tro and *in vivo* leaf extracts of *Gymnema estre* involve the protective effects and elopment of metabolic syndrome in High-Fat D Rats



a. Habit; b. A twig with flower;
c. Seeds

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ore than 1 billion adults worldwide are overweight, among 300 million e clinically obese, and nearly 43 million children are overweight (less than years old) in 2015 (WHO, 2011).

5-80% of diabetic patients are obese, showing that obesity is a strong ris ctor for developing type-2 diabetes mellitus (DM).

sulin resistance initially stimulates the secretion of insulin from the beta cells the pancreas.

owever, over time, the beta cells become exhausted resulting in increased ood glucose levels. As a result, people suffering from both acute and chroni ncreatitis become Type II diabetes.

he administration of certain drugs such as corticosteroids, ACTH, thiazide ggravates the obesity conditions.

Gymnema sylvestre is a woody climber and belongs to the family Asclepiadaceae.

t is well-recognized in traditional medicine as a remedy for diabetes mellitus stomachic, diuretic and used in folk, Ayurveda and homeopathic systems of medicine.

t maintained blood glucose through increased serum insulin levels provided by repair (or) regeneration of the endocrine pancreas (Shanmugasundaram *et al.,* 1990).

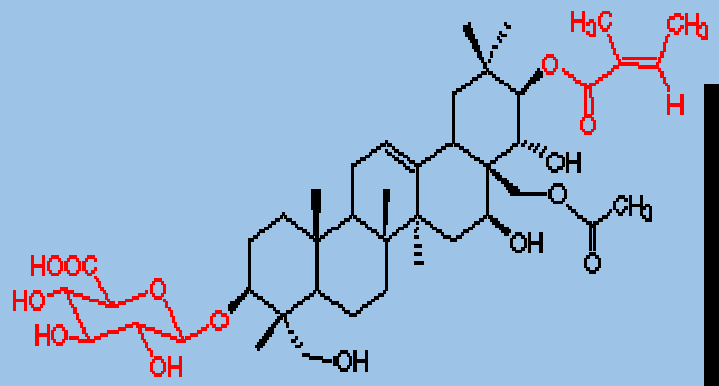
Gymnema sylvestre leaves (or) their extracts have been widely used as health foods in tea bags, tablets, and beverages in recent years in Japan.

The users of these health foods often expect weight reduction (or) improvement of against diabetes because of their ability to suppress the taste of sweetness and inhibit glucose absorption (Nakamura, 1988; Ueno, 1997).

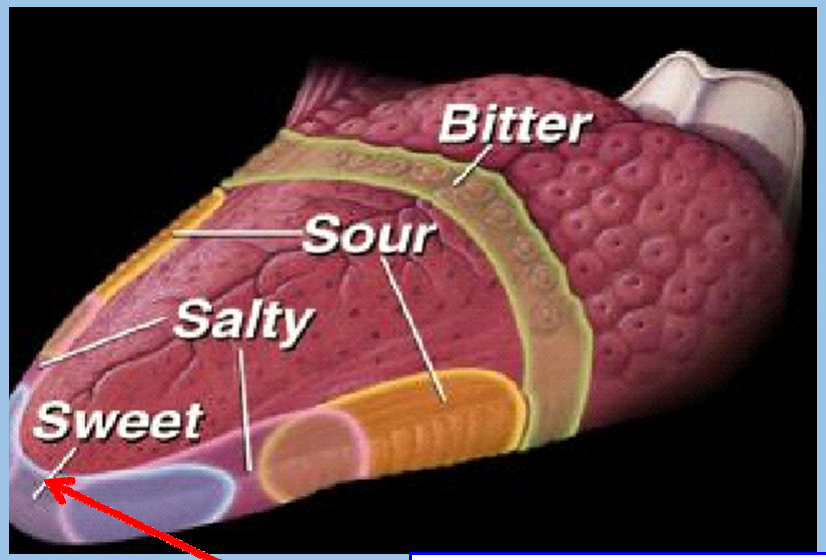


Prof. Shigenobu Arihara & Prof. Kazuko Yoshikawa
Kyoto Pharmaceutical University, Japan

Gymnemic acid ($C_{43}H_{66}O_{14}$)



Gymnemic acid action on taste bud nerves



"Inhibit sweet taste"
"Gymnemic acid" & "Gurmarin"

f action of Gymnemic acid

glycoside of gymnemic acid may block the absorption of
r from the intestine.

