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Hypoglycemic Effect of Extracts of Petai Papan *(Parkia speciosa, Hassk)*

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ABSTRAK

Pentadbiran extrak kloroform petai melalui mulut, dapat menurunkan dengan ketara ($p<0.01$) kandongan glukos dalam darah tikus yang di kencing manis oleh alloxan. Tindakan hypoglysaemik ini berkadaran dengan punca kuasa dua dos yang diberi. Tindakan hypoglysaemik adalah mixima selepas 2-5 jam pengambilan ekstrak tersebut melalui mulut dan kekal selama sekurang-kurangnya 24 jam.

ABSTRACT

The oral administration of the chloroform extract of Parkia speciosa *to alloxan-induced diabetic rats produced a significant ($p<0.01$) decrease in blood glucose levels. The hypoglycemic response was approximately proportional to the square root of the dose given. The hypoglycemic activity of the extract reached a maximum 2-5 hours after oral administration of the extract and lasted for at least 24 hours.*

Keywords: *Parkia speciosa*, antidiabetic, hypoglycemic, oral administration, rats, chloroform extract, dose-response

INTRODUCTION

Petai (*Parkia speciosa*) is a Southeast Asian legume of the Mimosae subfamily, whose seeds are consumed as a condiment or vegetable with rice, for its unique Shiitake mushroom-like flavour. When taken in excess it gives a strong onion-like smell, which is excreted by the body in the urine, the sweat and the faeces. Sometimes petai is eaten because it is believed to have anti-diabetic and anti-hypertensive activity.

Petai has been used in traditional medicine for its antibacterial effects on kidney, ureter and urinary bladder. The antibacterial and antifungal compounds were found to be cyclic polysulfides, whose structures were established as 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, 1,2,3,5,6-pentathiepane (lenthionine), 1,2,4,5,7,8-hexathionane and a pentathiocane (Gmelin *et al.* 1981). Dichrostachinic acid, djenkolic acid and thiozolidine-4-carboxylic acid were also identified (Holzman *et al.* 1982). Thiozolidine-4-carboxylic acid has been successfully used experimentally and clinically as an anti-cancer agent (Pandeya 1972). Djenkolic acid has been known to cause blockage of the urinary tubules due to its low solubility, resulting in pain, haematuria and even death. *P. speciosa* seeds also contain significant minerals, vitamins, protein and fat, while having a lower antinutrient content compared to soya bean (Suhaila *et al.* 1987).

This research was undertaken to investigate the hypoglycemic effect of *P. speciosa* on normal and alloxan-induced diabetic rats, because petai is eaten by diabetics for that purpose.

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MATERIALS AND METHODS

Preparation of Extracts

Ten kg of fresh petai pods were obtained from the local market. The seeds were separated from the pods. Both portions were air dried, ground to a powder and extracted sequentially and exhaustively with petroleum ether, diethyl ether, chloroform, dichloromethane, ammoniacal chloroform and methanol. The solvents were completely evaporated off with a rotary evaporator to obtain the extracts.

Experimental Procedure

Healthy Sprague Drawley rats of mixed sexes (weighing 200-450 g) were intravenously injected with 60 mg/kg alloxan (2,4,5,6-Tetra oxy pyrimidine) to induce diabetes within 40-48 hours (Lundquist and Rerupa 1967). The dry extracts of petai were orally fed to 24-hr-fasted normal and alloxan-induced diabetic rats at a dose level in the range of 25-500 mg extract/kg BW (body weight), together with 1 g glucose/kg BW of rat. Coadministration of glucose with the extract was done to cause hyperglycemia. Both diabetic and normal rats treated orally with 5 ml saline and 1 g glucose/kg BW were observed for comparison. Blood samples were taken hourly for the first 11 hours and again 24 hours after the administration of the extracts. Blood was obtained from the tail vein by using heparinised microhematocrit capillary tubes (Riley 1960).

