Determination of antidiabetic activity in
Allium cepa (onion) tissue cultures

S M Kelkar1*, G S Kakli2 and V A Bapat2
Radiation Biology Division1 and Nuclear Agriculture and
Biotechnology Division2
Bhabha Atomic Research Centre, Mumbai 400 085, India

Received 12 May 2000; revised 4 July 2000

Seedling, seedling parts and callus cultures of onion were
tested for their antidiabetic activity by feeding the tissue-extracts
to diabetic rats. The results indicated much higher antidiabetic
activity in callus cultures as compared to natural bulbs of onion.
These results may be of pharmaceutical significance since the
callus can be used as an alternative source for the isolation of
antidiabetic compounds.

Applications of plant cell cultures for production of
useful medicinal compounds have been reported from
several medicinal plants1,2. The advantages of plant
cell cultures such as rapid growth, relatively simple
cultivation requirements and amenability for scale-up
are well recognised3. Extensive in vitro work
pertaining to tissue cultures of onion has been carried
out4,5, however, there is no report on antidiabetic
activity from tissue cultures of onion.

Onion (Allium cepa L.) is one of the recognised
medicinal plants known to possess several medicinal
properties, including antidiabetic activity5,7. Sulphoxide
amino acids from onion inhibit cholesterol and other lipid(s) synthesis8. This is in
keeping with lower incidence of heart attack in east
Asian population regularly consuming Allium cepa in
their diet8. Thus, antidiabetic and antilipidemic
properties of onion are well recognised. The present
report describes elevated antidiabetic activity in
extracts of seedlings and callus from in vitro tissue
cultures of onion as compared to bulbs of onion.

Seeds of Phule Safed (White) variety obtained
from Mahatma Phule Krishi Vidyapeeth, Rahuri were
used for raising cultures. The seeds were washed
several times in running water before surface
sterilisation in HgCl2 for 5 min. Such surface
sterilised seeds were rinsed four times in sterile water
and were sown on 1/2 strength MS basal medium9
containing 3% sucrose. Segments from young leaf
base were excised from 3 week-old aseptic seedlings
and were cultured on MS medium supplemented with
auxins, cytokinins and other growth regulators. The
pH of the medium was adjusted to 5.8 before gelling
with 0.8% agar (Marine Chemicals, Kochi). The
cultures were incubated in growth room and exposed
to continuous day light fluorescent tubes (1000 lux)
at 25±2°C at relative humidity of 50-60%.

Callus formation from the cut ends of the cultured
leaf base explants was observed after about one week
inoculation on MS+2,4-dichlorophenoxyacetic acid
(2 mg/l)+ kinetin (0.2 mg/l). The proliferating callus
was separated from the explant and was subcultured
on the fresh medium of the same composition. The
callus was faint yellow, compact and nodular. The
tissue was maintained by periodic subcultures at
regular intervals.

Onions from local market were weighed and
homogenised in water, first in mixer-grinder (Sumeet
Company, Bombay, India) and then in Tri-R Stir-R
homogeniser (Tri-R Instruments Inc. NY, USA). The
callus or entire seedlings and the leaves/roots from
seedlings were weighed and homogenised in a
homogeniser. All extracts were filtered through nylon
wire mesh and the aqueous suspensions were used for
the bioassay.

Normal male Wistar rats 6-8 weeks old, weighing
180-200 g maintained on stock laboratory diet were
used. The diet constituted 70% wheat, 20% Bengal
gram, 4% yeast, 5% fish meal, 0.75% sesame oil,
0.25% shark oil (corresponding to 30% protein, 60%
carbohydrate, 5% fats/oils, 5% fibre) supplemented
with vitamins and mineral mixture.

Diabetes was induced in rats by a single
intraperitoneal injection of streptozotocin (60 mg of
STZ/kg body wt.) after 24 hr of fasting10. The
diabetic animals were stomach-fed with the extracts
using catheter tubes over a period of 4 days.

Blood was collected from tail-vein before first
feeding, and 3 hr after last feeding on the 4th day.
Throughout the experiments, rats were maintained on
a diet stated above. The blood was de-proteinsed by
using Ba(OH)2 and ZnSO4 and glucose was estimated
immediately in protein-free supernatant by Glucofix kit (Minarini Diagnostics, Italy). The hypoglycemic activity is expressed as % reduction in blood glucose. Data were subjected to statistical analysis using Student's 't'-test.

Table 1 shows that the feeding of callus-extracts resulted in 34% reduction in blood glucose. Extract of the entire seedling caused 31% reduction in blood glucose. Leaves isolated from seedlings were effective nearly to the same extent (32%) while roots did not show any effect. Two different varieties of onion bulbs. Literature survey cultures of these plants has already been report3,4,12 and now the foregoing observation brings out for the first time the presence of antidiabetic activity in callus and seedlings of onion bulbs. Literature survey pertaining to the presence of secondary products in plant cell cultures suggests that with notable exceptions, cultured plant cells do not produce high levels of secondary metabolites characteristic of that plant, and in general the level of desirable compound(s) in cell cultures are low5. Thus the high levels of antidiabetic activity in seedling and callus cultures as reported here, is of significance.

The extent of reduction in blood glucose in the present study appears to be comparable with the purified sulfoxide amino acids obtained from onion bulbs5. It may be noted that feeding of glibenclamide, a known antidiabetic compound in clinical practice, caused nearly 50% reduction in blood glucose in the same studies5. We have reported that feeding of chlorpropamide (Diabenese, Pfizer Company) mixed in a diet at a dose of 90-100 mg/rat/day for 5 days resulted in 50% reduction in blood glucose in STZ-diabetic rats7, while feeding of 270 mg callus-extract and 300 mg seedling leaf-extract for 4 days caused 33.6% and 30.7% reduction respectively. In conclusion, the potential of cell culture material as an alternative source to obtain biologically active components in general and antidiabetic compounds in particular is obvious.

References
7 Breu W & Dorsh W (1994) Economical and Medicinal Plant Research 6, 115-147