for 48 hours.

Preparation of *Artocarpus heterophyllus*

The leaf extract was prepared according to the method normally used by traditional medical

1. *Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.*
2. *Department of Biochemistry and Clinical Chemistry, University of Kelaniya, Talagolla Road, Ragama, Sri Lanka.*
* *Corresponding Author.*

practitioners for administration to diabetic patients. The methods quoted in medicinal plant books (1,2) were confirmed by discussion with several well-regarded traditional medical practitioners in Southern Sri Lanka. Mature leaves (100g), were cut into small pieces and boiled with distilled water (800 ml) for 3 hours, filtered, and the final volume reduced to 200 ml by boiling. The botanical identity of the plant was determined by using the descriptions of Jayaweera (2) and confirmed by comparison with authentic samples from the herbarium of the University of Peradeniya. The leaves were collected from trees growing in Galle in Southern Sri Lanka and activity was tested in leaves collected throughout the year.

Preparation of insulinase

A non-fasted rat was killed by decapitation and as much blood as possible was drained from the carcass. The liver was removed, rinsed in cold phosphate buffer (pH 7.8) and homogenized in four volumes of the buffer at approximately 4° C. The homogenate was then centrifuged at 18000 g for 30 min at 4° C. The supernatant containing the insulinase was siphoned off and collected (4).

Effect of *Artocarpus heterophyllus* on insulinase activity

Male Sprague Dawley rats (n=4) were randomly divided into 2 groups of 2 each. Group 1 was given distilled water (1 ml/100g body weight) and group 2 was given the aqueous leaf extract of *Artocarpus heterophyllus* (1 ml/100g body weight). The rats given distilled water were killed and a crude insulinase preparation prepared as described above. The rats given *Artocarpus heterophyllus* extract were killed at 3 hours after oral administration of the plant extract and a crude insulinase preparation made as described above. A standard insulin preparation (80 units/ml) was mixed with 1.5 ml of phosphate buffer or the crude insulinase preparation as indicated below and incubated at 37° C for 30 minutes;

Incubation mixture A: 1.5 ml phosphate buffer (pH 7.8) + 0.5 ml insulin;

Incubation mixture B: 1.5 ml boiled crude insulinase preparation + 0.5 ml insulin;

Incubation mixture C: 1.5 ml insulinase from distilled water treated animals + 0.5 ml insulin;

Incubation mixture D: 1.5 ml insulinase from *Artocarpus heterophyllus* treated animal + 0.5 ml insulin.

Male rats (n=24) were fasted overnight. After collecting blood samples for the determination of the fasting blood glocouse level, rats were randomly divided into four groups of six each. Group 1 was administered 0.25 ml (5 insulin units) of incubation mixture A by injection into the tail vein. Group 2 was administered 0.25 ml of incubation mixture B. Group 3, was given 0.25 ml of incubation mixture C. Group 4 was given 0.25 ml of incubation mixture D. Blood samples were collected after 30 min. and 60 min. for blood glucose estimation.

Effect of the aqueous extract of *Artocarpus heterophyllus* on glycogen synthesis in the liver and muscle and triacylglycerol synthesis in the adipose tissue

Male Sprague Dawley rats (n=12) were fasted overnight and divided into 2 groups of six each. Group 1 was given distilled water (1 ml/100 g body weight) 30 min, prior to receiving the oral glucose load (1ml/100 g body weight, 50% w/v). Group 2 was given the leaf extract of *Artocarpus heterophyllus* (1 ml/100 g body weight) 30 min prior to receiving a similar oral glucose load. All animals were killed 3 hours after administration of the leaf extract or distilled water because *Artocarpus heterophyllus* exerts maximal hypoglycaemic activity 3 hours after administration (5). As soon as the animals were killed by decapitation, their livers, muscle tissue and adipose tissue were collected. The glycogen content of the liver and muscle was estimated according to the method described by Kemp and Van Heijningen (6) after elimination of free glucose in the tissue by extraction into methanol (50% w/v). Approximately 100 mg of adipose tissue was homogenized in isopropanol (4 ml) and centrifuged. The supernatant was then mixed well with 4 ml isopropanol. Using this supernatant, the triacylglycerol content of the adipose tissue was estimated by the method of Varley *et al* (7).

