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Investigation of Anti-Hyperglycemic Activity of Micropropagated *Tinospora Cordifolia* in Alloxan Induced Experimental Diabetes

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Abstract

The aim of the present study was established for rapid clonal propagation of the valuable medicinal plant, *Tinospora cordifolia,* through *in vitro* culture using nodal explants. Best shoot induction was observed with 4.36 µM KIN produces 2.32± 0.1 cm length with 1.8 ± 0.1 numbers of shoots with 70% response. *Tinospora cordifolia* were cultured on MS medium with BA alone and in combination with NAA or IAA for multiple shoot proliferation. BA (8.82 µM) alone showed better growth response and produced 4.81± 0.2 numbers of shoots with length of 3.1± 0.1 cm after 20 days. Small shootlet were transferred to shoot elongation with 8.82 µM of BA alone. An average length of 4.82± 0.4 cm with 4.61± 0.2 numbers of shoots produced, 76% of response. The elongated shootlets transferred to half strength MS medium and 6.43 µM of IBA with 3% sucrose produce 5.2± 0.2 rootlets per plant with 3.2± 0.1 cm length after 27 days. Rooted plantlets were transplanted *ex vitro*, kept under green house conditions for one month followed by their field transfer. Approximately 80% of plantlets survived.

The micropropagated plants were assessing to hypoglycaemic effect of daily oral administration of methanol extract of *Tinospora cordifolia* in alloxan induced diabetic rats. A significantly decrease in blood glucose, glycosylated hemoglobin, cholesterol and urea ($p<0.01$), and increase in body weight and total protein ($p<0.01$) were observed in diabetic rats on treatment with *Tinospora cordifolia* methanol extract (500mg/kg body weight) when compared to normal. The activity of the hepatic enzyme hexokinase was significantly increased where as glucose 6-phosphatase and fructose 1, 6-bisphosphatase were significantly decreased ($p<0.01$) by the oral administration of *Tinospora cordifolia* methanolic extract in diabetic rats. When compared wild the micropropagated plant have greater signifying to control the blood glucose in alloxan induced diabetic rats.

Key words: *Tinospora cordifolia,* blood glucose, alloxan, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterised by common feature of chronic hyperglycaemia with disturbance in carbohydrate, fat, protein metabolism and sometimes ketonemia [1]. The widespread pathological changes lead to complications like retinopothy, microangiopathy and nephropathy [2]. In spite of the introduction of hypoglycaemic agents, diabetes and related complications continue to be a major medical problem. In India, this disorder is on alarming condition as compared to most of the developed countries. Despite advances in understanding of the disorder and the management, the mortality and morbidity due to this disease is increasing.

There is growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. The world health organization (WHO) has also recommended the evaluation of the effectiveness of plant in condition where we lack safe modern drugs. Therefore studies with plant extracts are useful to know their efficacy and mechanism of action and safety [3].

Many medicinal plants species are disappearing at an alarming rate, as a result of rapid agricultural and urban development, deforestation and indiscriminate collection. If this trend continues, mankind will, forever, loss some of the most important sources of drugs. The tissue culture technique has been proved very efficient in rapid mass propagation and conservation of important multipurpose medicinal plants [4,5].

Tinospora cordifolia (willd.) Miers Ex Hook.F. & Thoms. It is a large, glabrous, succulent, perennial deciduous twiner with succulent stems and papery bark, climbing shrub belonging to the family menispermaceae. It is distributed throughout tropical Indian subcontinent, Sri Lanka and China, ascending to an altitude of 1200m. *Tinospora cordifolia* (Guduchi) is Indian medicinal plant and has been used in Ayurvedic preparation for the treatment of various ailments throughout the centuries. Ancient Hindu physicians prescribed it for gonorrhoea. The plant is used in Ayurvedic, “Rasayanas” to improve the immune system and the resistance against infections. The whole plant is used medicinally; however, the stem is approved for use in medicine as listed by the Ayurvedic Pharmacopoeia of India [6]. In folk and tribal medicine the whole plant, powdered root and stem bark, decoction of root and stem, juice of the root and paste or juice of leaves or stem of *Tinospora cordifolia* are used to treat various ailments such as fever, jaundice, general debility, cough, asthma, leucorrhoea, skin diseases, bits of poisonous insects and venomous snakes and eye disorders [7].