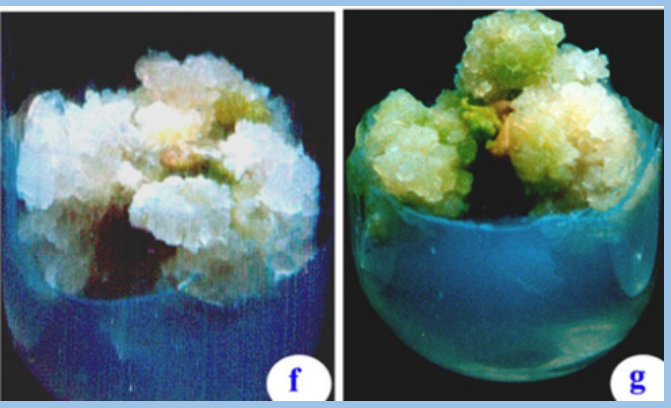
glycoside of gymnemic acid may block the sweet taste of
r.

s also shown the repair or regeneration of insulin producing
ls in pancreas and balance insulin level.

mic acid products - World market

ma sylvestre leaves (500kg) used to
1g Gymnemic acid ?

plant tissue culture approach?



Product name (Gymnemic acid)	Price ($)
Gymnema capsule 60	18.95
DIABETES SUPPORT FORMULA	29.95
Bio-shape	55.00
Thermo force metabolism 90 capsules	27.99
Gymnema caps	6.95
Gymnema Tea	15.50
DIA-BOTICA	8.95
Ayurvedic herbal extract	18.95
Glucobetic	29.95
SyndRx	49.95
Diaxinol	37.35
A to Z	15.98
Glucose balance (Beta – fast)	12.29
Glucochrom	15.00
Ex-Ell	41.99
Glucosamine plus	5.55
Shardunikha	20.95

ectives

timization of culture conditions for *in vitro* callus biomass production and gymnem

antification of gymnemic acid from *in vivo* leaf and *in vitro* raised callus and

omparative assessment of anti-obesity of methanol extract of *in vitro*
sed callus & *in vivo* leaf in High Fat Diet Fed rats.

Callus culture (Optimized Plant Growth Regulators)

(Leaf, Stem and Petiole)

↓

on (70% ethyl alcohol for 15 sec, 2% Sodium
ite for 3 min, 0.1% $HgCl_2$ for 3 min)

↓

SH and WPM medium

↓

IAA (Indole-3-acetic acid): 0.5-5.0 mg/l
IBA (Indole-3-butyric acid): 0.5-5.0 mg/l
2,4-D (2,4-dicholorophenoxyacetic acid): 0.5-5.0 mg/l
NAA (1-naphthaleneacetic acid): 0.5-5.0 mg/l

s :

BA (6-benzylaminopurine): 0.2-2.0 mg/l
KN (6-Furfurylaminopurine): 0.2-2.0 mg/l

:

Adenine sulphate: 5.0, 10.0 mg/l

↓

nt concentrations and combinations)

↓

ture, fresh and dry weight biomass
& growth period

Materials & Methods

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The ScientificWorldJOURN

Research Article

Production of Gymnemic Acid Depends on Medium, Explants, PGRs, Color Lights, Temperature, Photoperiod, and Sucrose Sources in Batch Culture of *Gymnema sylvestre*

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properly cited.

Gymnema sylvestre (R.Br.) is an important diabetic medicinal plant which yields pharmaceutically active compounds called gy
mic acid (GA). The present study describes callus induction and the subsequent batch culture optimization and GA quantific
determined by linearity, precision, accuracy, and recovery. Best callus induction of GA was noticed in MS medium combined
2,4-D (1.5 mg/L) and KN (0.5 mg/L). Evaluation and isolation of GA from the calluses derived from different plant parts, na
leaf, stem and petioles have been done in the present case for the first time. Factors such as light, temperature, sucrose, and ph
period were studied to observe their effect on GA production. Temperature conditions completely inhibited GA production. O
the different sucrose concentrations tested, the highest yield (35.4 mg/g d.w) was found at 5% sucrose followed by 12 h photope
(26.86 mg/g d.w). Maximum GA production (58.28 mg/g d.w) was observed in blue light. The results showed that physical
chemical factors greatly influence the production of GA in callus cultures of *G. sylvestre*. The factors optimized for *in vitro* pro
tion of GA during the present study can successfully be employed for their large-scale production in bioreactors.

