INTRODUCTION
Diabetes mellitus has been treated orally with herbal remedies based on folk medicine since ancient times. With the advancement of knowledge, the importance of plant constituents as source of unlimited natural wealth especially in the form of drugs and essential oils, has been increasingly recognised. The use of different plant constituents as drugs especially in Ayurvedic and Unani system of medicine is common in our country. However, systematic investigations on the clinical uses of the plant constituents on scientific lines have only recently been started in various laboratories. Nevertheless, no medicine capable of eliciting radical cure for diabetes has yet been discovered. Doubts about the efficacy and safety of present day oral hypoglycemic agents have prompted a search for safer and more effective drugs for the treatment of diabetes. Traditional herbal remedies, which are known, by experience, to be free from side effects, are still used by diabetic patients, especially in third world countries and may, therefore, represent new avenues in the search for alternative hypoglycemic drugs. The discovery of insulin in 1921, followed by oral hypoglycemic drugs (sulfonylureas in 1955 and biguanides a few years later) has simplified the control of hyperglycemia. India being a vast country having extreme geographical and climatic diversities provides innumerable plant species and is, therefore, ideally suited for scientific investigations on their clinical properties.

Insulin like activity in (-) epicatechin

Herbs and plant extracts have been traditionally used for controlling diabetes mellitus in folk medicine all over the
Fig. 13: Structure of (-)epicatechin.
Water extract of the bark of *Pterocarpus marsupium* Roxb is being used in Indian medicine for the treatment of diabetes since long. The active antidiabetic principle in the water extract has been suggested to be a benzopyran, (-) epicatechin (Devon et al., 1975; Sawhney et al., 1956). It protects normal rat islets from alloxan toxicity, brings about normalization of blood glucose level and helps B-cell regeneration after necrosis due to alloxan (Chakrawarthy et al., 1980, 1981). Recently Charles S Simon (1984) have reported that (-) epicatechin, when administered to rats, increases insulin secretion and also raises insulin content of the islets. However, its clinical trials on scientific basis have not been carried out.

The present studies describe some insulin like effects of (-) epicatechin when tested on insulin target tissues *in vitro*. With the view to understanding the nature of binding site of (-) epicatechin on the target cells, competitive binding studies with 125 I-insulin have been carried out. Studies on conversion of pro-insulin to insulin and cathepsin B activity in islets of Langerhans have also been described in this chapter.

**Coleus forskohlii**

It is perennial aromatic herb that grows throughout the plains of India and in subtropical Himalayan regions up to a height of 2400 m. It is also cultivated as ornamental plant. The leaves of this plant are used for stomach ailments. The dwellers of
Fig. 14: Structure of coleonol.
'Tawaghat' Pithoragarh (U.P., India) know this plant by the name of 'Baddh Ki Jarh' (Bhava Mishra, 1960). In abdominal pain, adults chew more than 2 gm of its fresh root and believe that it kills the worms of intestine. It is also used as anticonstipative.

Coleonol, a diterpenoid, was isolated from the roots of the plant independently by two different groups, one working at CDRI and the other at Hoechst Research Centre, Bombay (India) by the name of coleonol (Tandon et al., 1977) and forskolin (Bhat et al., 1977) respectively. The diterpenoid was found to activate hormone sensitive adenylate cyclase. Hence, this compound is an important tool for studying physiological effects of cyclic AMP. Primary biological screening of 50% ethanol extract of the roots indicated hypotensive and spasmolytic activities (Bhakuni et al., 1971; Dubey et al., 1974; Dubey et al., 1981). Systematic study of this extract has led to the isolation of a diterpenoid (Fig. 14) (Tandon et al., 1977; Bhatt et al., 1977), which can directly activate the catalytic subunits of adenylate cyclase (Hermansen, 1985). Adenyl cyclase-cAMP system is involved in the release of islet hormones (Sharp, 1979; Wollheim et al., 1976). In vivo adenylate cyclase may be stimulated to increase intracellular cyclic AMP levels in B-cell by hormones such as glucagon and this could result in insulin release. Forskolin has been found to change the electrical activity of B-cell membranes (Henquin & Meissner, 1984). The diterpenoid increases cyclic AMP content and insulin release in rat pancreatic islets (Wiedenkeller & Sharp, 1983).
We have studied the effect of coleonol in normal rats on insulin and glucagon secretion from islets \textit{in vitro}. The effects of oral feeding of coleonol on blood glucose and free fatty acid level as well as on liver glucose-6-phosphatase activity and glycogen level have also been studied.

\textbf{Effect of age and glucose concentration on the activity of CCK-4 and its synthetic analogues with respect to insulin and glucagon release from islets of Langerhans \textit{in vitro}}

Elevated blood glucose level is common in non diabetic elderly population and may be closely related to the development of type II diabetes mellitus (Nilson et al., 1964; Joffe et al., 1969; O’sullivan et al., 1971; Epstein, 1967; Hayner et al., 1965). Factors which play a role in the development of hyperglycemia in aging include (i) altered insulin biosynthesis and its release, (ii) altered insulin sensitivity of the target tissues and altered insulin actions. Similarly, the glucose tolerance on aging, which is primarily due to a post receptor defect in insulin mediated glucose disposal (Andres, 1975; De Fronzo, 1979; Rowe et al., 1983; Fink et al., 1983) and is accompanied by decrease in insulin clearance (Minaker et al., 1982), has been shown to be associated with defects in the primary B-cell functions. Glucose stimulated insulin secretion and insulin secretion per B-cell decreases with age (Kitahara et al., 1979; Reaven et al., 1983; Curry et al., 1984; Elahi et al., 1985), even if the animals are prevented from becoming
obese by exercise or caloric restriction (Reaven et al., 1983). There is diminished glucose stimulated insulin release from isolated pancreatic islets of Langerhans of the older animals as compared to younger controls, despite the fact that the size of the islets, the number of B-cells per islet and the number of secretory granules per B-cells increase with the increasing age without any considerable change in proportion of different secretory cells per islet (Reaven et al., 1979). Studies on biochemical mechanism responsible for the decreased glucose stimulated insulin release have demonstrated that there is an age related diminution in islet adenylate cyclase activity (Lipson et al., 1981a,b) islet's glucose oxidation (Reaven et al., 1980) and islets proinsulin biosynthesis and handling (Gold et al., 1981).

