

Effects of proanthocyanidin and flavonoid fractions of Jack leaves (*Artocarpus heterophyllus*) on the blood glucose levels in healthy, male Wistar rats.

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Abstract - Hot water extract of mature Jack leaves (*Artocarpus heterophyllus*) is recommended by Ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus. Previous studies have indicated that an extract of *Artocarpus heterophyllus* improves the glucose tolerance in normal human subjects and diabetic patients¹. Phytochemical screening has revealed that the hot water extract contains flavonoids, leucoanthocyanins, anthocyanins and tannins as components^{7,8}. The objective of this study was to investigate the hypoglycaemic effects of polysaccharide, proanthocyanidin and flavonoid fractions obtained from *Artocarpus heterophyllus* leaves using healthy male Wistar rats as the experimental model. These fractions were isolated by solvent extraction using ethanol and ethyl acetate as the solvents. There was a significant reduction in the blood glucose concentrations with both proanthocyanidin (54.8%, $p < 0.001$) and flavanoid (55.9%, $p < 0.001$) fractions in Wistar rats compared to the control. Medium Pressure Liquid Chromatography (MPLC) of the flavonoid fraction yielded seven sub fractions out of which four exhibited significant hypoglycaemic activity in Wistar rats. It was also evident that the hypoglycaemic effect of individual fractions is higher than the effect of the hot water extract (46%) indicating that crude extract contains inhibitory factor that can interfere with the hypoglycaemic effect.

Key words: *Artocarpus heterophyllus*, proanthocyanidin, hypoglycaemic activity.

Introduction

A great deal of attention has been paid recently to the use of herbal remedies for the treatment of diabetes mellitus. Several studies have been conducted in Sri Lanka to determine the hypoglycaemic effect of different plant materials^{1,2,5,9,10,11}. Hot water extract of mature Jack leaves (*Artocarpus heterophyllus*) is also recommended by Ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus^{3,4}. Previous studies have indicated that an extract of *Artocarpus heterophyllus* improves the glucose tolerance in normal human subjects and maturity onset diabetic patients¹. Phytochemical screening has revealed that the hot water extract contains flavonoids, leucoanthocyanins, anthocyanin and tannins as components^{7,8}. The objective of this study was to investigate the hypoglycaemic effects of the polysaccharides,

proanthocyanidin and flavonoid fractions of *Artocarpus heterophyllus* using healthy male Wistar rats as the experimental model.

Materials and Methods

Plant material

The botanical identity of *Artocarpus heterophyllus* was confirmed by Dr. P. Thisera of the Department of Botany, University of Sri Jayewardenepura.

Preparation of Polysaccharide,

Proanthocyanidins and flavonoid fractions

The hot water extract was prepared by boiling 200 g of fresh, mature Jack leaves with 1L of distilled water for 3h. The final volume was reduced to 200ml (1g/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.

The filtrate was concentrated in vacuum and extracted with ethyl acetate. The ethyl acetate soluble fraction (rich in flavonoids) and the aqueous fraction (rich in proanthocyanidin) were obtained. The 3 fractions (polysaccharide, flavonoid, and proanthocyanidin) were tested for hypoglycaemic activity using Wistar rats as the experimental model system.

2.3 Preparation of glucose

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

2.4 Biological assay

2.4.1 Experimental animals

Two months old male Wistar rats (average weight of 174 ± 8 g) obtained from the Medical Research Institute, Colombo, maintained under standard conditions, were used in this study.

2.4.2 Dosage and administration of extracts

The dosage administered was equivalent to 5.0 g of *Artocarpus heterophyllus* starting material/kg body weight. The control animals received an equal volume of water. All the extracts and glucose were orally administered using Sondi needles.

2.4.3 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.

2.4.4 Determination of the Effect of the hot water extract, proanthocyanidin, flavonoid and polysaccharide fractions on glucose challenge

The Rats were randomly divided into five groups (I, II, III, IV and V) with 6 rats in each, fasted for 16 h, and 0.1 ml venous blood was drawn under light anesthesia for determination of fasting blood glucose levels.

Group I was given distilled water (1ml) and groups II, III, IV and V were given the crude extract, proanthocyanidin, flavonoid and polysaccharide fractions respectively. After 30 minutes, a glucose load (3g/kg body weight) was administered to all the rats. Ninety minutes after administration of glucose, venous blood (0.1 ml) was drawn and serum glucose concentrations were analysed immediately using Reagent Kits (DMA, USA) employing the glucose oxidase method. Only the 90 minutes post glucose blood sugar level was determined since intermediate blood sampling is not necessary according to WHO criteria⁶.

2.4.5 Separation of Flavonoid fraction by Medium pressure liquid chromatography (MPLC)

As the Flavonoid fraction showed a significantly high hypoglycaemic activity when compared with the control, it was further separated using MPLC as follows;

1 g of the crude flavonoid fraction was dissolved in 5 ml of ethyl acetate and pre-absorbed to 5 g of column Silica gel. Ethyl acetate was removed using a rotary evaporator and applied to a MPLC silica gel (40-63 μ) column (7.5 x 1.5 cm). The fractions were eluted with a solvent gradient of Petroleum ether, Chloroform, Methanol in the order of 100% petroleum ether : petroleum Ether/CHCl₃ (1:1), 100% CHCl₃, CHCl₃/MeOH (9.5 : 0.5), CHCl₃/MeOH (9 : 1), CHCl₃ : MeOH (8 : 2), CHCl₃ : MeOH (5 : 5), Pure MeOH and pure EtOH. Fractions (5 ml each) were collected and analysed by TLC (Silica gel 60 F₂₅₄, 0.25 mm) in ethyl acetate: pet ether (2:3, v/v) and detection was carried out by illumination with UV light at both 254 and 366 nm. The seven sub fractions were pooled according to the TLC results. The fractions 1 (1-300 ml, 50 mg); 2 (300-400 ml, 12 mg); 3 (400-500 ml, 1 mg); 4 (500-600 ml, 8 mg); 5 (600-720 ml, 120 mg); 6 (720-900 ml, 454 mg); 7 (900-1050 ml, 9 mg) containing flavonoids (TLC) were obtained. These fractions were tested for the hypoglycaemic effect on Wistar rats.