1. Introduction

In vitro techniques are very useful in ensuring sustainable, optimized sources of plant-derived natural products. However, *ex situ* cultivation should be preceded by proper evaluation of the plants for their ability to produce the required bioactive constituents before commencing cultivation or introducing the technology to potential growers. The ability of plants to produce certain bioactive substances is largely influenced by the physical and chemical environments in which they grow. Plants also produce certain chemicals to overcome abiotic stresses [1]. Plants use light not only as an energy source for photosynthesis but also as an important environmental signal. Plants can detect almost all facets of light such as direction, duration, quantity, and wavelength by using three classes of photoreceptors: the red/far-red (600–750 nm) absorbing phytochromes, and the blue/UV-A (320–500 nm) absorbing cryptochromes. These photoreceptors perc
interpret, and transducer light signals, via distinct intra
lular signaling pathways, to mediate a broad range of phy
logical responses to light in addition to cell growth and de
opment [2]. Light can affect morphogenesis and the for
tion of plant metabolites as a signal and stress factor f
phytohormones. In most plant cell cultures, secondary m
bolism, including the production of phenolic terpenoid
alkaloid compounds, is stimulated by light [3]. On the o
hand, light had an inhibitory effect on the accumulatio
secondary metabolites in the case of nicotine and shik
[4]. The literature shows that the majority of pharm
logically important compounds of plant origin are prod
of defense and secondary metabolism [1, 5]. This abilit
plants to respond to physical and/or chemical stimuli ca
used for elicitation of pharmacologically active substance
subjecting an intact plant to stress factor(s) [1]. Growi

The Scientific World Journal (ISI publication)

UNI
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ulture – Under stress
ns

Explant (Leaf)

↓

Sterilization (70% ethyl alcohol for 15 sec, 2% Naocl$_2$ for 3 min, 0.1% HgCl$_2$ for 3 min)

↓

MS + Optimized growth regulators

↓

Different stress conditions:

ght : - Blue, Red, Green, white (16/8h)

ource:- Sucrose-2, 3, 4, 5 and 6%

iod:- 4h/20h, 8h/16h, 12h/12h, 16h/8h, 20h/4h, 24h light; 24h dark conditions

↓

lus growth curve (15, 25, 35, 45 and 55 days)

↓

Callus:- Nature, Fresh & Dry Weight



Peer-reviewed scientific article

Different wavelengths light to induce physiological changes callus for the biosynthesis of gymnemic acid in Gymnema sylvestre

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KEYWORDS: Callus culture; blue light; gymnemic acid.

ABSTRACT: A callus culture of Gymnema sylvestre was cultivated under different light conditions (blue, white, red and green light). Green-white coloured gymnemic acid containing cell groups were formed on the surface of the callus culture under blue light respectively. The phytohormone kinetin enhanced the gymnemic acid formation in blue light, but was unable to induce gymnemic acid in darkness. The phytohormone 2,4-D induced the formation gymnemic acid under all light conditions studied. Analysis of the callus growth phase under all treatments revealed that gymnemic acid accumulation was maximum (12.22 mg/g d.w) in the callus during stationary phase of 45 days, grown in MS medium with 2,4-D (1.5 mg/l) and KN 0.5 mg/l under white fluorescent light. Blue light enhanced the gymnemic acid accumulation up to 4.4 fold of that found under white fluorescent light and 2.8 fold of that found in intact leaves. Present findings concluded that blue light can be used as tool for enhancing pentacyclic tri-terpenoids in batch culture of G. sylvestre.

INTRODUCTION

Gymnema sylvestre (Retz) R.Br. Ex Roemer & Schultes is a woody climber belonging to the family Asclepiadaceae. It is distributed in Deccan peninsula parts of northern, western India, tropical Africa, Vietnam, Malaysia, Sri Lanka and it is available in Japan, Germany and USA as health food. It is a traditional medicine in India for diabetes mellitus, and used as a stomachic and as a diuretic (1). In Japan, there are Gymnema Teas and Gymnema chewing gum made from G. sylvestre leaves which are promoted as a natural method for controlling obesity, insulin secretion, diabetes and inhibiting the taste of sweetness (2). It helps to maintain blood glucose homeostasis through increased serum insulin levels provided by repair or regeneration of the endocrine pancreas (3). Several products are marketed under brand names such as Body Slatto Tea®, Gymnema®, Gymnema Tea®, Gymnema Diet®, Sugar Off®, Glucosef™, Cinndrome X™, and Pilisoft™. Gymnema sylvestre was propagated in vitro using axillary node and inter nodal explants; among that axillary node explants showed higher multiple shoots (57.2 shoots/ explant) than inter nodal explants (4). Furthermore, Veerashree et al. (2012) reported that yeast extract, pectin, and chitin elicitors enhanced the gymnemic acid via cell suspension culture in G. sylvestre (5). Current advances in plant biotechnology make cell and organ culture a promising approach and have been successfully applied to the production of valuable secondary metabolites, including pharmaceuticals, pigments, and other chemicals (6). Light is an important factor affecting growth, organogenesis and the formation of plant products including both primary and secondary metabolites. In addition, light is also involved in regulating the secretion mechanism of secondary metabolites (7). Light irradiation was established in which MS medium supplemented with plant growth regulators was used for callus growth, and modifying the shaking speed, pH and medium play an important role in the production of gymnemic acid (8). A series of experiments were carried out to investigate the influence of different wavelength lights on callus growth and gymnemic acid accumulation by varying the irradiation period and intensity under light supplied by illumination and radiation energy in the course of cultivation. The present report describes the establishment of callus from leaf explant of G. sylvestre and the enhancement of gymnemic acid accumulation using various wavelengths of light (blue, red and green light) as an inducing stress factor.