A selective impairment of A-cell response to glucose is seen in diabetic as well as in normal aging persons.

Insulin secretion is directly influenced by the central nervous system. Woods and Porte in 1974 showed that sympathetic and parasympathetic nervous system may be modulators of alpha and beta cell of islets of Langerhans. Some hormonal peptides such as gut hormones, gastrin and cholecystokinin play important role as neuroregulators. Cholecystokinin, a gastrointestinal hormone has been implicated as neurotransmitter influencing hormonal release from islets. Rehfeld et al. (1980) studied the molecular nature of CCK nerve terminals present in the pancreatic islets. The prominent form of CCK in these nerves seems to be the C-terminal tetrapeptide
amide of cholecystokinin (Trp-Met-Asp-Phe-NH₂) referred to as CCK-4. It was observed that amongst various cholecystokinsins, CCK-4 was considerably more effective in releasing islet hormones. Biological activity of cholecystokinin and gastrin is closely related due to the identical four C-terminal sequences.

With the view to introducing selectivity in CCK-4 molecule, congeners of CCK-4 were synthesized, which may selectively stimulate the release of insulin without affecting the release of other islet hormones. Structure-activity relationship studies of CCK-4 (Trp-Met-Asp-Phe-NI-L) and its synthetic congeners were carried out on the basis of side chain functionalities as well as by introducing stereoisomeric changes in amino acids.

Synthetic analogues of CCK-4 in which N-terminal Trp-residue was substituted by cyclic amino acids (Glp or Pro) retain the ability to stimulate the release of insulin from isolated islets of Langerhans (Ahmed et al., 1984) and have no effect on the release of glucagon (Khalid et al., 1986).

In continuation of these studies, seven different synthetic analogues of CCK-4 synthesized in this institute were studied for getting information regarding their specificity in releasing islet hormones. Modifications were introduced at 1st, 2nd and 4th position of parent CCK-4 molecule, while in three of the analogues C-terminal Pro residue was replaced by D-Pro, ^Pro and Thz (Thiazolidine 4-carboxylic acid).
Biological activities of CCK-4 and its seven now synthetic analogues were studied. Structural details have been given in Figs. 16-20.

CCK-4 \[\text{Trp-Met-Asp-Phe-NH}_2\]

Peptide I: \[\text{Trp-Met-Asp-Me Phe}\]
Peptide II: \[\text{Trp-Ser-Asp-Me Phe}\]
Peptide III: \[\text{Pro-Met-Asp-Phe-NHC}_{\text{H}_3}\]
Peptide IV: \[\text{D-Pro-Met-Asp-Phe-NH}\]
Peptide V: \[\text{Thz-Met-Asp-Phe-NH}_2\]
Peptide VI: \[\text{APro-Met-Asp-Phe-NH}_2\]
Peptide VII: \[\text{L-Pro-Met-Asp-Phe-NH}_2\]

In peptide I, the C-terminal residue Phe of CCK-4 is methylated (Trp-Met-Asp-Me Phe). Peptide II (Trp-Ser-Asp-Me Phe) has two modifications i.e. in addition to methylated C-terminal Phe residue, Met at position 2 is also replaced by Ser. The third analogue Pro-Met-Asp-Phe-NHCPL (Peptide III) has also two modifications, Trp being replaced by Pro at position 1 and the C-terminal amide is methylated. In peptide IV, Trp being replaced by D-Pro at position 1. In peptide V Trp at Position 1, being replaced by Thz (Thiazolidine-4-carboxylic acid). In peptide VI, Pro was substituted at position 1 in place of Trp, while in peptide VII, Trp was replaced by L-Pro at position 1 of the CCK-4. Peptide I and II were shown to have an inhibitory effect on the release of insulin and glucagon while peptide III has stimulatory effect.
on insulin release and inhibitory effect of glucagon release (Khalid et al., 1989).

Insulinotropic action of cholecystokinin and its congeners (Peptides IV, V, VI) have been studied at stimulatory and non-stimulatory concentrations of glucose on islets of Langerhans. Effect of peptides IV and VII were compared in 1 and 2 month old rats regarding insulin release.
C-Terminal tetrapeptide amide of cholecystokinin

[CCK-4]

Fig. 15: Structure of CCK-4.
Fig. 16: (i) Structure of peptide I. Met at position 2nd of the parent tetrapeptide is replaced by Ser and C-terminal is N-methylated.
(ii) Structure of peptide II. The parent tetrapeptide is N-methylated at C-terminal.
(iii) Structure of peptide III. Trp at position 1st of the parent tetrapeptide is replaced by Pro and C-terminal amido is substituted by methyl group.
Trp replaced by D.Pro at position 1st

Fig. 17: Structure of peptide IV.
Trp replaced by THz at position 1st

Fig. 18: Structure of peptide V.
Trp replaced by ^ Pro at position 1st

Fig. 19: Structure of peptide VI.
Trp replaced by L-Pro at position 1st

Fig. 20: Structure of peptide VII.