HYPOGLYCAEMIC ACTIVITY OF FLAVONOIDS

INTRODUCTION

Diabetes mellitus is one of the most widespread metabolic disorders in human beings and animals. It has been defined as a sustained state of hyperglycaemia and glycosuria. Other features associated with the diabetic state are, 'symptomatic', 'biochemical', 'functional' and 'morphological'. None of them however is constantly present. These additional features may precede, underlie or follow the detection of hyperglycaemia.

Willis described diabetic urine as tasting "wonderfully sweet as if combined with honey or sugar". Soon after, the adjective 'mellitus' which means honey was added.

Progress in the understanding of the disorder came slowly until the middle of 19th century. Endocrine pancreas in the form of islets of Langerhans was suggested to be involved in the development of diabetes by Von Mering and Minkowski, followed by...
the discovery of insulin by Banting and Best\textsuperscript{335}. This
led to the understanding that diabetes mellitus is
caused by insulin deficiency.

Insulin is the hormone secreted by the beta
cells of the islets of Langerhans. It is an anabolic
hormone which stimulates synthetic pathways including
glycogenolysis, protein degradation and lipolysis\textsuperscript{336}. The hormone stimulates glucose-uptake into the muscle
and fat cells. In muscle, a sufficient part of the
increased glucose taken up is converted into glycogen
while in fat cells it is mainly converted into
glycerol and fatty acid moieties of triglyceride\textsuperscript{337}.

One of the major functions of insulin is to
reduce the concentration of blood glucose by effecting
glucose utilisation in the peripheral tissues as well
as glucose production in the liver. In a fasting
animal, glucose concentration is a function of glucose
entering into plasma from the liver and its removal
from the plasma by the peripheral tissues\textsuperscript{338}.

The blood glucose haemostasis in the mammals is
the net result of the different specific activities of
insulin in consonance with glycaemic hormones (epinephrine and glucagon) on the metabolic activities mainly in the liver, adipocytes and skeletal muscles.

In insulin-dependent diabetes, there is a marked loss of insulin secretory capacity which is due to an actual loss of cell mass. In non-insulin-dependent diabetes, there is a considerable preservation of beta cell mass and the pancreas secretes a substantial amount of insulin into circulation. However, this is inadequate to prevent hyperglycaemia due to two major factors: i) enhanced hepatic glucose production in the basal state results in an elevated fasting blood glucose concentration. ii) impaired glucose disposal by the peripheral tissues results in elevated post-prandial blood glucose concentration. The disease is commonly preceded by impaired glucose tolerance and other metabolic disorders.

There are two different therapeutic measures in the control of diabetes in modern medical practice namely intramuscular injection of insulin and oral hypoglycaemic agents. Administration of insulin
remains as an ideal therapy for the treatment of diabetes in that it provides directly for the deficiency but auto-immunity and coronary complications appear and may affect longevity\textsuperscript{340}.

Oral hypoglycaemic agents play an important role in the treatment of diabetes\textsuperscript{341}. The pathogenesis of diabetes mellitus and the possibility of its management by the oral administration of hypoglycaemic agents have stimulated great interest in recent years.

The currently available oral hypoglycaemic drugs belong to two chemical classes viz. the sulphonyl ureas and the biguanides. The principal effects of hypoglycaemic therapy are, i) lowering fasting blood glucose concentration ii) improving glucose tolerance iii) promoting insulin release iv) lowering ketone body, free fatty acid and glycerol concentration and v) peripheral actions such as potentiating the tissue effects of insulin, reducing pancreatic glucagon secretion or decreasing the activity of hepatic insulinase\textsuperscript{14, 141}. 
In accordance with the recommendations of the WHO Expert Committee on Diabetes mellitus (1980), an investigation of hypoglycaemic agents of plant origin used in traditional medicine seems important. Many plant species are known in folk-medicine of different cultures which have been used for their hypoglycaemic properties. There is enough evidence to indicate that the plant kingdom is a fruitful hunting ground for obtaining effective oral hypoglycaemic agents. The efficacy of several indigenous plants of India alleged to exert hypoglycaemic effects has been reviewed. In an ethnobotanical survey of medicinal plants of Israel, a number of species have been found to be used for their hypoglycaemic effects.

Seventeen flavonoids isolated from plants have been claimed to possess antidiabetic activity through insulin release. Flavonoids are related to the interference with the normal working in the body of such phenolic substances as adrenaline, noradrenaline and 5-hydroxytryptamine. In most cases the flavonoids, like other foreign
LXXIX (±) Adrenaline

LXXX (±) Noradrenaline

LXXI 5-Hydroxytryptamine

LXXII

\[ R'^1 = H, R'^2, R'^3, R'^4, R'^5 = \text{Me, Eucalyptin} \]
phenolics, are converted into sulphates or glucurononates and excreted or they may be broken down into smaller phenolic acids\textsuperscript{24}. Flavonoids generally have a greater safety margin and minimal side-effects towards animals\textsuperscript{354}.

