



Research Paper

*Afr. J. Traditional,
Complementary and
Alternative Medicines*
www.africanethnomedicines.net

ISSN 0189-6016©2006

HYPOGLYCAEMIC ACTION OF THE FLAVONOID FRACTION OF *ARTOCARPUS HETEROPHYLLUS* LEAF

*Chandrika U. G¹, Wedage W. S., Wickramasinghe S. M. D. N¹ and Fernando W. S²

¹Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka, ²Department of Chemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

E-mail: chandri@sjp.ac.lk

Abstract

Hot water extract of mature jak leaves (*Artocarpus heterophyllus*) is recommended by Ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus. The leaf extract caused the hypoglycaemic effect at a dose of 50 mg/Kg, both in normal and alloxan-diabetic rats. The hypoglycaemic effect was at its maximum 2 h after flavonoid fraction administration, and multiple dosing maintained the activity for a week. The hypoglycaemic effect of the flavonoid fraction of leaf (49%) is higher than that of tolbutamide (27.0%), a sulphonyl urea drug commonly used for treatment of hyperglycaemia. Administering the flavonoid fraction for 3 months had no significant effects on liver function while the histology of liver, kidney and heart revealed no damage. These results indicate that the total flavonoid content of *A. heterophyllus* leaf exhibited a non-toxic and significant hypoglycaemic activity in male Wistar rats and may therefore be responsible for the previously reported antidiabetic activity.

Key words: *Artocarpus heterophyllus*, flavonoids, hypoglycaemic, rats

Introduction

Hot water extract of mature jack leaves (*Artocarpus heterophyllus* Lam, Family: Moraceae) is recommended by Ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus (Jayaweera, 1982). Previous studies have indicated that an extract of *Artocarpus heterophyllus* improves the glucose tolerance in normal human subjects and diabetic patients (Fernando *et al.*, 1991). Phytochemical screening has revealed that the hot water extract contains flavonoids, leucoanthocyanins, anthocyanins and tannins as components (Mahatantila and Chandrika, 1998). Recent studies showed that the flavonoid

fraction has the highest hypoglycaemic activity (Chandrika *et al.*, 2002). The value of any hypoglycaemic agent depends not only on its hypoglycaemic potency but also on its lack of toxicity. The objective of this paper was therefore to study the plant extract responsible for the hypoglycaemic activity of the flavonoid fraction of the extract using normal and alloxan-diabetic Wistar rats and determine any possible toxicological effects mediated by the long-term administration of active fraction.

Materials and methods

Plant material

The botanical identity of *Artocarpus heterophyllus* was confirmed by Dr. P. Mahagamasekara of the Department of Botany, University of Sri Jayewardenepura, Sri Lanka. A voucher specimen (USJP FMS 4) has been deposited at the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

Preparation of the Polysaccharide, Proanthocyanidins and flavonoid fractions

The hot water extract was prepared by boiling 200 g of fresh and mature leaves with 1 L of distilled water for 3 h. The final volume was reduced to 200 ml. The water extract was centrifuged and the supernatant was obtained. An excess of ethanol was added to the supernatant to precipitate the high molecular weight polysaccharide fraction (1g), which was filtered and concentrated in vacuum and extracted with ethyl acetate. The ethyl acetate soluble fraction (flavonoid fraction, 2g) and the aqueous fraction (rich in proanthocyanidin fraction, 7g) were obtained. This resulted 20% flavonoid fraction. (200g fresh weight leaves contained 2g flavanoids (w/w))

Preparation of glucose

Glucose (25 g) obtained from the Glaxo-Wellcome Co., Sri Lanka, was dissolved in 25 ml distilled water.

Experimental animals.

Two months old male Wistar rats (average weight of 200 ± 8 g) obtained from the Medical Research Institute, Colombo, maintained under standard conditions, were used in this study.

Determination of blood glucose levels.