Effect of the extract of *Artocarpus heterophyllus* on glucose absorption in the intestine

Male Sprague Dawley rats (n=24) were fasted overnight and randomly divided into 4 groups of six each. Group 1 and 2 were given distilled water (1 ml/100 g body weight, 50% w/v) 30 min prior to receiving the oral glucose load. Group 3 and 4 were administered the leaf extract of *Artocarpus heterophyllus* (1 ml/100 g body weight) orally 30 min prior to receiving the oral glucose load (1 ml/100 g body weight). The animals of groups 1 and 3 were killed 2 hours after the administration of distilled water or plant extract by decapitation and their intestines removed. The animals of group 2 and 4 were killed 3 hours after the administration of distilled water or leaf extract by decapitation and their intestines removed. Glucose content in the whole intestine was estimated by the glucose oxidase method (8).

Effect of the extract of *Artocarpus heterophyllus* on gluconeogenesis

***In-vivo* assessment**

Male Sprague Dawley rats (n = 12) were fasted for 48 hours and randomly divided into 2 groups of six each. Group 1 was given distilled water (1 ml/100 g body weight, orally). Group 2 was administered the leaf extract of *Artocarpus heterophyllus* orally (1 ml/100 g body weight, orally). The animals were killed 3 hours after the administration of distilled water or leaf extract by decapitation and their kidneys were removed. The kidneys were rinsed in cold saline, sliced and cut into small pieces of 30 - 40 mg. The gluconeogenesis of the test and control kidney tissues were then estimated by the method described by Tutwiler and Brentzel (9).

***In-vitro* assessment**

A male Sprague Dawley rat was fasted for 48 hours, killed by decapitation and then the kidneys were removed and rinsed in cold saline, sliced and cut into small pieces of 30 - 40 mg. These kidney slices were divided into 2 groups, a test group and a control group. The test group of kidney slices were pre-incubated in Krebs-Ringer bicarbonate buffer (4 ml) supplemented with *Artocarpus heterophyllus* leaf extract (3.4 ml buffer + 0.6 ml leaf extract). The control slices were pre-incubated in buffer alone. Subsequently, both test and control groups of kidney slices were individually incubated in 4 ml Krebs-Ringer bicarbonate buffer supplemented with pyruate (0.01 M final concentration) for 60 min. at 37° C with constant shaking. The medium was then removed for estimation of the glucose by the glucose oxidase method (8) and the tissues were dried and weighed as before (9).

Results and Discussion

The *Artocarpus heterophyllus* leaf extract caused a marked increase in the glycogen concentration in both liver and muscle tissue (Figure 1). The mean glycogen content in the livers of the *Artocarpus heterophyllus* treated rats (60 ± 6.5 mg equivalents of glucose/g liver tissue) was significantly higher ($p<0.001$) than that of the controls (35 ± 4.5 mg equivalents of glucose/g muscle tissue) and the mean glycogen content of muscle of the treated rats (32 ± 6.5 mg equivalents of glucose/g muscle tissue) was significantly higher ($p<0.01$) than that of the controls (16 ± 5 mg equivalents of glucose/g muscle tissue). The effects of *Artocarpus heterophyllus* on glycogen synthesis parallels that demonstrated by the juice of fruits of *Momordica charantia*, another plant reputed to have hypoglycaemic activity (4).

The Artocarpus heterophyllus leaf extract caused a significant increase in triacylglycerol synthesis in the adipose tissue as compared with water-treated controls (Figure 2). The mean triacylglycerol content in adipose tissue of the *Artocarpus heterophyllus* treated rats (890 ± 7 mg/g adipose tissue) was significantly higher ($p< 0.001$) than that in the controls (800 ± 10 mg/g adipose tissue). The effects of *Artocarpus heterophyllus* on triacylglycerol synthesis appears to be different to that demonstrated by *Momordica charantia*. An extract of *Momordica charantia* fruits did not have any significant effect on triacylglycerol synthesis in adipose tissue of rats (4). Some hypoglycaemic agents like biguanides and active hypoglycaemic principles isolated from *Salacia reticulata* are known to exert their hypoglycaemic actions via inhibition

of intestinal glucose absorption (10, 11). However, as evident from data in Table 2 the leaf extract of *Artocarpus heterophyllus* had no effect on intestinal glucose absorption when compared with treated controls. The mean glucose content in the intestines at 2 hours of the *Artocarpus heterophyllus* treated rats (1.91 ± 0.13 mg/rat) was not significantly different (P>0.5) from water-treated controls (1.75 ± 0.5 mg/rat). The mean glucose content in the intestines at 3 hours of the *Artocarpus heterophyllus* treated rats (0.66 ± 0.08 mg/rat) was also not significantly different (P>0.5) from water-treated controls (0.75 ± 0.14 mg/rat). The results demonstrate that the aqueous leaf extract of *Artocarpus heterophyllus* has no inhibitory effect on intestinal glucose absorption.

