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POSSIBLE MECHANISM OF ANTIHYPERGLYCEMIC EFFECT OF *AZADIRACHTA INDICA* LEAF EXTRACT: PART V

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Effect of *Azadirachta indica* leaf extract on adrenoreceptor blocking agents (propranolol and phentolamine) on serotonin inhibition in glucose mediated insulin release in rat pancreas was studied *in vitro* to elucidate the possible mechanism of antihyperglycemic effect of *A. indica* leaf extract. *A indica* leaf extract and phentolamine block significantly ($P<0.05$) the inhibitory effect of serotonin on insulin secretion mediated by glucose.

Key words: *A. indica*, Antihyperglycemic activity, Adrenergic blocking agents.

Introduction

Azadirachta indica A.Juss (Meliaceae, Neem) is an indigenous plant widely available in India and Burma. Different parts of this plant have been reported to possess antiseptic, wound healing, skin disease curing and antiulcer activity (Kirtikar and Basu 1933; Chopra *et al* 1956). Preliminary studies revealed that water soluble portion of alcoholic extract of *A.indica* leaves possessed significant antiinflammatory, antifertility, hypolipidemic and hepatoprotective activity (Chattopadhyay *et al* 1986, 1987, 1992; Chattopadhyay 1993a, 1995). Significant antihyperglycemic effect of *A.indica* leaf extract against glucose fed and adrenaline induced hyperglycemic rats and antiserotonin activity in rat uterus and fundal strip preparations have also been reported (Chattopadhyay *et al* 1986, 1987 b). Possible mechanism of antihyperglycemic effect of *A. indica* leaf extract on various experimental models have been studied (Chattopadhyay *et al* 1993, 1995; Chattopadhyay 1993 b, 1996). Results of important findings in relation to blood sugar lowering effect of *A. indica* leaf extract reveals that the antihyperglycemic effect of *A. indica* may be due to its antiserotonin activity.

Relevant literature reveals that serotonin may be present as an intracellular pool in the pancreatic islet cell which is effective in blocking insulin secretion (Feldman *et al* 1972). The literature further reveals that alpha adrenergic blocking agent like phentolamine, blocks the inhibitory effect of serotonin on glucose and dibutyl cyclic AMP mediated insulin release. This indicates that serotonin exerts inhibitory effect on insulin output via alpha adrenergic system inhibiting the generation of cyclic AMP (Feldman and Lebovitz 1970).

On the other hand, epinephrine and norepinephrine inhibit insulin secretion in response to various stimuli such as glucose, glucagon or tolbutamide, and this is inhibited by alpha adrenergic blocking agents. Beta adrenergic agents stimulate insulin output. It is suggested that beta adrenergic stimulation activates the adenylcyclase system leading to increased production of cyclic 3'5' AMP, which is responsible for insulin secretion. Alpha adrenergic stimulation, on the contrary, blocks insulin secretion by inhibiting the adenylcyclase system (Feldman and Labovitz 1970).

The present investigation has, therefore, been designed to study the effect of *A. indica* lead extract and adrenoreceptor blocking agents (phentolamine and propranolol) on serotonin inhibition in glucose mediated insulin release in rat pancreas *in vitro* with a view to elucidate the possible mechanism of antihyperglycemic effect of *A. indica* leaf extract.

Materials and Methods

Collection of plant material. Fresh matured leaves of *A. indica* were collected from Indian Statistical Institute's garden and were identified by a pharmacognosy expert. At the time of collection, standard herbarium record sheets were completed with the name of the collector, collection number, specimen number, date, locality and local name.

Extraction of plant material. Air-dried powder (1 kg) of *A. indica* leaves were extracted by percolation at room

temperature with 70% EtOH. Leaf extract of *A. indica* was concentrated under reduced pressure (bath temperature 50 °C) and finally dried in a vacuum dessicator. The residue was dissolved in distilled water and filtered. The filtrate was evaporated to dryness. The dried mass (yield=50.2g) was suitabley diluted with normal saline and used in experiments.