The blood samples were centrifuged at 3000 rpm for 15 min to separate the serum. The separated serum samples were analysed using Glucose Oxidase Reagent Kits (DMA,

USA). The absorbance of the coloured product produced was measured at 430 nm using Shimadzu UV-1201 UV spectrophotometer.

Effect of flavonoid fraction and tolbutamide on glucose loaded rats

The crude flavonoid fraction showed better activity than the crude extract and was therefore used for further studies, at the doses of 25, 50, 75 and 100 mg/kg body weight. Wistar rats (200 ± 11 g) were randomly assigned into 5 groups (Tests I - IV and Control), with five rats in each. Rats were fasted for 16 hrs and the doses (25, 50, 75, and 100 mg/kg) were administered to the groups I to IV respectively. To the control group (group V) 1ml of distilled water was given. After 30 minutes, a glucose load was administered. Ninety minutes after administration of glucose, venous blood (0.2 ml) was drawn and serum glucose concentrations were analysed immediately using a Reagent Kit (DMA, USA) employing the glucose oxidase method. Only 90 minutes post glucose blood sugar level was determined according to WHO criteria since intermediate blood sampling is not necessary (Kumar and Clark, 1994).

Time course for flavonoid fraction

The time of maximum activity of the flavonoid fraction was determined using twenty male Wistar rats (214 ± 10 g, 8 weeks old) divided randomly into a test and a control with 10 rats in each group (groups A and B). After an overnight (16 h) fast the test group was given 1ml of flavonoid fraction at a dose of 50mg/kg body weight while the control group was administered 1 ml of distilled water. After 30 minutes, a glucose load (3 g/kg body weight) was administered. Venous blood was drawn (0.2 ml) at 0, 1, 2 and 3 h post glucose administration for blood glucose level determination.

Effect of multiple doses of flavonoid fraction on blood sugar levels of Wistar rats

Twenty male rats, 10 weeks of age and weight 224 ± 10 g were divided randomly into 2 groups. Each rat in the test group was given 1ml of the flavonoid fraction (50mg/kg body weight/day) for one week and the control group was given distilled water in place of the extract for one week. At the end of one week rats were fasted for 16 h, the test group was administered 1 ml of the flavonoid fraction and the control group was given 1 ml of distilled water. After 30 minutes, a glucose load was administered. Venous blood was drawn after 90 minutes for glucose determination.

Comparison of the oral hypoglycaemic activity of flavonoid fraction with the oral hypoglycaemic drug tolbutamide

Twenty-four rats were randomly divided into three groups; the test, the positive and the negative control with eight rats in each. Rats were fasted for 18hrs and 0.5 ml of venous blood was drawn under anaesthesia for fasting blood glucose estimation. The test group and control were similarly given 1 ml of water and the flavonoid extract orally at a

dose of 50 mg/kg body weight. A suspension of the oral hypoglycaemic agent, tolbutamide, (obtained from the State Pharmaceutical Corporation) in distilled water at a dose of 15 mg/kg body weight. After 30 min, rats were orally administered a glucose load of 3g/kg body weight was the positive control. Sondi needles were employed for the administration of these solutions. Venous blood was drawn after one hour under anaesthesia and serum glucose concentrations were analyzed immediately using a Reagent Kit (DMA, USA) employing the glucose oxidase method (Hugget and Nixon, 1957)

Studies on alloxan-diabetic rats

The hypoglycaemic activity of the flavonoid fraction was tested in alloxan-induced diabetic rats. Rats were made diabetic by the intravenous injection of alloxan monohydrate dissolved in sterile normal saline at a dose of 40 mg/kg body weight. Rats were randomly assigned into two groups and the control group was given the standard feed and water *ad libitum*; while the test group was orally administered flavonoid fraction (50 mg/kg) in addition to the standard diet daily for one week. At the end of the week, the rats were fasted overnight and subjected to a glucose challenge test accordingly.

