Hypoglycaemia induced in fasted cats by aqueous extracts of *Pithecellobium Jiringa*

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Introduction

EVER SINCE JANBON and his co-workers (1942) discovered that a sulfonamide induced hypoglycaemia, the search has been on to discover other orally administered hypoglycaemic agents. This preliminary report shows that the seeds of Pithecollobium jiringa, which are eaten locally by Malays for their diuretic effect (Birkhill 1935), may have a hypoglycaemic action as well.

Materials and Methods

Samples of *Pithecellobium jeringa* ("Buah Jering" in Malay) were obtained from the market place at various times during the year. The pericarp was removed, and the seeds of the legume, together with the testa, were cut into small pieces using a razor blade. About 200 gms of cut seed were mashed in a Whering blender with 200 ml. of distilled water. The mash was then squeezed in a domestic fruit juicer and the extract collected. The resultant aqueous extracts had a pH which ranged from 5.3 — 5.8, close to isotonicity (approximately 240 mOsmoles). Aliquots (about 10 ml) were frozen and stored at — 20°C, and thawed immediately before use. A portion of each batch of extract was evaporated to dryness and the amount of solids per ml determined. The solid content ranged from 27.5 — 34 mg/ml.

The dose selected was 1 ml of extract per kg of body weight of the cat.

Fasted (24 hrs) cats of both sexes were anesthetised with Pentobarbital (Sagatal) at a dose of 35 mg/kg body weight by intraperitoneal injection. This was usually sufficient to maintain the animal under deep anesthesia for the entire experiment. Occasionally a booster injection of Pentobarbital had to be given. The femoral vein was cannulated and the animal was heparinised. A specially made T-form cannula of plastic tubing was inserted into the right carotid artery so that continuous flow of blood through the artery occurred after it was secured in place. The side-arm with a small rubber tube was clamped off, and released when samples of arterial blood were required. A small amount of blood was discarded each time before collecting samples in order to avoid contamination of samples by blood trapped in the dead space of the side-arm.

Haematocrits were determined at the beginning and end of experiments, and were found not to be significantly altered.

In pilot runs, the blood pressure was recorded. Apart from a rapid transient rise in blood pressure which occurred soon after the extract was given, no further changes occurred.

One ml samples were drawn from the carotid cannula each time. Samples were obtained 30 minutes before the injection of the extract, immediately after the injection and at hourly intervals for the total duration of the experiment. Control animals were given 1 ml/kg body weight of normal physiological saline but were otherwise treated in the same way.

The blood was centrifuged immediately after collection, the plasma transferred to a clean test tube, sealed with parafilm and stored at 4°C in a refrigerator until the end of the experimental run. Blood glucose was determined by the glucose oxidase method (modified from Raabo and Terkildsen 1960), using a Sigma glucose oxidase kit.

Paired statistical comparisons of the blood glucose levels of the experimental and control series at each sampling period were performed, using the "t" test. Only those results which showed a probability of $p = 0.05$ or less were deemed significant and those at $p = 0.01$ or less, highly significant.

Results

The results are shown in Figure 1 and Table I.

A regular pattern in the blood glucose levels is seen (Figure 1) in animals treated with the Buah Jering extract, i.e. a rise in blood glucose immediately after the extract was given (0 hr), followed by a slow steady reduction which reached a maximum at 4 hrs, and was still present 5 hours after the extract was given. The experiments were carried on, in a few cases until 7 hours, at which time this lower blood glucose continued to manifest itself.

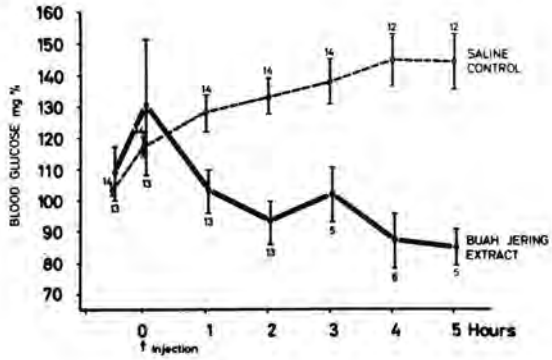
Figure 1 also shows a different pattern of blood glucose levels in saline-treated cats, viz. a slow rise in blood glucose throughout the experimental period. This result indicates that the apparent hypoglycaemic effect of the Buah Jering extract was not due to a spontaneous lack of glucose in these 24-hour fasted cats.

The paired comparisons between these two groups showed that the apparent hyperglycaemia at 0 hr was not statistically significant; while at one hour after the injection the probability that the slight hypoglycaemia was significant was only slightly greater than $p = 0.05$ (i.e. 0.07) but less than $p = 0.1$. This trend, however, became clearly established by 2 hours, and persisted in a highly significant manner throughout the remainder of the experimental period. It therefore appears that there is acting within cats treated with Buah Jering extract a factor clearly exerting a potent long-lasting hypoglycaemic effect.