Today the drug and a tincture prepared from *Tinospora cordifolia* are approved for use in the Indian pharmacopoeia. They are used for the treatment of general weakness, gonorrhoea, secondary syphilis, urinary diseases, impotency, gout and viral hepatitis [8]. A variety of compounds have been isolated from aerial parts and roots of *Tinospora cordifolia.* They belong to different classes such as alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoid, phenols, aliphatic compounds and polysaccharides.

Tissue culture has greatly enhanced the scope and potentiality of mass propagation by exploiting the regenerative behaviour in a wide range of selected horticultural and agricultural plants including the medicinal ones [9]. Many important medicinal herbs throughout the world have been successfully propagated *in vitro*, either by organogenesis or by callus formation.

The aim of the present study was undertaken with a view to establishing an efficient protocol for *in vitro* rapid propagation of this important medicinal plant and evaluate the hypoglycaemic activity of methanol extract of micropropagated *Tinospora cordifolia* in alloxan induced diabetes rats.

MATERIALS METHODS

Collection of Plant Materials

The healthy plants of *Tinospora cordifolia* were collected from two regions of Vellore district (Kalavai and Walajapet) and authentified by the herbarium of Botany Directorate in National Institute of Herbal Science, Plant Anatomy Research Center, Chennai. A voucher specimen (No: TC08) was deposited in the center. and raised in pots containing soil and farmyard manure (1:1) in greenhouse condition.

Explants Prepared and Surface Sterilization Methods

The explants were prepared from the nodal portion of stems, internodes and shoot tip, which were first washed thoroughly in running tap water for about half an hour to remove soil and other superficial contamination, and then allowed to stay for 3–4 hrs in double distilled water to facilitate the phenolic and a characteristic gummy substance of polysaccharide leaching out from explants. The disinfected explants were surface sterilized under aseptic conditions in a laminar flow chamber.

Later the explants (1cm each) with upper portion were washed with 1% sodium hypochlorite solution (v/v) for 5 min followed by thorough washing under running tap water for 15min. Then single bud explants (1cm each) with upper portion were washed with 1% Bavistin (w/v) for 5 min followed by 3 times washing in sterile distilled water. The explants were surface sterilized with 0.1% (w/v) mercuric chloride for 6 min. and later rinsed 4 or 5 times with sterile distilled water. Later the edges of the explants were trimmed with sterile blade to eliminate possible residue of sterilant and the explants were then used for culturing.

Micropropagation of *Tinospora cordifolia*

The *in vitro* shoot initiation from various explants of *Tinospora cordifolia,* the surface sterilized explants such as nodes, internodes and shoot tips were cultured on full strength MS medium supplemented with various concentration of Cytokinins BA (N6 – benzyladenine) (0.44 – 13.31µM), KIN (Kinetin) (0.46 – 13.94 µM) and 2ip (N6 -2- isopentenyl adenine) (0.49 – 14.76 µM) were tested individually for shoot induction. The cultures were incubated under a 16 hours photoperiod (50 $\mu m.m^2$/sec provided by cool-white day-light Sylvania fluorescent lamps, USA) at 25°C and 55% relative humidity in sterile environment condition. Different experiments were conducted to find out an optimum culture conditions for the maximum shoot initiation (budding) from the cultured explants. And use the different type of Phenolic exudation controlling substances such as polyvinylpyrollidone, activated charcoal, silver nitrate and ascorbic acid were used at the time of initiation.

Different experiments were conducted to find out an optimum culture conditions for the maximum shoot proliferation and elongation from the cultured explants. Shoot proliferation and elongations were cultured on MS medium supplemented with various concentration of Cytokinins BA (4.22 – 12.64µM), KIN (6.23 – 12.44 µM) and 2ip (2.11 – 13.32 µM) were tested individually and combine with Auxins like NAA (α- napthyl acetic acid) (4.22 – 12.88 µM) and IAA (Indole -3- acetic acid) (1.32 – 11.86 µM).

Root Initiation and Acclimatization

After 20 days the culture were used to rooting, 1 to 2 cm long shoots were transferred to half strength of MS medium amended with 3% sucrose with Auxins IAA (1.76 – 17.13 µM), IBA (Indole -3- butyric acid) (1.32 – 14.70 µM) and NAA (1.34 – 16.11 µM) tested individually. All the experiments were done in six cultures with ten replicated experiments.