Liver function test

Male rats (n = 20) were randomly assigned into two groups of ten each. The control group was given distilled water and the test group was given orally 50 mg/kg body weight of the flavonoid fraction daily for 12 weeks. At the end of one week, two weeks and 12 weeks, the blood was collected for liver function tests and at the end of experiment animals were sacrificed and organs were removed for the haematology. The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), L- γ - Glutamyl transferase (GGT) and alkaline phosphotase (ALP) were estimated using commercial reagent kits (Roch Diagnostics GmbH, D-68298 Mannheim, Germany)

Histological study of the effect of treatment

The organs (liver, heart, and kidney) were excised and fixed in formalin, buffered with sodium phosphate buffer for histological assessment of tissue damage, after heamatoxylin-eosin staining. The histological examination of sections was carried out in the Department of Anatomy, University of Sri Jayewardenepura, Sri Lanka.

Statistical analysis

All the results are presented as Mean \pm S.E.M. Data pairs, were compared by using the Student's t-Test in Microsoft Excel. A probability level of $P < 0.05$ was chosen as the criterion of statistical significance.

Results

Figure 1 shows the effect of the different doses of flavonoid fraction on the mean serum glucose concentrations in glucose loaded rats. All the groups treated with the extract had lower blood glucose than the control group while the maximal reduction ($P < 0.05$; 37%) was found at a dose 50-mg/Kg body weight. Table 1 shows there was a significant reduction in serum glucose concentrations in the test group, at 1 and 2 hours after administration of the plant extract compared to the control group. The maximal reduction ($P < 0.05$; 36.9%) in serum glucose was obtained at 2 h.

The effect of multiple doses of flavonoid fraction on serum glucose levels in a glucose loaded rats is shown in Table 2. There was a 40.9% reduction in the test group compared with the control and this reduction was statistically significant ($p < 0.05$). Table 2 also shows the effect of flavonoid fraction and tolbutamide on glucose loaded rats. There was a 49% reduction in the flavonoid fraction treated group compared with the control and this reduction was statistically significant ($p < 0.05$).

Table 3 shows the effect of multiple doses of flavonoid fraction on fasting levels and post-glucose blood glucose levels of diabetic rats. The fasting blood glucose level of the test group was significantly lower (29.7% reduction, $P < 0.05$) than that of control group. The serum glucose concentration after 90 min post glucose in the test group

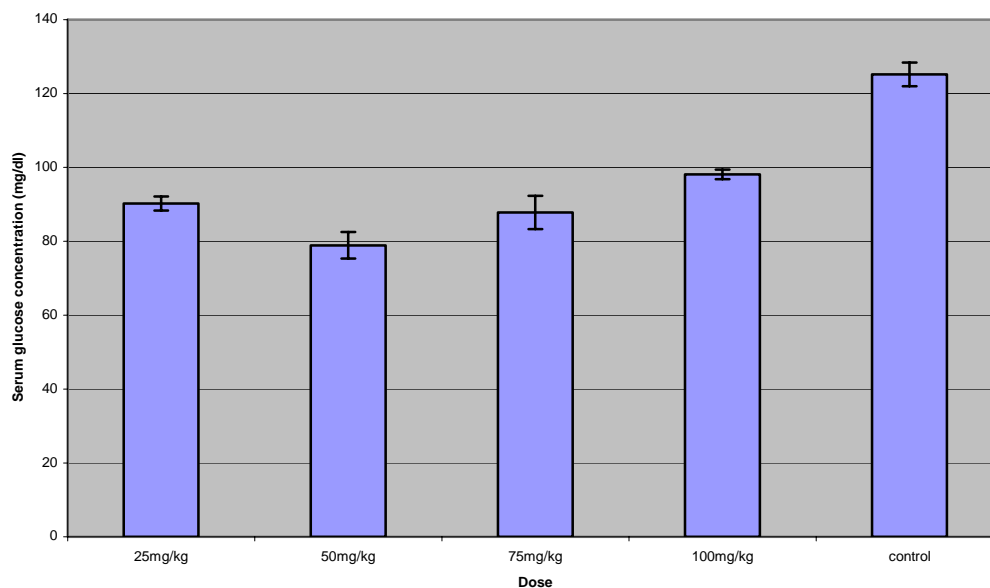


Figure 1. Effects of different doses of flavonoid fraction of *A. heterophyllus* on serum glucose concentration in glucose loaded rats (n = 5 per group).

showed a 51% reduction compared with the control and this reduction was statistically significant ($P < 0.05$).