The "active" principle is not only soluble in water but stable when kept frozen at — 20°C for several weeks — about 2/3 of the cats in this series were treated with thawed extracts which had been stored frozen. Its heat lability is suggested by results shown in Table I, where one cat given extract, portions of which had been heated over a steam bath for 30 minutes, failed to manifest any blood sugar lowering activity. Similarly, all such activity appeared to have disappeared in a cat treated with an extract of mash (Table I) which had been dried at 37°C for several days.

Discussion

The seeds of the *Pithecellobium Jeringa*, a species of the family Leguminosae, (Burkill 1935) are eaten by the Malays especially during the fasting month (puasa). They are used as flavouring, and eaten either boiled or in salads. Both immature and ripe seeds are used. According to Hooper (Burkill 1935), the seeds contained 70% starch, together with small amounts of fats, a volatile sulphuretted allyl compound, and an alkaloid.



It is common knowledge among local Malays that the seeds act as a diuretic. The allyl compound is excreted through the kidneys. In large amounts, it is reported to cause renal inflammation, and even "stricture" which may be fatal (Burkill 1935). There have been no reports on the hypoglycaemic effect.

The effect is mild in the doses used (i.e. 1 ml/kg body weight or 27-34 mg of solids/kg body weight). Low doses were chosen in order that any renal damage might be avoided.

Two mechanisms by which the *buah jering* extract can cause a hypoglycaemic effect are either by direct action of an active principle in the extract, or by a principle in the extract being first converted to an "active" intermediate *in vivo*. Figure 1 indicates that immediately after the extract is given, there is a rise in the mean blood glucose level, albeit not statistically significantly. It is known that hyperglycaemia triggers the release of insulin from the pancreas. Tolbutamide acts indirectly by stimulating the release of insulin from the pancreas (Loubatieres, 1957). All cats used in these experiments were normal, allowing such a mechanism of insulin release to operate in them. On the other hand, the biguanidines exert a hypoglycaemic action

TABLE I

Treatment	No. of Cats	Before	0 hr.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
Saline	13	103 ± —1.7	117 ± —4.3	129 ± —6.0	134 ± —6.8	139 ± —7.0	146 ± —9.5	145 ± —9.5
Steamed Extract	1	110	135	118	165	210	210	225
Dried Mash Extract	1	110	120	155	185	185	195	220

Blood glucose levels in cats treated with saline or Pithecollobium jiringa extracts which have been steamed or dried.

in alloxanised animals (Read & Fodden, 1954). Thus another possibility is that the active principle involved here might also act directly, independently of the pancreas as in the biguanidines. These avenues have not been explored so far.

In the light of the reported diuretic effects produced by *Pithecollobium jeringa,* the possibility that the active principle was causing a glycosuria was looked into. In two cats, the bladder was catheterised and the urine tested with Clinsticks during the entire experimental period for any sign of glucose in the urine. No trace was ever found.

There are indications that the active principle is not heat stable (Table I).

The saline-treated control series of cats displayed a slow but persistent rise in blood sugar level until it was quite noticeable at the end of 5 hrs. It is possible that the initial (immediate) rise in blood glucose observed at the time of the injection (0 hr) in these cats is an indication of the minor stress of the volume of the fluid injected (1 ml of physiological saline/kg body weight), but that the steady later rise is a reflection of the lightening anesthesia.

Suskind and Rahn (1954) have shown that following pentobarbital administration (intraperitoneally) to dogs there is also a rise in alveolar and arterial pCO_2. The increase in sympatho-adrenal activity in response to hypercapnia has been demonstrated in conscious man by Sechzer et al. (1960). The importance of the sympatho-adrenal system in cardiovascular responses to hypercapnia in the dog and cat has been demonstrated by Honig and Tenney (1957).

It seems possible then that in these cats the rising hypercapnia activates the sympatho-adrenal system leading to a hyperglycaemia, especially in view of the lightening anaesthestic state. From the above, it does appear the prevention of this rise in blood glucose by the extract may, in fact, be more pronounced in normal unanesthetised cats.

Summary

Aqueous extracts of the ripe and immature seeds of a Malaysian legume, *Pithecellobium Jeringa,* appear to prevent a rise in the blood glucose level in fasted cats under pentobarbital anesthesia. The effect is not an immediate one, but develops gradually during the first hour after injection of the extract, and persists for at least 5 hours. The effect is apparently not due to a lowering of the renal threshold for glucose. This property of the extract appears to be heat labile, but the fresh extract can be kept frozen at — 20°C for several weeks without loss in activity.

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