MATERIALS AND METHODS

Plant materials and in vitro culture conditions

Gymnema sylvestre plants collected from Pachamalai hills, Tiruchirappalli, India were maintained in the botanical garden of Department of Plant Science, Bharathidasan University, Tiruchirappalli. The young leaf explants collected from G. sylvestre were sterilized with alcohol 70 percent (v/v) for 30 s, 3 percent sodium hypochlorite (v/v) for 2 min and 0.1 percent mercuric chloride (w/v) for 3 min, followed by a three times rinse with sterile distilled water. Sterilized leaf explants were cultured on MS medium (9) supplemented with various concentrations and combinations of growth regulators, i.e. 1-naphthaleneacetic acid (NAA), 2,4-dicholaraphenoxyacetic acid (2,4-D) and kinetin (KN) in 100 ml culture tubes and were maintained at 25°C under control white fluorescent light (380-780 nm, Philips Company, India;16 h photoperiod) for callus induction and gymnemic acid production.

***IN VITRO* CALLUS GROWTH CURVES**

Callus cultures were optimized and evaluated quantitatively for their appearness nature, biomass and gymnemic acid content at the end of their respective growth cycle. MS media treating with various combinations of auxins, cytokinins, and in combinations, fresh and dry weight of the calli was determined at 15, 25, 35, 45 and 55 days.

Food technologies | AgroFOOD Industry hi-tech | May/June 2012 | vol 23 | n 3

ISI Publication: AgroFood Industry Hi Tech, Vol-23, Issue-2, 2012

nemical screening and identification of emic acid

raised callus & *in vivo* leaf

↓

ction with methanol

↓

ol extract centrifuged in 5000 rpm for 10 es

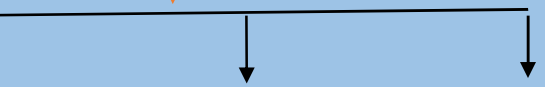
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tant was used for further studies

↓

ation of Gymnemic acid and compare with rd

↓



C HPTLC HPLC

Acta Chromatographica
DOI: 10.1556/AChrom.25.2013.2.10

HPTLC/HPLC and Gravimetric Methodology for the Identification and Quantification of Gymnemic Acid from *Gymnema sylvestre* Methanolic Extracts

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Summary. Gymnemic acid (GA), a well known anti-diabetic compound has been detected in methanol extracts of intact leaves and *in vitro* callus cultures derived from leaf explants of *Gymnema sylvestre*. Callus biomass was developed in MS medium with optimum plant growth regulators (OPGRs) of 2,4-D (1.5 mg L^{-1}) + KN (0.5 mg L^{-1}) under abiotic stress conditions at 45 days determined by growth curve analysis. GA detection and quantification were carried out using thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and gravimetric techniques. GA detection peak area and their absorption spectra were evaluated through HPTLC and HPLC with the standard GA. Quantification of GA had showed the linearity, accuracy, robustness and precision by HPLC. GA content was significantly higher in gravimetric method than HPLC. All these methods were found to be simple, accurate, selective and rapid and could be successfully applied for the determination of GA. It could have potential as a pharmaceutical drug for Type 1 diabetes mellitus (IDDM) and obesity.

Key Words: *Gymnema sylvestre*, gymnemic acid (GA), abiotic stress, HPTLC, HPLC, gravimetric method

Introduction

Type 1 diabetes, or insulin-dependent diabetes mellitus (IDDM), is a common pediatric chronic disease, affecting an increasing number of children every year. IDDM occurs due to autoimmune destruction of insulin-producing β-cells in the pancreas, resulting in low or no production of insulin, a hormone necessary for survival [1]. According to World Health Organization, obesity has reached epidemic proportions globally, with at least 2.6 million people dying each year as a result of being overweight or obese [2].