The *in vitro* rooted shoots were carefully removed from the culture vessel and they were gently washed with sterile distilled water to remove every trace of media. Thereafter plantlets were dipped in 0.05% Bavistin (systemic fungicide) for 10 seconds to minimize the microbial infection. Again a second wash was given with sterile distilled water. The treated plantlets were then transferred aseptically to small earthen pots containing mixture of vermiculite, sterilized red soil and farmyard manure at 1:1:1 ratio in growth chamber with controlled temperature, light and humidity to acclimatize with the outside environment. Half strength liquid medium was added periodically. The plantlets were covered with polythene bags to ensure a relative humidity of 70 to 80%. The acclimatized complete plantlets were then transferred to the field.

Media Composition and Culture Conditions

For all the above studies, MS medium were used as sole basal medium. Four individual stock solutions *viz.*, Macro, Micro, Fe, EDTA and Vitamins were prepared and stored in refrigerator; whereas meso-inositol and Plant Growth Regulator (PGR) stock solutions were freshly prepared for use. Three percentage of sucrose (30 g/l, w/v), meso-inositol (100 mg/l, w/v) and required amount of PGR were added to the MS medium and the pH was adjusted to 5.6 with 1N HCl or 1N NaOH. The gelling agent, agar at 0.8% (w/v) was added to the prepared media and mixed well before dispensing into glassware. The contents were labelled and sterilized in an autoclave at 15 lb pressure for 15 minutes at 121°C. The cultures were incubated under a 16 hours photoperiod (50 $\mu m.m^2$/sec provided by cool-white day-light Sylvania fluorescent lamps, USA) at 25°C with 55% relative humidity in sterile environment condition.

Preparation of plant extracts from wild and micropropagated *Tinospora cordifolia*

Tinospora cordifolia plant materials collected from wild and micropropagated matured stem were dried and powered, were allowed to pass through ss sieve (20mesh). It was defatted by treating with petroleum- ether (60-80°C) and then extracted to exhaustion (soxhlet) with methanol. The solvent was removed under vacuum (35-40°C) to get the some solid mass. It was used for the further activities.

Animals

Healthy adult cross breed of male *wistar albino* rats (weighing 180- 210g) were used in the experiments. Animals were housed in polypropylene cages at 22±2^oC with relative humidity of 45- 55% under 12 hour's light and dark cycle. They were feed with standard laboratory animal feed (Hindustan lever Ltd., India) and water ad libitum. Ethical clearance was obtained from the Institutional Animal Ethical Committee (Approval No.115/ac/07/CPCSEA).

Acute toxicity studies

Acute toxicity study was performed according to Organisation for Economic Co-operative and development guidelines No. 423 [10]. *wistar albino* rats of either sex were divided into six groups with six animals each. The micropropagated *Tinospora cordifolia* methanol extract was administered orally as single dose at different dose levels of 50, 250, 500, 1000, 1500, and 2000mg/kg b.w. Animals were observed individually during the first 30 minutes and periodically during 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 days.

Induction of diabetes mellitus

The experimental animal in this model is the male, adult *wistar albino* rats, weighing 180- 210g. After a 12-hour fast, the rats were weighed and a solution of 2% alloxan monohydrate (S.D. Fine Chemicals, Mumbai) diluted in saline (0.9%) corresponding to 80 mg of alloxan per kg body weight was

administered intraperitoneally in a single dose. Food and water were given to the rats after 30 minutes of drug administration [11]. After two week rats blood glucose levels of 200-260mg/dl. were used for the study. Blood was taken from eyes (Venous pool) and glucose was estimated by Sasaki method [12]. All the biochemical and chemicals used in the experiment were of analytical grade.

Experimental designs

The rats were divided into four groups with six in each group (06 Normal rats and 18 diabetic surviving rats). Group-I Animals were administrated a single daily dose of 0.5 ml of saline served as healthy control group. Group-II Animals served as alloxan control, which received alloxan corresponding to 80 mg/kg in a single dose. Group-III Alloxan induced diabetic rats received wild plant methanol extract of TCS in 500 mg /kg of body weight, once in every day using an intragastric tube for 6 weeks. Group–IV Alloxan induced diabetic rats received micropropagated plant methanol extract of TCS in 500 mg /kg of body weight, once in every day using an intragastric tube for 6 weeks. Group – V Alloxan induced diabetic rats received standard drug Glibenclamide 25mg/kg of body weight, once in every day using an intragastric tube for 6 weeks.

