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LETTER TO THE EDITOR

EFFECT OF EXTRACT OF LEAVES OF *VINCA ROSEA LINN.* ON GLUCOSE UTILIZATION AND GLYCOGEN DEPOSITION BY ISOLATED RAT HEMIDIAPHRAGM

Sir,

Vinca rosea ('Nayantara'; fam. apocynaeceae) is a medicinal plant commonly grown in Indian gardens and a native of West Indies. Parts of this plant have been reported to exert medicinal properties, such as antidiabetic, antileukemic and hypotensive activity (1-3). The water soluble portion of alcoholic extract of Vinca rosea leaves has been shown to possess significant hypoglycemic and antihyperglycemic effect in rats (4). The present investigation on isolated rat hemidiaphragm was therefore designed to ascertain whether the leaf extract had any direct insulin like activity or any effect which can augment the effect of insulin.

The water soluble portion of alcoholic extract of leaves of Vinca rosea was prepared by the method described earlier (4). Albino rats of either sex (80-100 g), maintained on a standardised diet (water *ad libitum*), and fasted overnight were used. The animlals were killed by decapitation and diaphragms were taken out quickly avoiding trauma and divided into two halves. The hemidiaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and were placed in small conical flasks containing 2 ml Tyrode solution with 2000 mg% glucose and incubated as follows.

(A) For glucose uptake :

The hemidiaphragms were incubated for 30 min at 37°C in an atmosphere of 95% 0_2-5% CO_2 with shaking at 140 cycles/min. Four sets of experiments were performed. The hemidiaphragms were exposed to:

i) Tyrode solution with 2000 mg% glucose only. This group served as control.

ii) Tyrode solution with glucose (2000 mg%) + insulin (0.25 I.U./ ml).

iii) Tyrode solution with glucose (2000 mg%) + Leaf extract (25 mg/ml).

iv) Tyrode solution with glucose (2000 mg%) + insulin (0.25 I.U./ml) + Leaf extract 25 mg/ml).

Two hemidiaphragms from the same animals were not used for the same set of experiment. Following incubation, the hemidiaphragms were taken out and glucose content of the incubated medium was measured by glucose oxidase method (5). Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium.

(B) For glycogen content :

The hemidiaphragms were incubated in Tyrode solution with glucose (2000 mg%) in the similar way as described for glucose uptake : only the time was extended to 90 minutes. Following incubation, the hemidiaphragm was rinsed for 10-15 sec in 0.9% NaCl at 0° to wash off external glucose and stop enzyme activity. It was blotted, frozen on dry ice and the glycogen content of the tissue was measured by the method of Carroll et al (6). The glycogen content was expressed as micromoles glucose equivalent/g of tissue.

Results were statistically analysed by Student's 't' test.

Using this methodology Gemmill (7) demonstrated the *in vitro* stimulating effect of insulin on glucose utilization and glycogen synthesis of an isolated muscle. In our experiments we also have found that insulin increases the glucose utilization and glycogen synthesis of an isolated rat hemidiaphragm (Table Ib), but failed to observe such type of effect of Vinca rosea leaf extract on glucose uptake and gly-

cogen synthesis (Table Ic). These findings suggest that Vinca rosea leaf extract probably has no direct insulin like effect which can enhance the peripheral utilization of glucose. It was also noted that at 25 mg/ml, the leaf extract rather inhibited to activity of insulin *in virto* (Table Id). The mechanism of this inhibition is not clear. It may be that the leaf extract binds or absorbs the insulin during incubation period or it might be due to non-specific inhibition of different tissue enzymes as observed with sulphonylurea derivatives and sulphonamide drugs (8). The exact mechanism is still obscure. Although these experiments do not indicate the mechanism of blood sugar lowering effect of Vinca rosea leaf extract reported earlier they do tend to eliminate a direct insulin-like effect.

TABLE I : Effect of Vinca rosea leaf extract on glucose uptake and glycogen synthesis by isolated rat hemidiaphragm.

Incubation medium	*Glucose uptake (mg/g/ 30 min)*	*Glycogen content µmoles (glucose equivaent)/ g tissue*
(a) Tyrode solution with glucose (2000 mg%). Control group	4.00±0.49	18.08±0.01
(b) Tyrode solution with glucose (2000 mg%)+ Insulin (0.25 IU/ml)	7.33±0.82**	29.46±2.63**
(c) Tyrode solution with glucose (2000 mg%) + Leaf extract (25 mg/ml)	3.86±0.27	19.31±1.60
(d) Tyrode solution with glucose (2000 mg%)+ Insulin (0.25 IU/ml) + Leaf extract (25 mg/ml)	4.27±0.63*†	21.27±47*†

Results are Mean ± SEM from 6 experiments
**P< 0.01 in comparison with Ia
†P<0.025 in comparison with Ib.

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