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ISI publication: Acta Chromatographica, Vol-25, Issue-2, 2013

Experiment groups and given treatment in Wistar rats

oup 1 → Normal rats fed with control diet

oup 2 → High Fat-fed animals received fat-enriched diet for 40 days

oup 3 → High Fat-fed animals treated with standard drug simvastatin (3 mg/kg/day)

oup 4 → High Fat-fed+ *G. sylvestre* leaf extracts - 200 mg/kg rats body Weight (Shanmugasundaram *et al.,* 1990)

up 5 → GCE_1: Blue light treatment callus + MS + (2,4-D 1.5 mg/l) + KN(0.5 mg/l)

oup 6 → GCE_2: 5% sucrose treatment + MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

oup 7 → GCE_3: 12 h photoperiod with MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

oup 8 → GCE_4: 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

Optimum gymnemic acid production treatment

The composition of the experime (g/kg diet)

Ingredients	Control diet	High-f
Casein	200	
Corn starch	397.5	
Sucrose	100	
Dextrinated starch	132	
Lard	-	
Soybean oil	70	
Cellulose	50	
Mineral mixture	35	
Vitamin mixture	10	
Choline	2.5	
L-Cystine	3	

dlts

Callus culture

↓

Leaf explant

↓

MS medium

Auxins:-

MS + NAA (1.0 mg/l); 2,4-D (1.5 mg/l)

Auxins + Cytokinins:-

MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

MS + NAA (1.0 mg/l) + KN (1.5 mg/l)

MS + NAA (1.0 mg/l) + KN (1.0 mg/l)

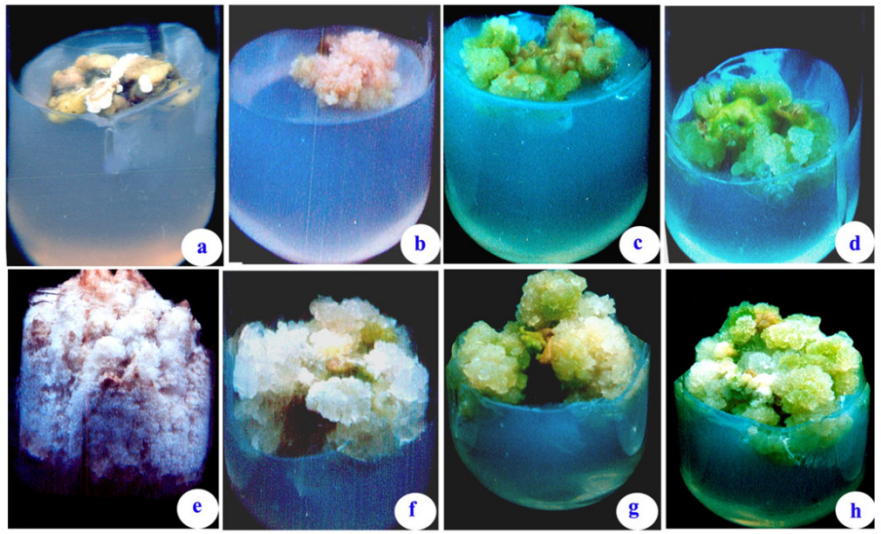
Auxins + Cytokinins + Ads:-

MS + 2,4-D (1.5 mg/l) + BA (1.0 mg/l) + 5 mg/l Ads

MS + 2,4-D (1.0 mg/l) + KN (1.0 mg/l) + 5 mg/l Ads

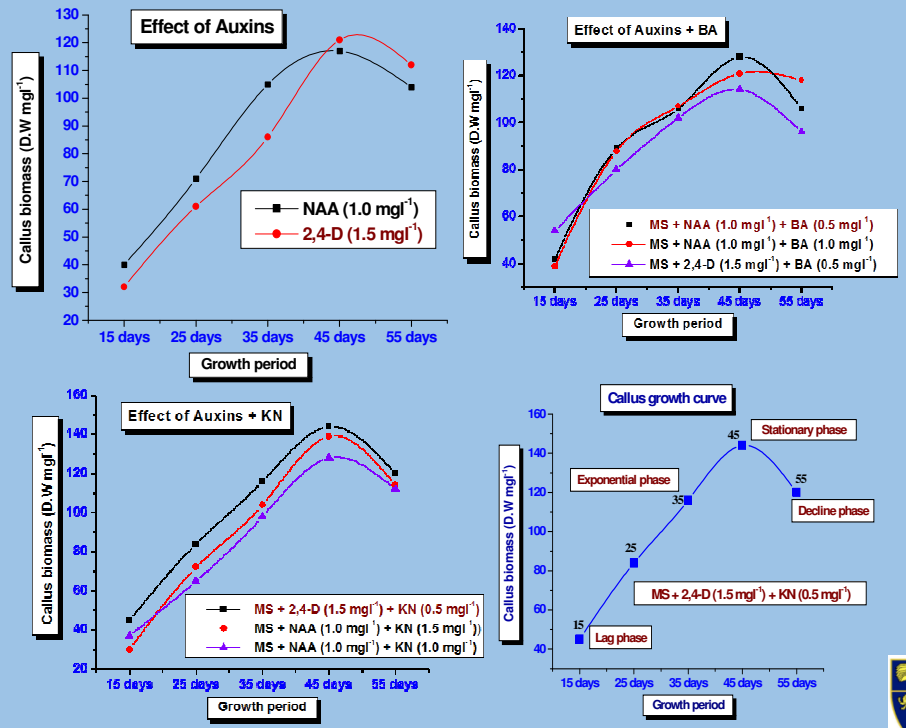
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Green callus + Maximum dry weight biomass callus production at 45th day