Analysis of Blood Glucose

The plasma glucose level was determined by glucose oxidase method (Roche Glucose test kit No 07 1011 3) where D-glucose is specifically oxidised to gluconic acid and hydrogen peroxide by glucose oxidase. The generated hydrogen peroxide converts O-dianisidine, by the catalytic action of peroxidase to the red-brown semiquinone. The colour intensity is directly proportional to the glucose concentration and is measured spectrophotometrically. 0.02 ml of serum was used for glucose assay and compared with 0.02 ml standard D glucose solution.

Statistical Analysis

The data were statistically analysed using analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and regression analysis on MSTAT computer program.

RESULTS AND DISCUSSION

Results showed that only the chloroform extracts (1 g/kg body weight) from both the empty pods and seeds of petai had a strong hypoglycemic activity on diabetic rats *(Fig. 1)*. Blood glucose level at time zero is the blood glucose level just after the oral administration of extracts/saline and glucose. ANOVA analysis showed significant differences between chloroform extracts of both the seeds and pods ($p<0.01$), and extracts from other solvents

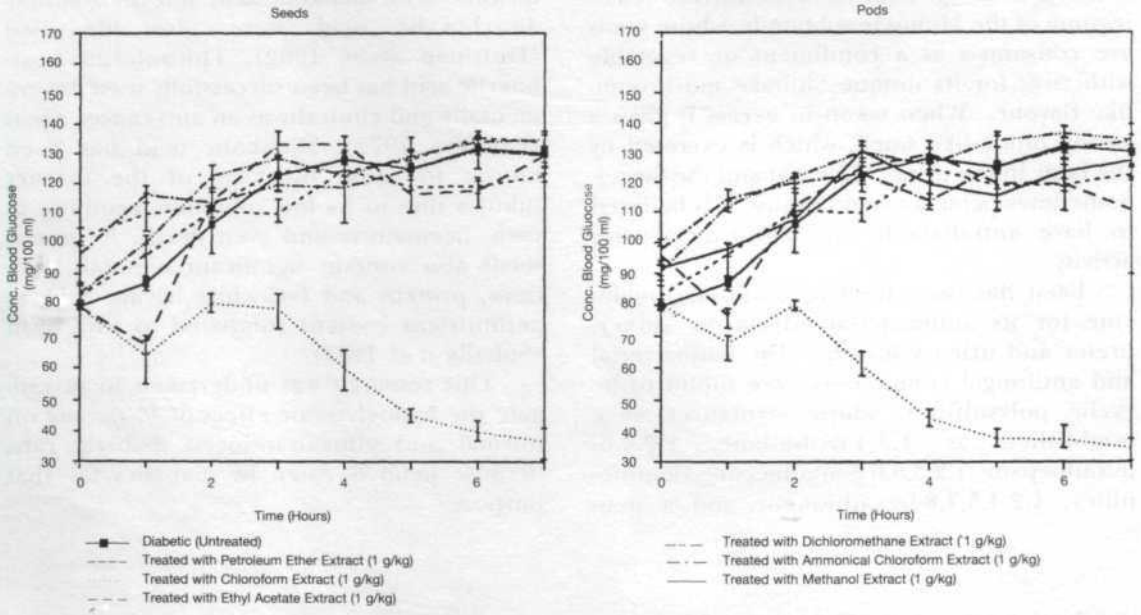

Fig. 1. Effect of different chemical solvents extracts of P. speciosa *on blood glucose levels in alloxan-diabetic rats. Data are means ± SE (n = 4)*

(1 g/kg body weight) or the control (treatment with saline). Further work therefore concentrated only on the chloroform fraction.

Fig. 2 shows that there was insignificant increase in the blood glucose levels of normal rats fed with 0.4 g ground seeds or pods together with 1 g glucose/kg body weight. The normal rats had an average blood glucose content of 124 mg/100 ml, while the alloxan diabetic rats had an average blood glucose level of 379 mg/100 ml after ingesting 1 g glucose/kg body weight.