An aqueous alcoholic extract of \textit{Allium cepa} containing quercetin and its glucosides\textsuperscript{355} has been reported to exhibit hypoglycaemic activity in human beings and experimental animals\textsuperscript{356,357}. \textit{Chimaphila umbellata} containing the flavonoids-hyperoside and kaempferol has been reported to elicit hypoglycaemic activity in laboratory models\textsuperscript{358}. \textit{Trigonella Foenum-graecum} reported to contain many flavonoids, vitexin, vitexin 7-O-glucoside, homoorientin saponaretin, quercetin, luteolin and vitexin cinnammate has been proved to show hypoglycaemic effect in animals\textsuperscript{359-361}. A crude extract of \textit{Eucalyptus globulus}, rich in phenolic glycosides like quercetin, quercitrin, rutin, hyperoside and eucalyptin(LXXII) has been reported to have antihyperglycaemic activity in rabbits\textsuperscript{362}. \textit{Coriandrum sativum} reported to contain quercetin 3-O-glucuronide,
isoquercitrin and rutin, has been recorded to possess hypoglycaemic activity\textsuperscript{363}.

An aqueous alcoholic extract of the leaves of \textit{Gymnema sylvestre}, a herb belonging to the Asclepiadaceae has been found to control blood glucose level in alloxan-treated diabetic rats. This is associated with normal blood glucose and serum insulin level during oral glucose tolerance test in treated rats\textsuperscript{364}.

\textit{Cyamopsis tetragonoloba} has been reported to lower the postprandial blood glucose in human beings. \textit{Panax ginseng} and \textit{P. quinquefolius} have been reported to lower blood glucose level and liver glycogen content\textsuperscript{365}. \textit{Innula helenium} has been recorded to exhibit hyperglycaemic effect in larger doses and hypoglycaemic effect in smaller doses in experimental animals\textsuperscript{366}.

Hypoglycaemic properties of the extracts of \textit{Cimicifuga racemosa}\textsuperscript{367}, \textit{Arctium lappa}\textsuperscript{368}, \textit{Taraxacum officinale}\textsuperscript{368}, \textit{Allium sativum}\textsuperscript{369}, \textit{Cassia auriculata}\textsuperscript{370}, \textit{Vinca rosea}\textsuperscript{371}, \textit{Eugenia jambolana}\textsuperscript{371}
and Cassia alata\textsuperscript{372} have been reported. The leaves of Rivea cuneata\textsuperscript{373} have afforded an antidiabetic principle.

A decoction prepared by boiling the roots of Inula racemosa has been reported not only to lower the fasting blood sugar level in normal rabbits, but also to protect the animals against glucose-induced hyperglycaemia\textsuperscript{374}. Boyadzhieva\textsuperscript{375} has reported on the antidiabetic activity of Lepidium ruderale in experimental hyperglycaemia. An oral administration of the decoction of Clerodendron phlomidis could inhibit the adrenaline-induced hyperglycaemia in rabbits\textsuperscript{376}. An extract of the roots of Eleutherococcus senticosus has effectively reduced the high blood sugar level in rabbits with adrenaline hyperglycaemia and in humans with alimentary hyperglycaemia\textsuperscript{377}.

The studies presented in the subsequent pages are based on the analysis of oral glucose tolerance test in the rats treated by some flavonoid-isolates as described in Chapter I. The observations are presented and discussed in the light of available literature.
Healthy female albino rats of the Wistar strain (Rattus norvegicus) aged about 90 days and weighing approximately 100-150 g were obtained from Fredrick Institute of Plant Protection and Toxicology, Padappai, Madras. They were kept in clean cages and housed in a well ventilated animal house. Standard rat pelleted diet (Gold Mohur, Hindustan Liver Ltd., India) and clean water were made available ad libitum.

The animals were divided into eleven groups, each consisting of nine animals and each group was further divided into three sub groups and each subgroup consisting of three animals. The animals were allowed to fast overnight prior to each experiment. Animals placed in Group 1 were normal control animals which received the vehicle (distilled water) through a gastric catheter, while those in Group 2 were glucose control animals which received glucose orally through a gastric catheter at a dose of 150 mg/100 g BW, as 15% glucose (Dextrose anhydrous AR, Chemco Fine Chemicals, Bombay) aqueous solution.
Animals in Groups 3-9 were experimental ones which received the flavonoid isolates as follows.