Samples Collection

At the end of 6^{th} week the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in fresh vials. Liver is dissected out and washed in ice-cold saline immediately.

Evaluation of effect on biochemical variables

Fasting blood glucose [12], total protein [13], cholesterol [14], liver glycogen [15], blood urea [16] were estimated and glycosylated haemoglobin [17] was estimated by using the hemolysate obtained during the isolation of erythrocyte membrane [18]. The liver supernatant was extracted and used for the assay of hexokinase [19], fructose- 1, 6- bi- phosphatase [20] and glucose- 6- phosphatase [21]. Biochemical determinations were carried out using Shimadzu Spectrophotometer.

Statistical Analyses

All the plant experiments were done in six cultures with ten replicated experiments. The data were subjected to ANOVA and means were performed by using SPSS (SPSS ver.16.0). The animal studies results are expressed as mean$\pm$SD. The data were analyzed by one way ANOVA followed by Dun net's test at level of significance was expressed as $P<0.05$ and $P<0.01$.

RESULTS AND DISCUSSION

After surface sterilization the node, internodes and shoot tips explants were inoculated on MS supplemented with different concentration of BA, KIN and 2iP showed different response according to the hormonal concentration used. In the present study, the auxiliary buds on the nodal cuttings showed visible growth after five days in culture and most of them were grow into shoots within 20 days. Shoots formation was affected by the concentration of hormones used in the medium. Among the different Cytokinins (BA, KIN, and 2iP) the better result was produced only in nodal explants with KIN 4.36µM, produces 2.23 ± 0.1 cm length with 1.8 ± 0.1 numbers of shoots with 70% response when compared with other Cytokinins [Table 1]. The Primitive role of KIN for shoot initiation has been documented in many other medicinal plants too, such as *Saussurea obvallata* [22] and *Holarrhena antidysenterica* [23].

In initiation of shoot induction with nodal explants responded better than other explants such as internodes and shoot tips.

Table: 1. Influence of Cytokinins on shoots induction from nodal explants of *Tinospora cordifolia* in 20 days culture.

BA (µM)	KIN(µM)	2iP(µM)	No. of Shoots	Shoot length (cm)	% of Response
00.44	0	0	Callus	0	0
03.21	0	0	0	0	0
06.42	0	0	0	0	0
13.31	0	0	1.0 ± 0.0^{d}	0.34 ± 0.1^{e}	30^{d}
0	00.46	0	1.2 ± 0.3^{bc}	1.34 ± 1.1^{c}	40^{bc}
0	04.36	0	1.8 ± 0.1^{a}	2.23 ± 0.1^{a}	70^{a}
0	08.46	0	1.4 ± 0.1^{b}	2.01 ± 1.1^{b}	43^{b}
0	13.94	0	1.0 ± 0.1^{d}	0.62 ± 0.3^{cd}	32^{d}
0	0	00.49	Callus	0	0
0	0	04.26	1.2 ± 0.0^{bc}	0.21 ± 0.1^{f}	20^{e}
0	0	08.42	1.4 ± 0.1^{b}	0.23 ± 0.0^{f}	21^{e}
0	0	14.76	0	0	0

Explants were cultured on MS basal media supplemented with BA, KIN and 2iP. Data were recorded after 20 days of culture. Results represent mean ± SD of six cultures, ten replicated experiments. Values denoted by different letters in each column differ significantly at $p < 0.05$.

After a month of shoot induced cultures, creates a phenolic exudation problem in shoots formation, media had discoloration and explants were browning and the buds were break, so further analysis was needed to control the phenolic leaching out, the protocols were found by treating with different phenolic exudation controlling substances.

The different mediums used were polyvinyl pyrollidone (0.01%), activated charcoal (1.5%), silver nitrate (20%), PVP (0.01%) + ascorbic acid (0.01%) and with 4.36 µM of KIN in MS medium for controlling of phenolic exudation. The results were given in Table 2**.** Among these combinations, silver nitrate (20%) with KIN (4.36 µM) has shown 100% response with 3.01± 0.1 cm length of shoots with 2.01± 0.1 numbers of shoots within 16 days of cultures. It indicates that Silver Nitrate at 20% along with KIN (4.36µM) will control the Phenolic exudation in shoot induction.