Some hypoglycaemic agents lower the blood glucose concentration by inhibiting gluconeogenesis (12). The effect of the oral treatment with *Artocarpus heterophyllus,* on the gluconeogenic capacity of the kidney slices obtained from 48 hours fasted rats are illustrated in Figure 3 (columns A and B). It is evident from the results that there is no difference between the gluconeogenic capacities of the test and control animals. Similar observations (Fig. 3, columns C and D) were made with the kidney slices pre-incubated *in vitro* with *Artocarpus heterophyllus* leaf extract. The overall data from these experiments show that alteration of gluconeogenesis is not one of the mechanisms by which *Artocarpus heterophyllus* exerts its hypoglycaemic action.

The results of the effects of aqueous leaf extract of *Artocarpus heterophyllus* on insulinase activity are shown in Table 1. As evident from Table 1, insulin, even after incubation for 30 min with phosphate buffer can markedly lower the blood glucose levels from a fasting blood glucose concentration in group A of 85 ± 2.9 mg/dL to 17 ± 2.8 mg/dL at 30 min and 25 ± 3.1 mg/dL at 60 min respectively. A similar lowering of fasting blood glucose level is also produced by insulin that was incubated with boiled insulinase preparation (in group B, fasting blood glucose concentration was 79 ± 3.8 mg/dL and at 30 min and 60 min it was 19 ± 4.1 mg/dL and 24 ± 4.5 mg/dL respectively). This establishes that there is no non-enzymic interference (with insulin) by the liver homogenate. The mean fasting blood glucose concentration of group C was 82 ± 2.6 mg/dL. The blood glucose concentration at 30 min and 60 min were 37 ± 4.6 mg/dL and 40 ± 2.8 mg/dL respectively. The hypoglycaemia produced in group C by the insulin that was incubated with the control insulinase preparation (from the livers of the distilled water treated rats) was comparatively low. Statistical analysis by the student's t-test revealed a significant difference between the group A and group C (0.05>P>0.02). This reduction in the potency of insulin is supposed to be due to the destruction of insulin by insulinase present in the liver homogenates (14). The hypoglycaemia produced by the insulin that was incubated with insulinase preparation from the livers of the *Artocarpus heterophyllus* treated rats was similar to that produced by the insulin that was incubated with phosphate buffer (21 ± 5.1 mg/dL and 28 ± 4.9 mg/dL at 30 min and 60 min respectively). There was no statistical difference between group A and group D (P>0.5). The low reduction in the potency of insulin that was incubated with insulinase preparation from the livers of the *Artocarpus heterophyllus* treated rats may be due to the destruction or inhibition of insulinase in the liver by the aqueous leaf extract of *Artocarpus heterophyllus.* The half life of circulating insulin is 3-5 minutes (13). The liver and kidney are the two main organs that remove insulin from the circulation by hydrolysis through the action of insulinase. The fact that insulinase activity is markedly inhibited by the aqueous leaf extract of *Artocarpus heterophyllus* suggests that one of the mechanisms by which this extract achieves its hypoglycaemic effect is by preventing breakdown of insulin in circulation. The increased glycogen synthesis in the liver and muscle and increased triacylglycerol synthesis in the adipose tissue following prior administration of the aqueous leaf extract of *Artocarpus heterophyllus* could well have been mediated via increased concentrations of insulin resulting from an inhibition of insulinase activity by the leaf extract. The result obtained in the present study indicates that the aqueous leaf extract of *Artocarpus heterophyllus* can exert significant extra-pancreatic effects through which the hypoglycaemic actions of this leaf extract may be achieved.

