

Determination of hypoglycemic activity in *Morus indica* L. (mulberry) shoot cultures

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The aqueous extract from the leaves of shoot cultures of two elite varieties of mulberry, viz. S-34 and S-36, showed the presence of hypoglycemic activity when fed to the diabetic rats. The hypoglycemic activity of the extracts of leaves from the shoot cultures was higher and better as compared to the field-grown plant leaves. Based on these results, the usefulness of *in vitro* plantlets of mulberry, as a source of antidiabetic compound (S) is discussed.

MEDICINAL plants and their extracts have gained increasing importance over the past several years, since they can be used as a source for the preparation of herbal drugs¹. Studies on *in vitro* shoot cultures of medicinal plants have shown the presence of known and new pharmacologically active compounds².

Mulberry is valued for its foliage which constitutes the chief feed for silkworms. Besides its use in silk industry, mulberry leaves are useful as cattle fodder. They are nutritious and palatable and are known to improve milk yield when fed to dairy animals³. Analysis of mulberry leaves collected from different parts of India has revealed the presence of proteins, carbohydrate, calcium, iron, ascorbic acid, β -carotene, vitamin B-1, folic acid and vitamin D. All these are essential for the growth of the silkworm⁴.

Apart from their use as animal and insect feed, mulberry leaves have been shown to possess medicinal applications as they contain diuretic, hypoglycemic and hypotensive agents³. Thus, non-toxic mulberry leaves would be an ideal source for testing the presence of hypoglycemic activity. The present report describes results of the presence of hypoglycemic activity in extracts of leaves from *in vitro* shoot cultures and field-grown plants of mulberry.

Shoot cultures of mulberry (*Morus indica* L. var. S-34 and S-36) were used for experiments. The cuttings from mature trees of these two varieties were collected from Central Silk Research and Training Institute, Mysore, and were planted in experimental station at Trombay, Mumbai. These two varieties were selected on the basis of their good quality foliage throughout the year, their amenability to vegetative propagation with fast regenerative capability, high nutritional value and more resistance to stress, diseases and pests⁵. From such field-grown plants, buds which sprouted within 4–6

weeks were used for the establishment of shoot cultures. Auxiliary buds were surface-sterilized with 0.1% HgCl₂ for 5 min. After rinsing 5–6 times with sterile water the auxiliary buds were cultured aseptically on medium⁶, supplemented with IAA (5.71 μ m) containing 3% wt/vol sucrose on which they produced extensive shoots and roots. The detailed protocol for establishment of shoot cultures from such plantlets has been described earlier⁷. Among these two varieties, S-36 grew very well on solid medium (i.e. MS supplemented with 22.10 μ m BAP), whereas S-34 variety produced ample shoots in liquid medium of the same composition. From a single cultured auxiliary bud of S-34, within a period of three weeks 30–35 shoots were formed. The pH of the medium was adjusted to 5.8 before gelling the medium with 0.8% agar (HiMedia, 201 HiMedia Laboratories Pvt Ltd, Mumbai, India). Both solid and liquid cultures were maintained in a culture room at 25 \pm 2°C at a relative humidity of 50–60% and were exposed to continuous fluorescent light (ca. 1000 lux). The liquid cultures were kept on a shaker at 70 rpm for continuous agitation.

The leaves from field-grown S-36 or S-34 variety were cleaned with water, blotted, weighed and homogenized in ice-cold water, first in mixer-grinder (Sumeet Company, Mumbai, India) and then in Tri-R Stir-R homogenizer (model S63C, Tri-R Instruments Inc., NY, USA). Similarly leaves from shoot cultures of S-36 or S-34 variety growing in test-tubes were taken, weighed and homogenized. All extracts were filtered through nylon wire mesh and the aqueous suspensions, having pH 6.1 to 6.4, were used for the bioassay.

Normal male Wistar rats weighing 190–210 g maintained on stock laboratory diet were used. The diet constituted 70% wheat, 20% bengal gram, 4% yeast, 5% fish meal, 0.75% til oil, 0.25 shark oil (corresponding to 60% carbohydrate, 30% protein, 5% fats/oils, 5% fibre), supplemented with vitamins and minerals mixture.

Diabetes was induced in rats by a single intramuscular injection of streptozotocin (60 mg of STZ/kg body wt) after 24 h of fasting⁸. The diabetic animals were stomach-fed with extracts using catheter tubes over a period of 3 days.