Pancreas incubation system. Rats were anaesthetised with pentobarbital (40 mg kg^{-1}, i.p.). The pancreas was removed and cut into segments weighing 20-30 mg. Each piece of pancreas underwent two sequential 15 minutes incubation periods. The first incubation was carried out in basal media (initial incubation) while the second incubation was carried out in media containing the test substance to the basal media [*A.indica* leaf extract, 25 mg ml^{-1}; propranolol (10^{-4}M); phentolamine (10^{-4}M)]. The basal media is Kreb-Ringer bicarbonate buffer with 0.005 M pyruvate, 0.005 M fumerate, 0.005 M glutamate, 60 mg 100 ml^{-1} glucose and 400 mg 100 ml^{-1} bovine serum albumin. All incubations were performed at 37 °C in an atmosphere of 95% O_2-5% CO_2. The pH of the buffer was 7.4. At the end of each incubation, aliquots from incubation media were placed in chilled tubes and assayed for insulin by RIA technique.

The difference in insulin release between the treatment and initial incubations for each piece was designated as Δ insulin release. This method of expressing the data (Δ insulin) was used because of the nonuniform distribution of the islet cells throughout the pancreas. Therefore, each piece served as its own control and the data were more meaningful, since each response was relative to the initial secretion of insulin from that piece of tissue.

Statistical analysis. The data were expressed as mean ± S.E.M. of 6 observations and statistically assayed by analysis of variance (ANOVA) F-test. The difference between treated and control animals were made either by Student's or Dunnet's t-test (Scheffe 1953).

Results and Discussion

Figure 1 shows the insulin release induced by 3 mg ml^{-1} of glucose and the significant inhibition of this insulin release produced by serotonin (10^{-4}M). Propranolol (10^{-4}M) did not produce any inhibitory effect of serotonin on glucose mediated insulin release.

Figure 2 shows the insulin release produced by 3 mg ml^{-1} of glucose and the inhibition of this insulin release by serotonin (10^{-4}M). Phentolamine (10^{-4}M) by itself did not alter the glucose mediated insulin release but prevented the inhibitory effect of serotonin on glucose mediated insulin secretion.

Figure 3 illustrates the insulin release induced by 3 mg ml^{-1} of glucose and the inhibition of this insulin release by serotonin (10^{-4}M). *A. indica* leaf extract (25 mg ml^{-1}) potentiates the glucose mediated insulin release and prevented the inhibitory effect of serotonin on insulin release induced by glucose.

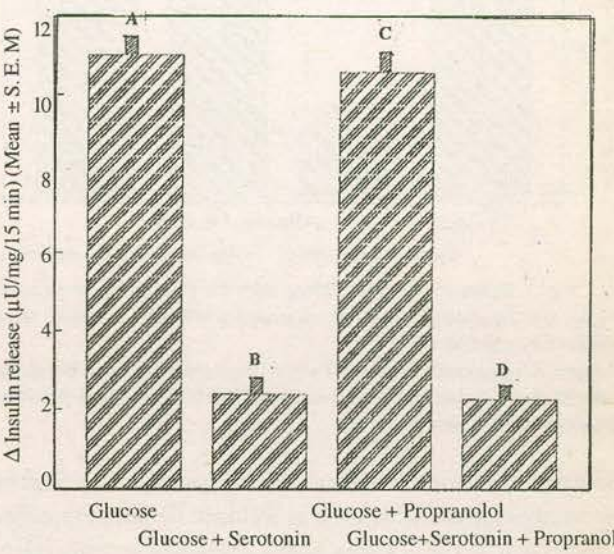

Fig 1. Effect of propranolol (10^{-4} M) on glucose mediated insulin secretion and inhibition of this secretion by serotonin (10^{-4}M). n = 6.

Different groups B and D are significantly different (P<0.05) from control group A but C is not. Pairs of groups AC and BD are also not significant.

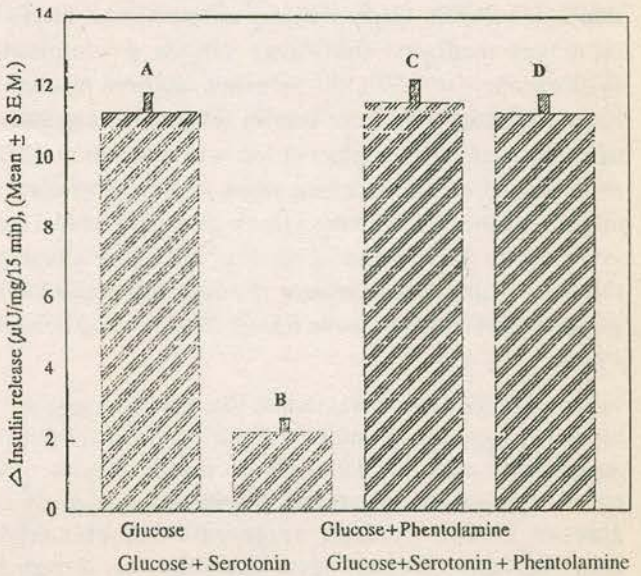

Fig 2. Effect of phentolamine (10^{-4} M) on glucose mediated insulin secretion and the antagonistic effect on serotonin inhibition in insulin release mediated by glucose. n = 6.