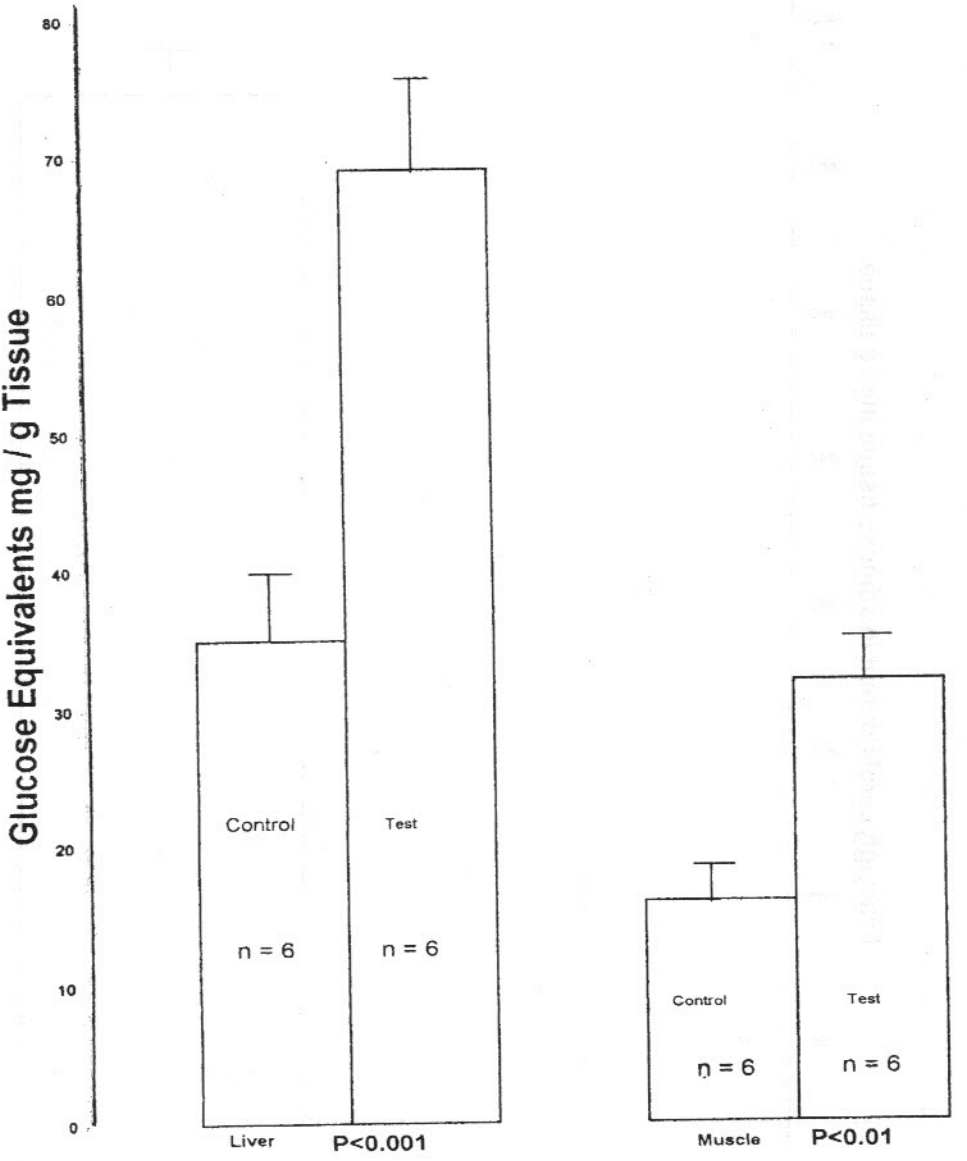

Figure 1: Effect of the aqueous leaf extract of *Artocarpus heterophyllus* on liver and muscle glycogen synthesis. After overnight fasting rats were given the aqueous leaf extract of *Artocarpus heterophyllus* (10 mL/kg body weight) 30 min prior to receiving a glucose load (50% w/v, 10 mL/kg body weight). The control animals were given an equivalent dose of distilled water prior to the glucose load. The animals were killed 3 hours after leaf extract or distilled water administration and their livers and muscle tissues removed for glycogen assay. The results are given as glucose equivalents mg/g wet tissue. The bars show standard errors.