The results shown in Table 4 indicate that administering the extract for one or two weeks and three months respectively had no effect on the serum levels of alanine transaminase, aspartate transaminase, L- γ -Glutamyl transferase and alkaline phosphotase enzymes. Likewise, comparison of histological sections of the liver, heart and kidney of the treated and control animals with those of animals treated with flavonoid fraction for three months, showed no difference between the two groups.

Discussion

At all the tested doses (25 to 100 mg/kg body weight), the extract effectively reduced the blood glucose levels, but the dose that exerted the optimal effect was 50 mg/kg body weight. Hence this dose was chosen for further determination of the antidiabetic activity of the leaf. The optimal time of the hypoglycaemic activity of the flavonoid fraction was found to be 2 h from the time of administration of the plant extract (Table 1) showing the activity to be of a short duration. However in the multiple dose experiment (Table 2), glucose challenge was performed 24 h after the administration of the last dose and a significant reduction in the serum glucose levels was still observed in the test group as compared with the control group may provide evidence for the long-term activity of the flavonoid fraction. The results shown in Table 2 indicates that the hypoglycaemic effect of

Table 1. Effect of flavonoid fraction (150 mg/kg) of *A. heterophyllum* on glucose loaded rats at 0, 1, 2 and 3h after administration^b

Time (h)	Mean serum glucose concentration \pm S.E.M. (mg/dl)		% Reduction in test compared with control
	Test	Control	
0	75.6 \pm 5.7	78.6 \pm 4.9	-
1	100.5 \pm 2.9	121.9 \pm 3.1	17.5*
2	84.9 \pm 2.1	134.7 \pm 5.6	36.9**
3	101.1 \pm 4.4	107.1 \pm 3.1	5.5

^b n = 5 *, Significantly different from Control at $P = 0.005$; **, $P = 0.00007$.

Table 2. Effect of multiple doses of the crude flavonoids from jak leaf extract on serum glucose concentration

Group	Multiple dose		Single dose	
	Fasting	90 minutes post glucose	Fasting	90 minutes post glucose
Flavonoid fraction	95.6 ± 2.3	100.4 ± 2.1*	66.5 ± 8.9	74.0 ± 2.5*
Tolbutamide	96.3 ± 2.8	130.6 ± 4.6	70.3 ± 2.4	106.8 ± 4.1*
Control	97.2 ± 1.5	141.3 ± 3.7	69.5 ± 5.5	146.0 ± 2.1

the flavonoid fraction of *Artocarpus heterophyllus* (49%) is higher than that of tolbutamide (27.3%), a salphonyl urea drug commonly used for treatment of hyperglycaemia.

Data provided in Table 3 shows that the flavonoid fraction was effective in reducing the fasting blood glucose level (29.7% reduction, $P < 0.05$) as well as the post –glucose load blood sugar level (51% reduction, $P < 0.05$) in alloxan-induced diabetic rats.

The therapeutic value of any isolated plant extract depends not only on its pharmacological potency but also on its lack of toxicity. This is important in the case of hypoglycaemic drugs, which have to be administered over a relatively long period of time. Administering the flavonoid fraction for 3 months had no significant effects on the liver function and the histology of various body organs. The general condition of the animals

Table 3: Effect of multiple doses of flavonoid fraction (100 mg/kg) on serum glucose concentration of diabetic Wistar rats.