After, the determination of best treatments for shoots induction. The effect of different concentration of Cytokinins (BA, KIN, and 2iP) alone and combination of BA+ NAA and BA + IAA on shoot proliferation in nodal explants of *Tinospora cordifolia* in MS medium. Among the different experiment, BA (8.82 µM) alone showed better growth response and produced 4.81± 0.2 numbers of shoots with an average length of 3.9±0.1cm after a 20 days of culture with 80% of response [Table 3].

Table: 2. Standardization of controlling in Phenolic exudation in shoot induction from nodal explants of *Tinospora cordifolia* in 20 days of culture.

KIN(µM)	Gelling agent	Control measures	No. of shoots	Shoot length (cm)	% of Response
4.36	Agar	Polyvinylpyrollidone (PVP, 0.01%)	1.01 ± 0.3^{c}	2.34 ± 0.0^{b}	60^{d}
4.36	Agar	Activated Charcoal (AC, 1.5%)	1.22 ± 0.1^{b}	2.23 ± 0.2^{c}	85^{b}
4.36	Agar	Silver Nitrate ($AgNO_3$, 20%)	2.01 ± 0.1^{a}	3.01 ± 0.1^{a}	100^{a}
4.36	Phytagel	PVP (0.01%) + Ascorbic Acid (0.01%)	1.12 ± 0.1^{b}	2.42 ± 0.2^{b}	75^{b}

Explants were cultured on MS basal media supplemented with KIN and different gelling agents. Data were recorded after 20 days of culture. Results represent mean ± SD of six cultures, ten replicated experiments. Values denoted by different letters in each column differ significantly at $p < 0.05$.

Similar observations have been reported for *Bauhinia variegate* [24] and *Holarrhena antidysenterica* (**Fig. 1. E; F and G)** [25,26]. Other experiments of KIN and 2iP different concentrations produced less number of shoots, whereas 8.82 µM BA with different concentration of NAA produced very less number of shoots and high concentration of NAA produced dark green cultures. The same results of dark green were obtained in BA 8.86 µM with different concentration of IAA (data was not showed). Between the three Cytokinins tried, BA had more positive effective on KIN and 2iP for shoot multiplication.

The multiple shoots obtained from our experiments were short (below 2cm) and had condensed nodes. Therefore, transfer of these shoots to shoot elongation medium contains Cytokinins (BA, KIN and 2iP) alone or combinations. The best result was produced [Table 4] with the combination treatment of BA (6.88 µM) + KIN (8.22 µM) produces 86% of response with 4.8 ± 1.4 numbers and 6.8 ± 0.1cm length of shoots produced within 20 days of cultured (**Fig. 1. H, I & J**). Higher concentration of KIN show increase in shoot length 4.8cm and better growth with enlargement of single leaves.

The regeneration of shoots were excised and cultured on half strength of MS supplemented with 3% sucrose and different concentration of Auxins in IAA (1.76 – 17/13 µM), IBA (1.32 – 14.70 µM) and NAA (1.34 – 16.61 µM) were used in alone. The results were provided in Table.5. Rootining was noticed in all the concentrations of Auxins used, however, maximum number of shoots rooted in 6.43 µM IBA at 85% of response [Fig. 1. K] followed by 10.21 µM which produces 80% of response when compared with other concentration of Auxins at 27 days on medium. Several authors reported that IBA was an effective Auxin in the induction of roots in different ornamental, medicinal and fruit plants like chrysanthemum [27], carnation [28], neem [29], apple [30]. After 30 days of growth, lateral roots were produced from main root. The rooted plants were transplanted *ex vitro* and raised in pots [Fig. 1. L and Fig. 1. M] containing red soil, vermiculite and farmyard manure in 1:1:1 ratio, kept under green house conditions for one month followed by their field transfer. Approximately 80% of plantlets survived [Fig. 1. N].

Table: 3. Effect of different concentration of Cytokinins on shoot proliferation in nodal explants of *Tinospora cordifolia* on MS medium.