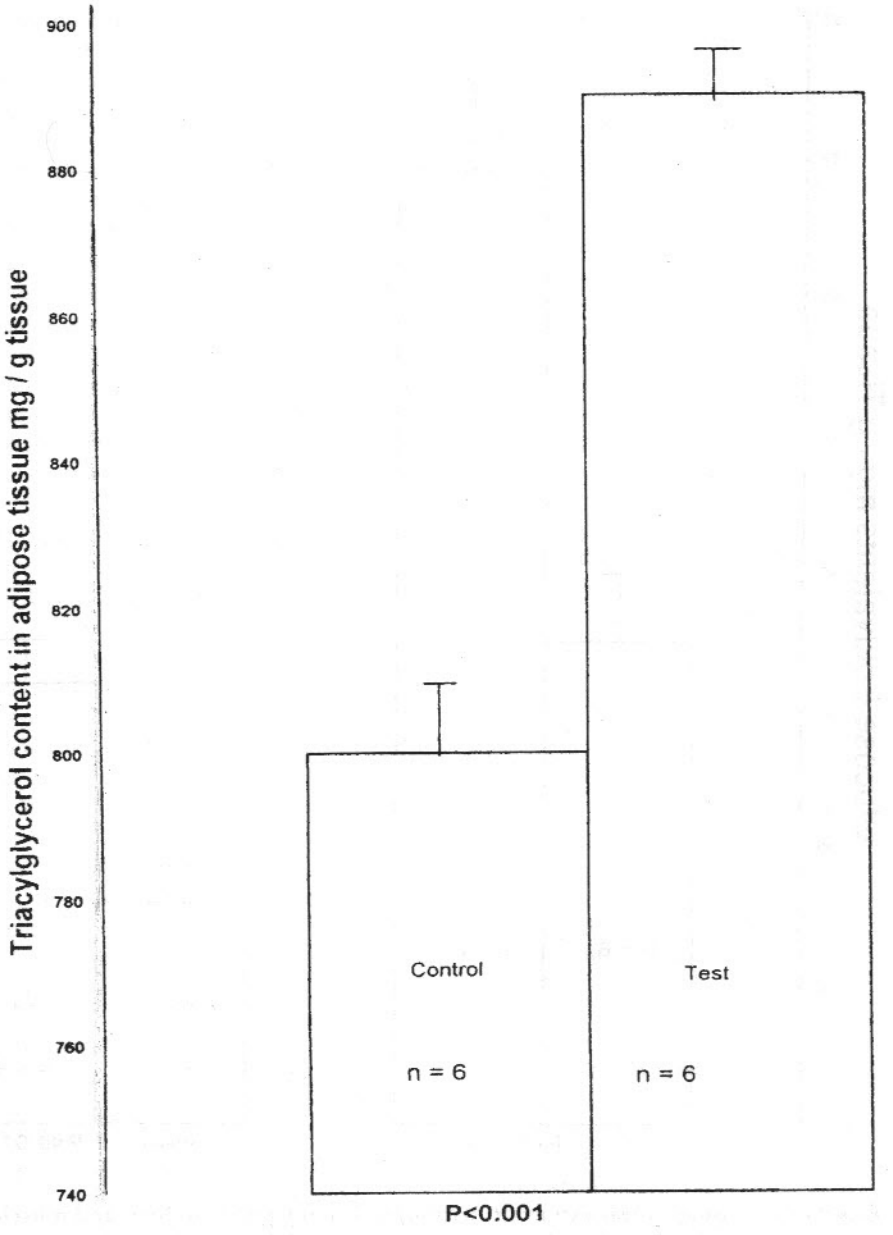

Figure 2: Effect of the aqueous leaf extract of *Artocarpus heterophyllus* on triacylglycerol synthesis in adipose tissue. After overnight fasting rats were given the aqueous leaf extract of *Artocarpus heterophyllus* (10 mL/kg body weight) 30 min prior to receiving a glucose load (50% w/v, 10 mL/kg body weight). The control animals were given an equivalent dose of distilled water prior to the glucose load. The animals were killed 3 hours after drug or distilled water administration and their adipose tissues removed for triacyglycerol assay. The results are given as mg/g wet tissue. The bars show standard errors.