Group B is significantly different (P<0.05) from control group A; Group D is also significantly different (P<0.05) from group B; pairs of group AC, AD and CD are not.

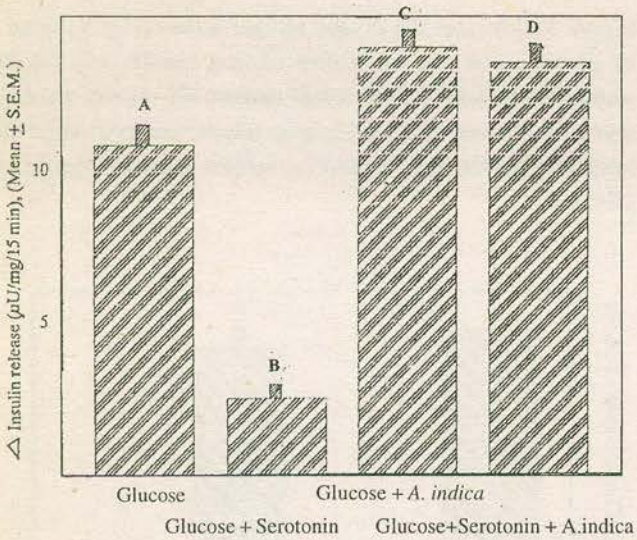

Fig 3. Effect of *A.indica* (25mg ml^{-1}) on glucose mediated insulin release and the antagonistic effect on serotonin inhibition in insulin release mediated by glucose. n = 6.
Group B is significantly different ($P<0.05$) from control group A but group C is not; Group D is also significantly different ($P<0.05$) from group B; but pairs of group AC, AD and CD are not.

Release of insulin from beta cells of pancreas is influenced by various factors which are as follows: (i) parasympathetic stimulation via the vagus nerve increases insulin release (Best and Tailor 1986), (ii) sympathetic stimulation via splanchnic nerve inhibits insulin release. This effect is exerted via alpha receptors. Agonists that affect primarily the beta receptors actualy stimulates the release of insulin via cAMP. Under ordinary circumstances, the alpha receptors mediated inhibitory effects predominates (Vishwanath *et al* 1978), (iii) serotonin has been reported to have inhibitory effect on insulin release in response to adrenaline injection or glucose load (Feldman et al 1972), (iv) alpha adrenergic blocking agent, such as phentolamine and beta adrenergic blocking agent such as propranolol have given rise to the hypothesis, the beta cell alpha adrenergic receptors inhibit insulin release and beta cell beta adrenergic receptors stimulate insulin release (Feldman and Lebovitz 1970).

In the observations, it was noted that beta adrenoreceptor blocking agent, propranolol did not by itself inhibit the antagonistic effect of serotonin on insulin release. The result supports the hypothesis that insulin release due to glucose is not via beta adrenergic stimulus.Alpha adrenoreceptor blocking agent phentolamine, though by itself failed to alter the glucose mediated insulin release, it significantly inhibited the inhibitory action of serotonin on insulin release mediated by glucose. *A. indica* leaf extract by itself blocked significantly the inhibitory effect of serotonin on insulin release mediated by glucose.

The present studies emphasize the striking similarities between the action of *A. indica* and phentolamine (alpha adrenoreceptor blocking agent) on serotonin inhibition in glucose mediated insulin release. Thus *A. indica* and phentolamine are truely a manifestation of their alpha receptor blocking action and at least one locus of the alpha receptor is in the mediation of cAMP action.

Chemical analysis revealed that the extract contains the following six compounds: (i) quercetin - 3 - O - β - D - glucoside, (ii) myricetin-3-O rutinoside, (iii) quercetin-3-O-rutinoside,(iv)kaempferol-3-O-rutinoside,(v) kaempferol-3-O-β-D-glucoside and (vi) quercetin-3-O-αL-rhamnoside.

These compounds were found to be collectively responsible for blood sugar lowering activity.

In spite of these results indicating the involvement of alpha adrenoreceptors in the antihyperglycemic activity of A.indica, further studies are needed both on the extract and or its chemical constituents.

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