2.4.6 Effect of MPLC separated flavanoid sub fractions on glucose challenge

Seven pooled fractions were tested for hypoglycaemic activity on Wistar rats (n=48) as described in section 2.4.4. The dosage administered was equivalent to 5 g of *Artocarpus heterophyllus* starting material/kg body weight. The control rats received an equal volume of water.

2.5 Statistical analysis

Statistical analysis was carried out in Microsoft Excel. All the results are presented as mean ± SEM. The significance of the differences between the means of the tests and controls was established by student t-test. p-value less than 0.001 was considered to be significant.

Group	Blood glucose concentration ± SEM (mg/dl)		% Reduction in test compared to control
	Fasting	90 minutes post glucose	
Control	73.5 ± 4.7	166.8 ± 8.1	
Hot water extract(crude extract)	65.4 ± 4.1	90.00 ± 2.1*	46%
Proanthocyanidin fraction	75.2 ± 6.2	75.29 ± 4.7*	54.8%
Flavonoid fraction	73.4 ± 7.0	73.5 ± 5.3*	55.9%

Table 1: Effect of different fractions of the hot water extract of mature Jack leaves (*Artocarpus heterophyllus*) on blood sugar levels of glucose loaded Wistar rat.

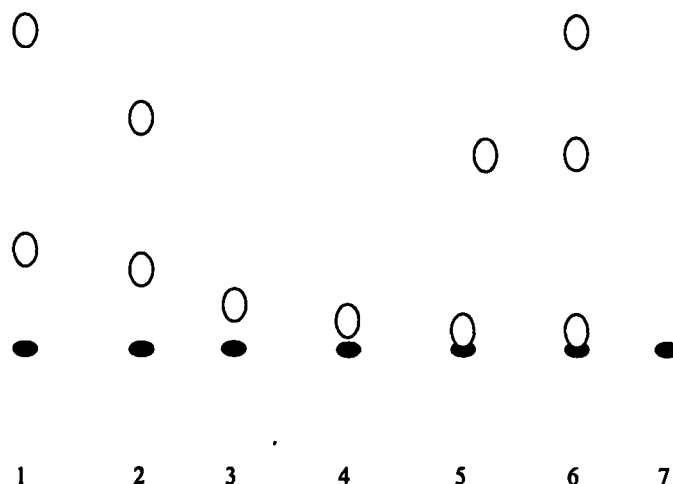


Figure 1: Diagrammatic representation of TLC profiles of flavanoid sub fractions 1-7 on Silica gel 60 F₂₅₄, 0.25 mm plates (detection under UV 366nm, solvent system was ethyl acetate: pet ether (2:3, v/v)).

3. Results

The effects of the hot water extract, proanthocyanidins, flavonoids and polysaccharides are shown in Table 1. Figure 1 is a diagrammatic representation of TLC profiles of flavonoid sub fractions 1-7 on Silca gel plates. Table 2 shows the effects of each sub fraction of flavonoids on hypoglycaemic activity.

4. Discussion

The groups which received the proanthocyanidin and flavonoid fractions of the hot water extract of Jack leaves (*Artocarpus heterophyllus*) showed a significant improvement in their ability to utilize the external glucose load. It is possible that these two fractions contain active pharmacological compounds, which exert hypoglycaemic activity.

It is also evident that the hypoglycaemic effect of each of the two individual fractions is higher than the effect of the crude extract. It is possible that the crude extract contains inhibitory factor(s) that can interfere with the hypoglycaemic effect. The polysaccharide fraction did not produce any hypoglycaemic effect. When subjected to further isolation by MPLC, the flavonoid fractions yielded 7 sub fractions. MPLC fractions 1, 5, 6, and 7 showed considerable hypoglycaemic activity. The preliminary acute toxicity studies (not included in this paper) have revealed no visible signs or symptoms of toxicity of the flavonoid or proanthocyanidin in normal Wistar rats.

Therefore the present studies have further encouraged the possible use of the total flavonoids and proanthocyanidins isolated from *A. heterophyllus* leaves for future evaluation as a routine treatment for diabetes mellitus. Furthermore, detailed chemical studies followed by pharmacological and toxicological investigations are required for this purpose. Further research to isolate the active principle(s) responsible for the hypoglycaemic activity of *A. heterophyllus* leaves and to elucidate their mode(s) of action are underway.

5. Conclusion

Our results show that flavonoid and proanthocyanidin fractions of *Artocarpus heterophyllus* exert hypoglycaemic activity in normal Wistar rats. Furthermore, the presence of more than one active principle responsible for hypoglycaemic activity is also indicated.

6. Acknowledgements

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7. References:

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