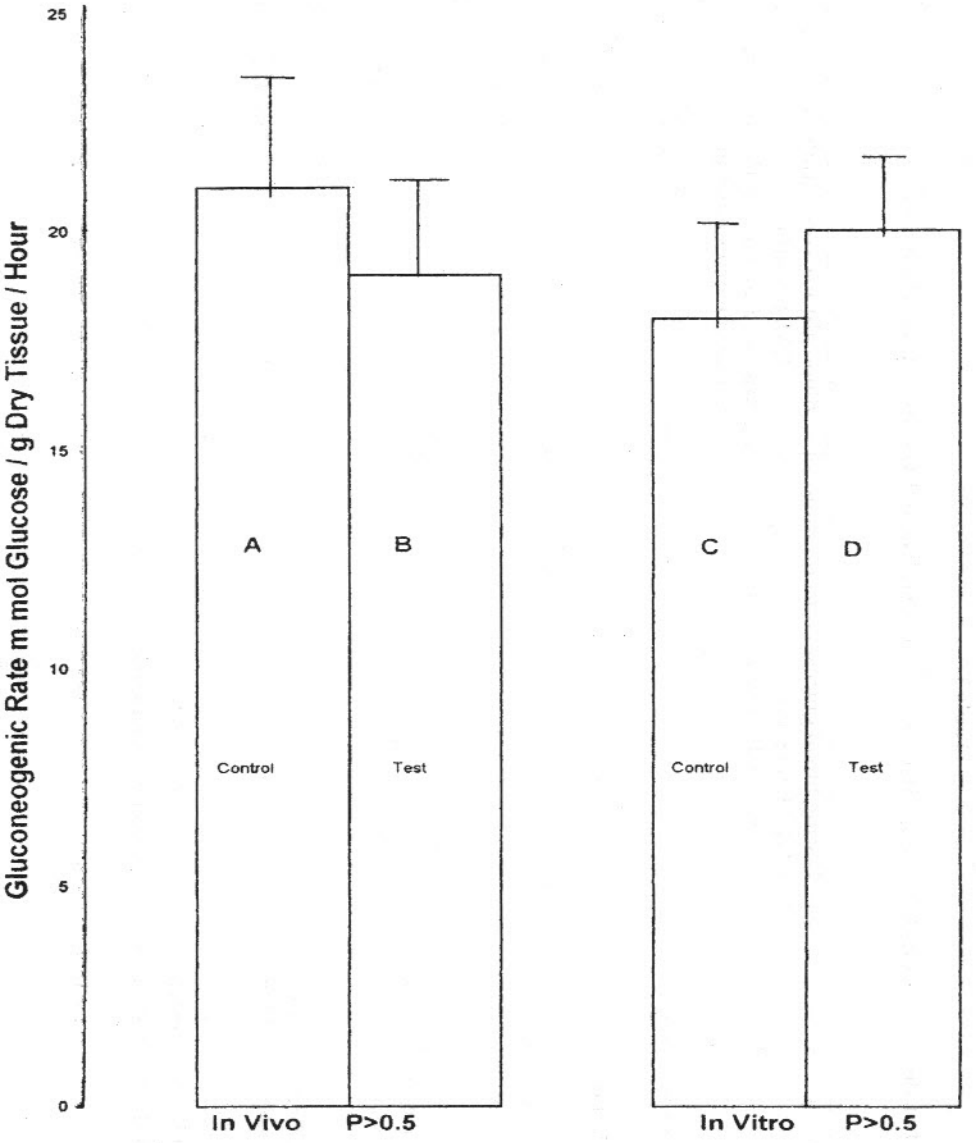

Figure 3: Effect of the aqueous leaf extract of *Artocarpus heterophyllus* on gluconeogenesis. Columns A (n = 6) and B (n=6) illustrate the effect of pre-treatment of 48 hours fasted rats with distilled water and *Artocarpus heterophyllus* extract respectively (10 mL/kg body weight) on the gluconeogenic capacity of kidney slices. Columns C (n=6) and D (n=6) illustrate then effect of pre-incubation of kidney slices with buffer and *Artocarpus heterophyllus* extract, repectively, on their gluconeogenic capacity. In each instance gluconeogenesis was estimated by incubating the kidney slices in Krebs Ringer bicarbonate medium supplemented with pyruvate substrate (0.01 M), for 60 min at 37° C. The results are given as m mol glucose/mg dry tissue/hour. The bars show standard errors.

Table 1

Effect of the aqueous leaf extract of *Artocarpus heterophyllus* on liver insulinase activity

Substance injected to rats (dosage, 0.25mL/rat	**Fasting blood glucose concentration ± S.E.M. (mg/dL) (mean of 6 determinations)**	**Blood glucose concentration ± S.E.M. (mg/dL) After injecting incubation mixtures (mean of 6 determinations)**	
		+ ½ h	**+1 h**
Incubation mixture A (buffer, 1.5 mL + Insulin, 0.5 mL) Group 1	85 ± 2.9	17 ± 2.8	25 ± 3.1
Incubation mixture B (boiled insulinase from water treated rats, 1.5 mL + insulin, 0.5 mL) Group 2	79 ± 3.8	19 ± 4.1[a]	24 ± 4.5[a]
Incubation mixture C (insulinase from water treated rats, 1.5 mL + insulin, 0.5 mL) Group 3	82 ± 2.6	37 ± 4.6[b]	40 ± 2.8[b]
Incubation mixture D (insulinase from *Artocarpus heterophyllus* treated rats, 1.5 mL + insulin, 0.5 mL Group 4	86 ± 3.1	21 ± 5.1[a]	28 ± 4.9[a]