Callus biomass production from leaf explant of *Gymnema sylvestre* on MS medium supplemented with auxins, after 45 days

a & b - IBA treatment (1.5x), c & d - NAA treatment (1.5x),
e & f - IAA treatment (2.5x), g & h - 2,4 - D treatment (2.1x)



Callus culture: – Under stress conditions

MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

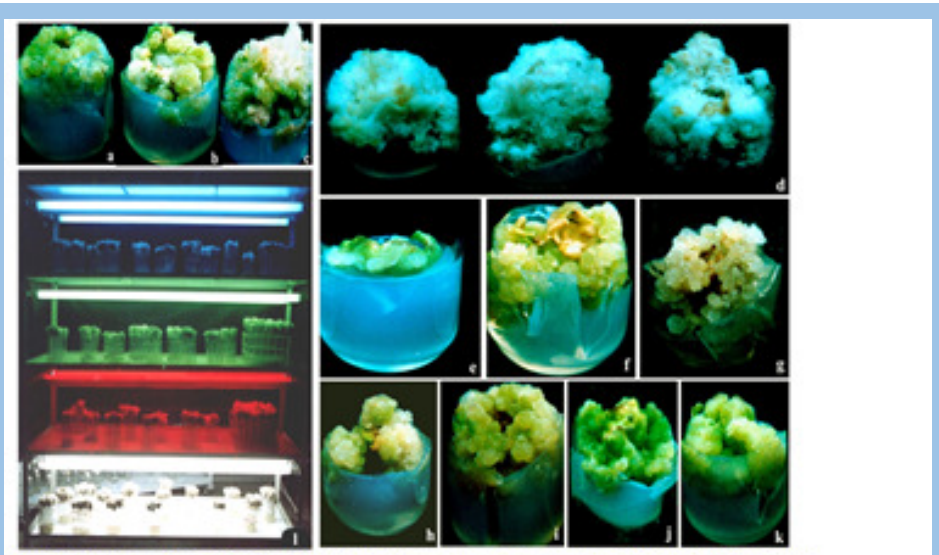
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Stress treatments

- **Light** – Blue light
- **Carbon source** – 5 % sucrose
- **Photoperiod** – 16/8h, 12/12h (light/dark)