The blood glucose level of alloxan diabetic rats was reduced by 36±6 % to 288 mg/100 ml with the oral treatment of 0.4 g/kg BW pericarp (pod), and by 57±6 % to 236 mg/100 ml after the oral treatment with 0.4 g/kg BW petai seed. The treatment could be seen to take effect within less than an hour and lasted for at least 24 hours. The maximum fall was observed 2 hours after oral administration. However, there was an initial rise in blood glucose level between 0-3 hours, showing that the glucose was rapidly absorbed from the alimentary canal and that the extract of petai took effect only two hours after ingestion. The blood glucose level of healthy and diabetic rats fed with saline plus 1 g/kg BW glucose is shown for comparison.

The seed had a higher activity than the pericarp. *Fig. 3* shows the dose-response relationship of petai seed on blood glucose level in diabetic rats. A dose of 25 mg/kg BW decreased the blood glucose by 24±4 %, yet a 4-fold increase in dosage (100 mg/kg BW) only decreased the blood glucose by 43±5 %. Increasing the dosage 20-fold (500 mg/kg BW) decreased the blood glucose by 77±12 %. Further increasing the dosage (3 g seed/kg BW) decreased the blood glucose level by 116±12 % i.e. bringing the glucose level below that of a normal healthy rat.

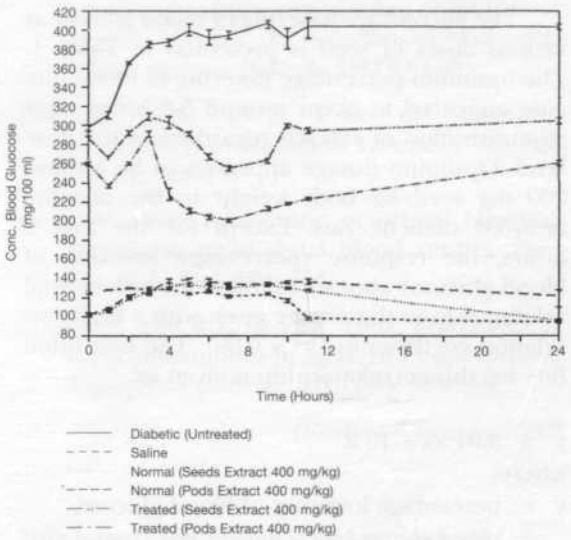

Fig. 2. Effect of chloroform extracts of P. speciosa *on normal and diabetic rats. Data are means ± SE (n = 4)*

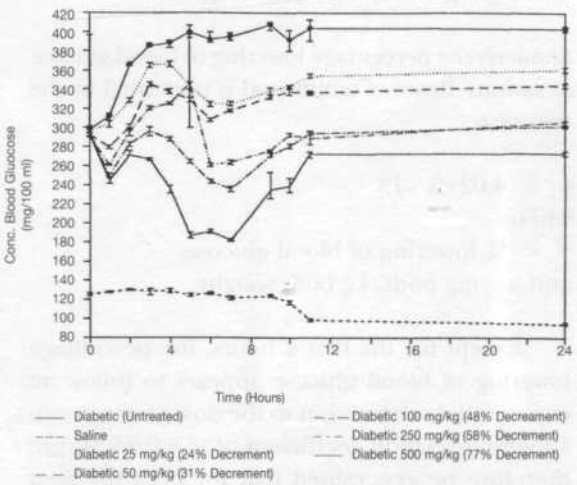

Fig. 3. Dose-response relationship of fresh and ground seeds on blood glucose levels of alloxan-diabetic rats. Data are means ± SE (n = 4)

TABLE 1
Reduction in blood glucose (%) of
diabetic rats following the administration of chloroform extract of petai seeds

dose hr (mg/kg body wt)	50	100	150	200	250	300	350	400	450	500
1	25.41	51.76	53.17	55.52	56.47	57.41	58.35	59.29	58.35	57.41
3	20.70	30.11	32.94	36.70	37.64	37.64	38.58	39.05	43.76	48.94
5	25.4	34.82	43.29	50.82	55.52	59.29	64	67.76	72	75.76
8	25.41	46.11	53.17	60.23	64	73.88	66.35	68.70	69.17	70.11
11	13.17	30.11	36.70	44.23	47.05	49.41	51.76	53.64	44.70	36.70
24	8.941	21.64	27.29	33.88	34.35	34.82	35.76	36.70	34.82	33.88