[a] When examined by the student's t-test, not significantly different from group 1 ($p>0.5$)

[B] When examined by the student's t-test, significantly different from group 1 ($0.05 > p > 0.02$)

Table 2

Effect of the aqueous leaf extract of *Artocarpus heterophyllus* on glucose absorption in the intestine

Substance administered (dosage 10 mL/kg)	Glucose content in the intestine ± S.E.M. (mg) at 2 h and 3 h after drug administration	
	2 h	3 h
Distilled water (control group)	1.75 ± 0.5	0.75 ± 0.14
Artocarpus heterophyllus leaf extract	1.91 ± 0.13*	0.66 ± 0.08*

Aqueous leaf extract of *Artocarpus heterophyllus* or distilled water (10 mL/kg, orally) were administered to rats 30 min prior to receiving the oral glucose load (10 mL/kg of 50% w/v glucose). At +2 hours and ± 3 hours after the glucose load, the animals were killed and the glucose content in the intestines of the four groups estimated. Each value is the mean of 6 determinations ± S. E. M. When examined by student's t-test, not significantly different from control; *$p > 0.05$.

References

1. Attygalle J. *Sinhalese Materia Medica*, 2nd Edn. Colombo: M.D. Gunasena and Co. Ltd., 1952; 44-45.

2. Jayaweera, D.M.A. *Medicinal plants used in Ceylon.* Colombo: National Science Council of Sri Lanka 1982; 9.

3. Fernando, M.R., Wickramasinghe, S.M.D.N., Thabrew M.I., Ariyananda, P.L. and Karunanayake, E.H. Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. Journal of Ethnopharmacology 1991; 31: 277-282.

4. Welihinda, J. A Biochemical study of the oral hypoglycaemic activity of Momordica charantia. Doctoral dissertation, Faculty of Medicine, University of Colombo, Sri Lanka 1987.

5. Fernando, M.R., Thabrew, M.I. and Karunanayake, E.H. Hypoglycaemic activity of some medicinal plants in Sri Lanka. General Pharmacology 1990; 21: 779-782.

6. Kemp, A. and Heinjningen, A.K.J.M. A colorimetric micro-method for the determination of glucose in tissues. British Medical Journal 1954; 56: 646-648.

7. Varley, H., Gowenlock, A.H. and Bell, M. Practical Clinical Biochemistry, London, William Heinemann Medical Books Ltd., 1980; 673-674.

8. Hugget, A.St.G. and Nixon, D.A. Use of glucose oxidase, peroxidase and O-dianisidine in determination of blood and urinary glucose. Lancet 1957; 2: 368-381.

9. Tutwiler, G.F. and Brentzel, H.J. Effect of the oral hypoglycaemic agent pirogliride on gluconeogenesis, Biochemical Pharmacology 1980; 29: 1421-1427.

10. Bosello, O., Cominacini, L., Zocca, I., Garbin, V., Ferrari, F. and Davoli, A. Effect of guar gum on plasma lipoproteins and apolipoproteins C-11 and C-111 in patients affected by familial combined hyperlipoproteinemia. American Journal of Clinical Nutrition 1984; 40: 1165-1175.

11. Ruvin Kumara, N.K. V.M. An investigation of the hypoglycaemic properties of *Salacia reticulata*. Ph.D thesis, Faculty of Medicine, University of Ruhuna, Sri Lanka 2000;

12. Patrick, S.J. Effect of phenformin and hypoglycin on gluconeogenesis of rat tissues. Canadian Journal of Biochemistry 1966; 44: 27-33.

13. Mirsky, I.A. and Broh-Kahn, R.H. The inactivation of insulin by tissue extracts. The distribution and properties of insulin inactivating extracts (insulinase). Archives Biochemica 1949; 20: 1-9.

14. Mirsky, I.A., Diengott, D. and Perisutti, G. The inhibition of insulinase by hypoglycaemic sulfonamides. Metabolism 1956; 5: 156-161.