Group 3- Rutin (G₂)
Group 4- Kaempferol 3-0-(6"-0-acetyl)-gluco-7-0
-rhamnoside (G₃).
Group 5- Luteolin 7-0-rutinoside (G₄)
Group 6- Isorhamnetin 3-0-sophoroside (G₆)
Group 7- 3',4',6,8-Tetrahydroxyflavonol-5'-methylether
-7-0-neohesperidoside (G₇)
Group 8- Quercetin 5-0-rhamno-3'-0-arabinoside (G₉)
Group 9- 3',4'-Dihydroxyflavone-7-0-glucuronide (G_{10})
Group 10- Myricetin 5'-methylether-3-0-
-neohesperidoside (G₁₂)

The flavonoids were administered orally through a gastric catheter at a dose of 15mg/100g BW as 1% aq. solutions of the concerned flavonoid. Two hours later glucose was given orally as for the second Group of animals. The last group was treated as flavonoid control which received the flavonoid (G₆) only as before and was not given glucose, to study the effect of the flavonoid on the normal glycaemia of the animals.
Exactly after 30, 60, 120 min after glucose administration each sub-group of animals (Groups 2-10) was sacrificed by decapitation, blood was collected and the plasma separated and stored at -4°C until used for estimation of blood glucose. The last group of animals was sacrificed after the administration of flavonoid G6 at the above mentioned time intervals. Blood was collected and treated as before.

Blood glucose was estimated by the method of Asatoor. The proteins were precipitated by tungstic acid. The alkaline copper solution was reduced and the amount of cuprous oxide formed was colorimetrically estimated at 680nm (Spectronic 20D Colorimeter) by reacting with phosphomolybdic acid. A blank experiment was also carried out. Each sample of blood was estimated in duplicate. Altogether six observations were available for each sub-group.

The effect of the flavonoid isolates on glucose-induced hyperglycaemia is presented in Table II-1 and in Figs. II-1, 2 and 3. Blood glucose level is expressed as mg/100ml. The data were analysed statistically using the Student's t-test.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Glucose in 100 ml blood (mg)</th>
<th>+ 30 min</th>
<th>+ 60 min</th>
<th>+ 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>93.30±5.61</td>
<td>--</td>
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<td></td>
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<tr>
<td>2.</td>
<td>Glucose control</td>
<td>144.58±1.64</td>
<td>125.83±1.05</td>
<td>95.83±1.39</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>G2</td>
<td>114.17±1.90***</td>
<td>92.92±3.95***</td>
<td>101.67±4.69(NS)</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>G3</td>
<td>115.75±4.55***</td>
<td>88.33±4.46(NS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>G4</td>
<td>110.00±3.54***</td>
<td>114.58±7.17(NS)</td>
<td>75.00±1.83***</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>G6</td>
<td>80.00±1.94***</td>
<td>56.67±2.94***</td>
<td>39.17±1.05***</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>G7</td>
<td>120.83±0.53***</td>
<td>108.33±2.30***</td>
<td>102.08±4.67(NS)</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>G9</td>
<td>127.50±1.58***</td>
<td>90.42±8.50**</td>
<td>92.92±7.57(NS)</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>G10</td>
<td>121.25±4.51***</td>
<td>116.67±6.88(NS)</td>
<td>57.50±3.16***</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>G12</td>
<td>91.67±6.54***</td>
<td>89.58±3.73***</td>
<td>84.58±2.84**</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>G6 control</td>
<td>52.50±0.50***</td>
<td>68.33±1.39***</td>
<td>103.75±0.50***</td>
<td></td>
</tr>
</tbody>
</table>

As compared with control, *p < 0.05; **p < 0.01; ***p < 0.001.

Groups 3-10 were compared with the corresponding subgroup of glucose control and group 11 was compared with normal control animals.

N.S. - Statistically not significant.
Figure II-3
RESULTS AND DISCUSSION

Ingestion of glucose is followed in normal subjects by a rise in blood sugar. Figs. II-1, 2 and 3 as also the data presented in Table II-1 show the effect of flavonoids on glucose-induced hyperglycaemia. In the Figures, mean values of fasting blood glucose levels of the animals expressed in mg/100 ml are plotted against time in min. Each sub-group of Groups 3-10 which form the flavonoids-treated glucose induced hyperglycaemic animals was compared with that of the corresponding sub-group in glucose control animals. All the flavonoid isolates have been found to cause a significant decrease ($p < 0.001$) in the fasting blood glucose level 30 min after glucose load administration, when the glycaemic values were the highest in the controls with apparently G6 being the most active. The hypoglycaemic effect of G6 significantly sustained even after 120 min. It was therefore selected to study its effect on normal glycaemia of animals. Each sub-group of Group II was compared with normal control viz. Group 1. G6 caused
a significant decrease (p < 0.001) in normal glycaemic condition too. However fasting blood glucose level registered a marked increase (p < 0.001) 120 min after the ingestion of G6 leading to a hyperglycaemic state.

The above results demonstrate the hypoglycaemic effects of the flavonoid isolates on hyperglycaemic and normoglycaemic animals though the mechanisms of action in the two cases are different due to the difference in control states.

The fasting blood glucose level of animals treated with bioflavonoids have been found to be significantly different from those of the controls of the same weight and age. The flavonoids are able to lower the fasting blood glucose concentration and improve glucose tolerance. The antidiabetic activity of the flavonoids may then be suggested to proceed through release of insulin.