	Mean serum glucose concentration ± SEM (mg/dl)	
	Test	Control
Fasting	260.3 ± 8.5	258.2 ± 10.5
Fasting after one week	100.5 ± 5.9	142.8 ± 5.3
90 min Post glucose	125.7 ± 12.4*	257.3 ± 12.1

$P < 0.05$; $n = 10$ Values shown are the mean ± SEM of six determinations while those in parentheses represent % reduction of glucose with reference to control

Table 4: Effect of flavonoid fraction on liver enzymes

	Alanine amino transferase (Units/liter)		Aspartate amino transferase (Units/liter)		Alkaline phosphatase (Units/liter)		L- γ -Glutamyl Transferase (Units/liter)	
	Test	Control	Test	Control	Test	Control	Test	Control
One week	38.4 \pm 2.9	37.4 \pm 2.4	45.8 \pm 1.5	44.9 \pm 2.7	164.3 \pm 8.9	163.3 \pm 8.9	1.6 \pm 0.2	1.94 \pm 0.1
Two weeks	46.1 \pm 3.8	42.9 \pm 3.4	78.1 \pm 4.9	74.1 \pm 4.1	289.4 \pm 15.5	300.4 \pm 19.1	4.6 \pm 0.3	5.1 \pm 0.4
Three months	46.3 \pm 3.8	47.2 \pm 2.7	46.1 \pm 1.3	44.7 \pm 2.7	246.1 \pm 22.4	253.2 \pm 34.3	1.4 \pm 0.4	1.3 \pm 0.3

also did not change and all of them remained in good health through out the experimental period. These results indicate that flavonoid fraction of the hot water extract of *A. heterophyllus* leaf therefore appears to free from major toxic or unacceptable effects when administered for a period of three months. However, for a more definitive conclusion with regards to non-toxicity of the flavonoid fraction, a greater variety of animal species should be studied.

In contrast to oral antidiabetic drugs such as tolbutamide, exogenous insulin is well known to produce hypoglycaemia both in normal and alloxan-diabetic subjects (Goth, 1985). Therefore the flavonoid fraction, which produce hypoglycaemic effects both in normal and diabetic rats seems to act like insulin. In diabetic rats, the flavonoids are unlikely to act by stimulating the release of insulin as alloxan-treatment cause permanent destruction of β -cells. Further studies are needed to elucidate the precise mode of action.

Acknowledgement

This work was supported by University of Sri Jayewardenepura, Sri Lanka (Grant Asp/G/RE/2001/6).

References

1. Chandrika, U. G., Fernando, W. S., Wickramasinghe, S. M. D. N. and Wedage, W. S. (2002). Effects of proanthocyanidin and flavonoid fractions from hot water extract of jak leaves (*Artocarpus heterophyllus*) on the blood glucose levels in healthy male Wistar rats. *Chemistry in Sri Lanka* **19** (2): 10
2. Fernando, M. R., Wickramasinghe, S. M. D. N., Thabrew, M. I., Ariyaratne, P. L. and Karunanayake, E. H., (1991) *Journal of Ethnopharmacology* **31**, 277-282
3. Goth, M. D. Medicinal Pharmacology (9th edn) pp 471-480. C. V. Mosby, St. Louis 471-480.

Afr. J. Trad. CAM (2006) **3** (2): 42 - 50

4. Hugget, A. S. G., and Nixon, D. A., (1957). Use of glucose oxidase, peroxidase and O-dianisidine in determination of blood and urinary glucose. *Lancet* **2**, 368-370
5. Jayaweera, D. M. A. (1982). Medicinal plants used in Ceylon 4-89. Part 11, National Science Council: Sri Lanka, Colombo. 4-89.
6. Kumar P. and Clark M, (1994). Clinical Medicine. A test book for medical students and doctors. 3rd edition. W. B. Saundern : London; 834.
7. Mahatantila, K. C. P. and Chandrika, U. G., (1998). Ayurveda Sameekshawa, **50**: 243-245.