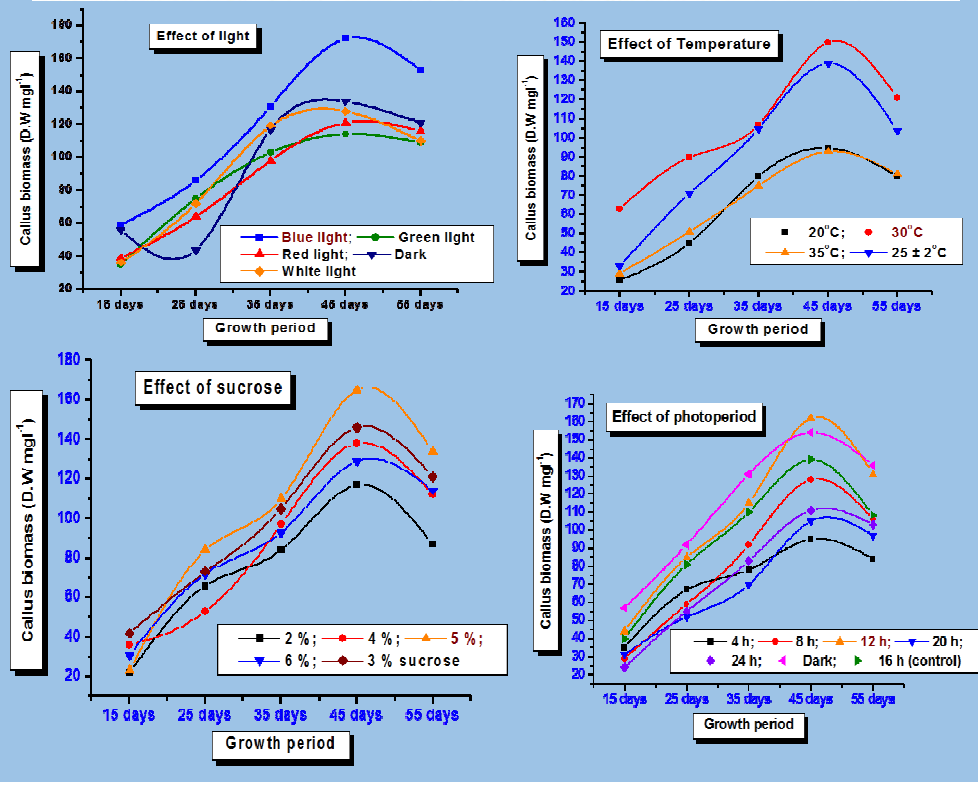
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Green callus + Maximum dry weight biomass callus at 45th day



Callus biomass production from leaf explant of *Gymnema sylvestre* grown on MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l) under various stress conditions, after 45 days

a - d Different light sources (a - Blue light (1.8x), b - Red light (1.8x), c - Green light (1.6x), d - Dark (1.6x), e - g Temperature (e - 20°C (1.8x), f - 30°C (1.7x), g - 35°C (1.6x), h - k Carbon source (h - 2% (1.4x), i - 4% (1.4x), j - 5% (1.2x), k - 6% (1.2x) and l - Different colour lights.



Dried leaf and callus

Methanol extract

Experiment I.

value compared with standard Gymnemic acid

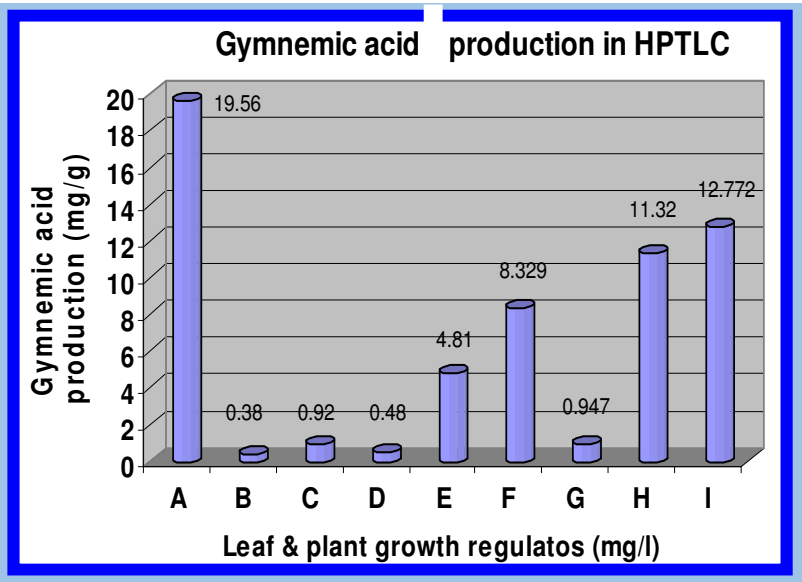
wn spot (Isopropyl alcohol: oform: Methanol: Acetic acid - : 3: 1: 0.5)- Gymnemic acid

Experiment II.

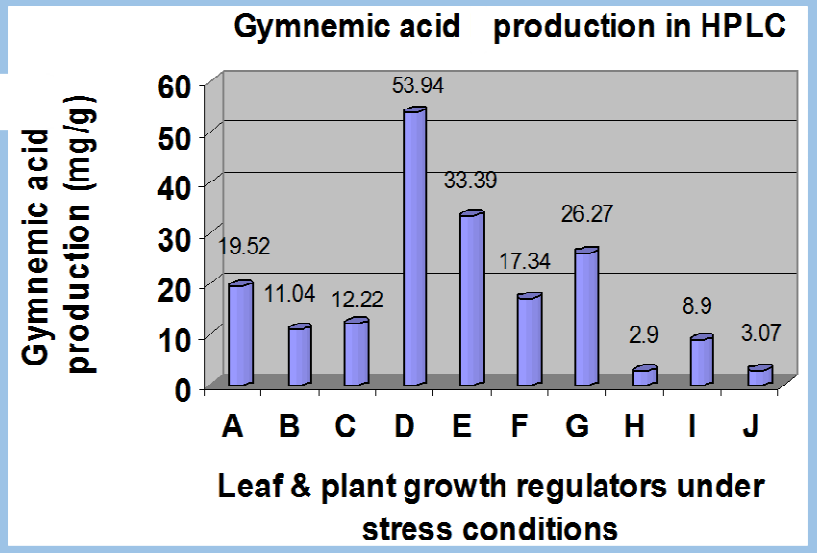
C – Leaf and callus extract Rf ue compared with standard Gymnemic acid

Experiment III.