The blood was collected from tail-vein before feeding, 2.5 h after first feeding and after 5th feeding on the 3rd day. During this period (otherwise also), rats were maintained on the diet stated above. The blood was deproteinized⁹ and glucose was estimated immediately by Glucofix kit¹⁰ (Minarini Diagnostics, Italy) in protein-free supernatant. The hypoglycemic activity is expressed as percent reduction in blood glucose. Data was subjected to statistical analysis using Student's *t* test.

The aqueous extracts of mature leaves from field-grown S-36 variety exhibited hypoglycemic activity in STZ-diabetic rats within 2.5 to 3 h as shown in Table 1. The extent of reduction in blood glucose level decreased

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Table 1. Hypoglycemic activity in leaf-extracts of S-36 and 34 varieties of *Morus indica* grown in field or in cultures

Source	Maturity	Dose/feed	n	Reduction in blood glucose (%)		Reduction/g (%)*	
				2.5 h	3rd day	2.5 h	3rd day
S-36							
FGL	Old	250	15	26.5 ± 1.2	14.9 ± 1.4	21.2	11.9
FGL	Young	260	11	nil	nil	—	—
TCL	Old	130	13	16.6 ± 1.4	26.0 ± 1.2	25.6	40.1
TCL	Young	200	11	nil	nil	—	—
S-34							
FGL	Old	300	11	37.7 ± 1.2	9.3 ± 0.9	25.1	6.2
TCL	Old	700	15	34.4 ± 1.3	40.2 ± 1.1	49.2	57.5

The dose is indicated as extract corresponding to mg of leaves/feed/animal. Total of five feeds were given. Diabetic rats were 8–10 post Stz-days. Zero hr blood glucose were exceeding 550 mg/dl and were taken as control. *P* values were <0.005 for S-36 and <0.001 for S-34 variety compared to zero hr blood glucose. FGL: field-grown leaves; TCL: leaves of shoot cultured plants; Young leaves – weight <1.0 g/leaf. Old leaves – weight >1.5 g/leaf.

*per gram of leaves.

on the 3rd day in spite of giving feeds twice daily. This partial loss of hypoglycemic effect could possibly be attributed to rapid metabolism of the compound(s) responsible for the observed effect. However, the extract of young leaves from field-grown S-36 variety did not show any effect. The presence or absence and concentration of any bioactive compound are known to be influenced by the age of tissue, age of plant, season and location or area of plant. The difference in hypoglycemic activity of old and young leaves in the present study, could be attributed to the age of leaves. Nearly half the dose of leaves from cultured plantlets of S-36 variety caused 16% reduction in blood glucose after 2.5 h which increased to 26% on the 3rd day.

The leaves from field-grown S-34 variety showed 38% reduction in blood glucose after 2.5 h which decreased to nearly 9% on the 3rd day (Table 1). This decrease was similar to that observed with extract of S-36 variety leaves stated above. It can be seen that feeding 700 mg of extract of leaves of S-34 shoot cultures resulted in 34% reduction in blood glucose after 2.5 h which increased to 40% after 3 days feeding.

The total number of feeds were the same for all the groups, though the amount in each feed varied. Therefore, comparative hypoglycemic efficacy per gram of field-grown and *in vitro* shoot cultured leaves is given in Table 1. It is evident that the response of leaves from shoot cultures of both S-34 and S-36 was better than field leaves possibly due to the *in vitro* and *in vivo* stability of compounds responsible for blood glucose reduction. The retention of hypoglycemic response up to 3rd day in case of leaves from shoot cultures is in contrast to that observed for leaves from field grown plants of the same variety. This could presumably be ascribed to the differences in the nature and/or composition of hypoglycemic compound(s) present in natural/field grown plant and cultured plantlet(s).

The foregoing observations bring out for the first time the presence of hypoglycemic activity in field-grown mulberry leaves using diabetic rats as a model for testing. Moreover, hypoglycemic activity from cultured leaves appeared to have better efficacy. It is difficult to compare the efficacy of the extract used in the present studies with the known sulphonyl ureas employed in practice. Nevertheless, a gross comparison can be made. Feeding of chlorpropamide (Diabinese, Pfizer Company) mixed in a diet at a dose of 90–100 mg/rat/day for 5 consecutive days resulted in 50% reduction in blood sugar in STZ-diabetic rats⁸. However, feeding of 700 and 130 mg extract of leaves from plantlets of S-34 and S-36, for 3 days caused 40% and 26% reduction in blood glucose respectively. In fact, the efficacy may be significantly more after purification of the compound(s) from the extract. These studies are underway.

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