BA (µM)	KIN(µM)	2iP(µM)	No. of shoots	Shoot length (cm)	% of Response
04.22	0	0	2.42± 0.1 c	3.6± 0.3 b	60 b
08.82	0	0	4.81± 0.2 a	3.9± 0.1 a	80 a
12.64	0	0	3.21± 0.0 b	3.4± 0.1 c	52 b
0	06.23	0	2.11± 0.1 c	2.5± 0.4 e	55 cd
0	09.46	0	2.32± 0.2 c	2.2± 1.0 fg	63 b
0	12.44	0	2.0± 0.1 d	2.1± 1.1 g	60 b
0	0	03.34	1.26± 0.3 e	2.5± 0.5 e	55 cd
0	0	08.68	1.32± 0.5 e	2.8± 0.3 d	54 cd
0	0	13.32	2.0± 0.1 d	2.1± 0.2 g	50 e

Explants were cultured on MS basal media supplemented with BA, KIN and 2iP. Data were recorded after 20 days of culture. Results represent mean ± SD of six cultures, ten replicated experiments. Values denoted by different letters in each column differ significantly at $p < 0.05$.

Table: 4. Effect of BA and KIN in different concentrations on elongation of shoot regenerates from nodal explants of *Tinospora cordifolia* on MS medium.

BA (µM)	KIN(µM)	No. of shoots	Shoot length (cm)	% of Response
2.44	4.66	3.4 ± 1.1 b	5.2 ± 0.0 b	80 c
6.88	8.22	4.8 ± 1.4 a	6.8 ± 3.1 a	86 a
8.82	12.44	3.6 ± 1.3 b	4.87 ± 1.1 c	72 b

Explants were cultured on MS basal media supplemented with BA and KIN. Data were recorded after 20 days of culture. Results represent mean ± SD of six cultures, ten replicated experiments. Values denoted by different letters in each column differ significantly at $p < 0.05$.

Micropropagated *Tinospora cordifolia* plant methanol extract administered at a dose of 2000mg/kg did not show any sign or symptoms of mortality during the observation period. Based on this study the dose was selected as 500 mg/kg. for the experiment.

Figure. 1. Micropropagation of *Tinospora cordifolia* in various Stages

A. Shoot induction from nodal explant of *Tinospora cordifolia* on shoot induction medium containing silver nitrate allowing with supplementary of 4.36 µM KIN. B. Shoot induction with 4.36 µM KIN in M.S. media after 20 days. C. Second sub culturing of the growing initiation culture along with leaves. D. Elongation culture with supplementary of KIN (9.46 µM) to form single large leaf. E. Initial stage of multiplication in shoot culture medium with BA (8.82 µM). F. Multiple shoot proliferation from nodal explants on shoot induction medium. G. multiplication of shoot in M.S. culture medium allowing with BA (8.82 µM) after 4 weeks. H. Shoot elongation on M.S. medium with BA (6.88µM) and KIN (8.22 µM). I & J . Elongated shoot supplementary with BA (6.88 µM) and KIN (8.22 µM) after 12 and 28 days respectively. K. *In vitro* rooting on Half strength M.S. medium with IBA (6.43 µM). L & M. Acclimatized plantlets in pots. N. Five month old tissue cultured *Tinospora cordifolia* plants in pot.

The present study was conducted to conform the hypoglycaemic activity of micropropagated *Tinospora cordifolia* was compared to wild plant and standard drug Glibenclamide. The mature stem of micropropagated and wild plant methanol extracts were administrated for oral daily 500mg/kg of body weight up to 6 weeks for analysis of biochemical variables in experimental animals. In diabetic rats shows the increased fasting blood glucose, decreased the body weight and decreased the liver glycogen in Table 6. Daily oral administration of micropropagated *Tinospora cordifolia* and wild plant bringing back to near normal blood glucose and significantly increased Body weight and liver glycogen levels. The plants extracts were found to help in the maintenance of glucose homeostasis in alloxan induced diabetic rats. The *Tinospora cordifolia* methanol extract has proved to protect massive body weight loss due to enhanced glucose transport across the cell membranes and glycogen synthesis [31]. When compared to normal animals, the concentration of blood urea was doubled in liver of alloxan induced

diabetic rats, [Table.7]. The increased may be attributed to the enhanced catabolism of body liver and plasma proteins. The total cholesterol level also increased two fold in diabetic rats when compared to normal animals.