The percentage lowering of blood glucose at various doses of seed is presented in Table 1. The optimum percentage lowering of blood glucose appeared to occur around 5-8 hours after administration of extracts regardless of the dose level. Optimum dosage appeared to be around 200 mg seed/kg body weight in the alloxan-induced diabetic rats. Except for the first 2 hours, the response (percentage lowering of blood glucose) appears to follow an exponential relationship to the dosage given with a high correlation coefficient of $r^2 = 0.99$. The best fitted line for this correlationship is given as:

$$y = 3.01 \sqrt{x} + 10.2$$

where:

y = percentage lowering of blood glucose,

$$= \frac{(\textit{blood glucose level of diabetic rats - treated rats}) \times 100}{(\textit{blood glucose level of diabetic rats - healthy rats})}$$

and x = mg seeds/ kg body weight.

Similarly the percentage lowering of blood glucose at various doses of empty pod is presented by the equation

$$y = 4.02 \sqrt{x} - 13$$

where:

y = % lowering of blood glucose,

and x = mg pods/kg body weight.

Except for the first 4 hours, the percentage lowering of blood glucose appears to follow an exponential relationship to the dosage given with a high correlation coefficient of $r^2 = 0.94$. It can therefore be generalised that for both the seed and pericarp, the response is approximately proportional to the square root of the dose given. The time taken for the pericarp to take effect and the duration of the hypoglycemic activity are shown in *Fig. 4*. The pericarp had a lower activity than the seed. At 25 mg/kg BW there was no significant activity. At 50 mg/kg BW the lowering of blood glucose was 18±4 % and the activity at 100 mg/kg and 250 mg/kg was 31±5 % and 47±5 % respectively. This is about half the activity of the seed.

The fact that the blood glucose response to petai seeds and pods is square root to the dose may indicate that the mechanism of action of the

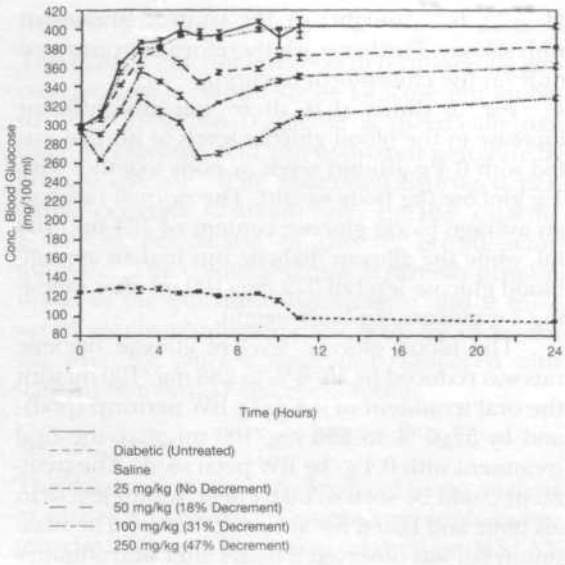

Fig. 4. Dose-response relationship of fresh and ground empty pods on blood glucose levels of alloxan-diabetic rats. Data are means ± SE (n = 4)

active compounds in petai is peripheral. This is based on comparison of dose response curves of peripherally-acting compounds to centrally-acting ones (those causing the pancreas to increase insulin production and release). Peripheral-acting compounds act directly on all the cells in general, enabling more glucose to enter the cells. Chemical studies on the active compounds of petai showed them to be sterols (results to be published) which can readily affect the lipoprotein part of cell membranes. Further work is being carried out to determine the mechanism of action.

Even though the activity of the pericarp (empty pod) and mesocarp (testa) are half of that from the seed, extraction of compounds from the empty pod is viable because it constitutes 57 % of the whole pod and only the seeds are normally eaten while the outer skin is less palatable although edible. In Malaysia, canned petai seeds with anchovies and chilli sauce are available in the market. The empty pods are therefore a waste product which can be used as a raw material for the extraction of hypoglycemic material.

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