Table: 5. Effect of different Auxins on rooting from *in vitro* elongated shoots of *Tinospora cordifolia* in half strength MS medium.

IAA (µM)	IBA (µM)	NAA (µM)	No. of roots	Root length (cm)	% of Response
01.76	0	0	4.3 ± 1.1^{d}	3.0 ± 0.4^{bc}	79^{c}
04.32	0	0	4.2 ± 1.1^{ef}	2.9 ± 0.3^{c}	78^{c}
08.56	0	0	4.3 ± 0.3^{d}	2.2 ± 0.1^{ef}	74^{d}
17.13	0	0	4.5 ± 0.4^{c}	3.2 ± 0.2^{a}	79^{c}
0	01.32	0	4.3 ± 1.1^{d}	2.1 ± 1.3^{g}	74^{d}
0	06.43	0	5.2 ± 0.2^{a}	3.2 ± 0.1^{a}	85^{a}
0	10.21	0	5.0 ± 1.1^{bc}	3.2 ± 0.4^{a}	80^{bc}
0	14.70	0	4.7 ± 1.0^{c}	2.3 ± 0.2^{ef}	78^{c}
0	0	01.34	3.2 ± 1.0^{g}	2.1 ± 1.0^{g}	60^{f}
0	0	05.43	4.1 ± 0.3^{f}	2.1 ± 0.1^{g}	60^{f}
0	0	10.22	4.6 ± 1.1^{c}	2.5 ± 0.0^{d}	62^{e}
0	0	16.11	3.5 ± 0.2^{g}	2.8 ± 1.0^{c}	61^{e}

Explants were cultured on half strength MS basal media supplemented with IAA, IBA, NAA and 3% of Sucrose. Data were recorded after 27 days of culture. Results represent mean ± SD of six cultures, ten replicated experiments. Values denoted by different letters in each column differ significantly at $p < 0.05$.

The elevated levels of blood urea and cholesterol observed in diabetic conditions is found to be corrected to near normal in the micropropagated and wild methanol extract of *Tinospora cordifolia.* When compared to wild the micropropagated have the capability to controls significantly.

The total protein was decreased in alloxan induced diabetic rats [Table.8] because of the insufficiency and absence of insulin leading to increased protein degradation and decreased protein synthesis. Glycosylated haemoglobin was found to increase in diabetic rats and the amount increased was found directly proportional to the fasting blood glucose level. During diabetes the excess glucose present in blood reacts with haemoglobin, therefore, the glycosylated haemoglobin level is increased [31], the administration of micropropagated and wild methanol extract of *Tinospora cordifolia* prevents significantly elevation in glycosylated haemoglobin and improved the significant near normal in protein levels [Table.8].

The activity of hexokinase enzyme decreased in liver of alloxan induced diabetic rats (Table.9) and the activity of fructose 1,6, bi phosphatase and glucose 6-phoaphatase were found to be increased in diabetic rats liver [32]. The micropropagated and wild *Tinospora cordifolia* methanol extracts administration to alloxan induced rats resulted in an increased activity of liver hexokinase leads to increased glycolysis and increased utilization of glucose for energy production.

Table: 6. Effect of *Tinospora cordifolia* extracts on animal body weight, blood glucose and liver glycogen in normal, diabetic, micropropagated *Tinospora cordifolia* treated and Glibenclamide drug treated diabetic rats.

Groups	Initial body weight (g)	body weight (g) 6th week	blood glucose in initial (mg/dl)	blood glucose (mg/dl) in 6th week	Liver Glycogen (mg/g wet tissue)
Normal	182.3 ± 0.4	194.6 ± 0.2	81.1 ± 0.4	82.4 ± 1.0	46.3 ± 2.29
Diabetes	181.4 ± 0.5	142.7 ± 0.3*	215.5 ± 0.5	226.4 ± 1.2*	31.48 ± 0.0*
Wild *Tinospora cordifolia* treated diabetes (500mg/kg)	184.8 ± 0.5	191.6 ± 0.2**	210.8 ± 0.5	102.7 ± 0.3**	40.86 ± 1.07**
Micropropagated *Tinospora cordifolia* treated diabetes (500mg/kg)	183.6 ± 1.3	192.6 ± 0.1**	221.4 ± 1.3	106.6 ± 1.0**	42.42 ± 1.34**
Standard drug Glibenclamide (25mg/kg)	184.3 ± 0.4	173.2 ± 0.1**	222.2 ± 0.4	112.3 ± 1.2**	38.12 ± 0.01**

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

Table: 7. Effect of *Tinospora cordifolia* extracts on cholesterol and blood urea in normal, diabetic, micropropagated *Tinospora cordifolia* treated and Glibenclamide drug treated diabetic rats.

Parameters	Normal	Diabetes	Wild T*inospora cordifolia* treated diabetes (500mg/kg)	Micropropagated *Tinospora cordifolia* treated diabetes (500mg/kg)	Standard drug Glibenclamide (25mg/kg)
Serum cholesterol (mg/dl)	162.0 ± 1.44	243.4 ± 0.24*	176.3 ± 0.24*	172.4 ± 0.24**	181.2 ± 0.21**
Blood urea (mg/dl)	25.60 ± 1.04	49.03 ± 1.24*	29.30 ± 0.20*	28.23 ± 1.02**	30.10 ± 2.11**

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

Table: 8. Effect of *Tinospora cordifolia* extracts on Serum total protein and glycosylated haemoglobin in normal, diabetic, micropropagated *Tinospora cordifolia* treated and Glibenclamide drug treated diabetic rats.

Parameters	Normal	Diabetes	Wild *Tinospora cordifolia* treated diabetes (500mg/kg)	Micropropagated *Tinospora cordifolia* treated diabetes (500mg/kg)	Standard drug Glibenclamide (25mg/kg)
Serum total protein (g/dl)	7.3 ± 0.23	6.2 ± 0.054**	6.8 ± 0.24*	6.9 ± 1.34**	6.7 ± 1.01**
Glycosylated Haemoglobin (mg/gHb)	0.266 ± 0.004	0.779 ± 0.003*	0.409 ± 0.002**	0.366 ± 0.001**	0.482 ± 0.002**

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

Table: 9. Changes in *Tinospora cordifolia* extracts on hexokinase, fructose 1, 6 –bisphosphatase and glucose - 6 – phosphatase in normal, diabetic, micropropagated *Tinospora cordifolia* treated and Glibenclamide drug treated diabetic rats.

Parameters	Normal	Diabetes	Wild T*inospora cordifolia* treated diabetes (500mg/kg)	Micropropagate d *Tinospora cordifolia* treated diabetes (500mg/kg)	Standard drug Glibenclamide (25mg/kg)
Hexokinase [1]	264.68 ± 0.83	115.43 ± 1.46*	238.83 ± 1.83*	240.73 ± 1.32**	228.11 ± 1.21**
Glucose - 6 – phosphatase [2]	1032.4 ± 0.42	1236.8 ± 0.22*	1123.5 ± 1.32*	1119.3± 1.26**	1131.2 ± 2.02**
Fructose 1, 6 – bisphosphatase [2]	474.14 ± 1.63	757.42 ± 1.82*	563.56 ± 0.83*	542.32 ± 0.13**	552.14 ± 1.32**

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

1. μ – moles of glucose - 6 – phosphate formed/h/mg protein.
2. n moles of phosphorous liberated/h/mg protein.

Tinospora cordifolia methanol extracts was proved the decreased level of blood glucose in 500mg/kg body weight. The activity of fructose 1,6, biphosphatase and glucose 6 – phosphatase were found to be increased in diabetic condition in liver [33], the oral administration of micropropagated and wild *Tinospora cordifolia* plant methanol extract to bring back to significantly near normal [Table.9].

CONCLUSION

As a result of the over-exploitation of plant material from natural stands for traditional medicinal purposes, the standardization of the regeneration protocols for *Tinospora cordifolia* medicinal plant was becoming important. The protocols will facilitate conservation of the species and could also serve as an alternative source of materials for use. The regeneration protocol described herein would benefit the conservation of *Tinospora cordifolia*, which was extensively used in traditional medicine. The methanol extract of micropropagated and wild *Tinospora cordifolia* was found to exhibit a signifying anti hyperglycemic activity in alloxan induced experimental diabetic rats.

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