LC – Leaf and callus extract tention time compared with standard Gymnemic acid



A- Leaf; B - MS + NAA (1.5 mg/l); C - MS + 2, 4-D (1.0 mg/l); D - MS + 2,4-D (1.5 mg/l) mg/l); E - MS + NAA (1.0 mg/l) + BA (0.5 mg/l); F - MS + NAA (1.0 mg/l) + KN (1.0 mg/ NAA (1.0 mg/l + BA (1.0 mg/l); H - MS + NAA (1.0 mg/l) + KN (1.5 mg/l); I – MS + 2,4- + KN (0.5 mg/l).

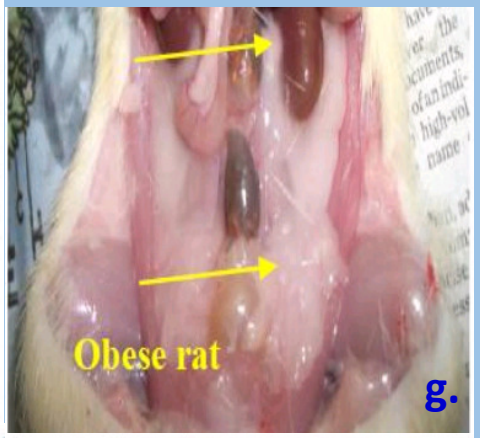


A - Leaf; B – MS + NAA (1.0 mgl^{-1}) + KN (1.5 mgl^{-1}); C - MS + 2,4-D (1.5 mgl^{-1}) + KN (0.5 m Effect of blue light; E - Effect of 5% sucrose; F - Effect of 3mM $NH_4 NO_3$; G - Effect of 12h photoperiod; H - Effect of 30°C temperature; I - Effect of red light; J - Effect of green light

Experimental animal stages



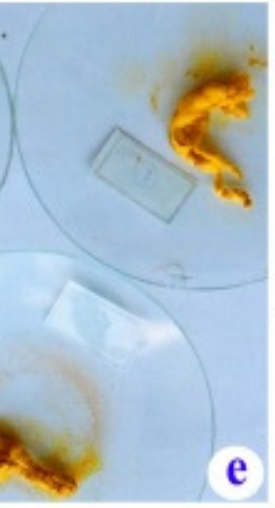


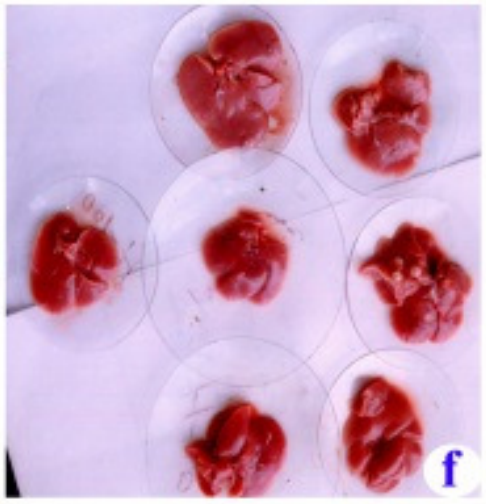














a. Control rats

b. Control rat after 40 days

C. *G. sylvestre* leaf extracts and callus extra
mg/kg) given by oral.

d. standard drug simvastatin injection (3 mg/

e. Collected pancreas after 40 days

f. Collected liver after 40 days

g. High Fat diet rat after 40 days (obese rat

h. Standard drug simvastatin after 40 da

i. *Gymnema sylvestre* leaf treated rats
days

fect leaf and Callus extracts of *G. sylvestre* on Body weight, Blood glucose, and cholesterol in high-fat fed Wister rats

Body weight (g)				Blood glucose (mg/dl)				Cholesterol (mg/dl)			
10th day	20th day	30th day	40th day	10th day	20th day	30th day	40th day	10th day	20th day	30th day	40th da
205±16.5	220±30.2	245±24.6	260±14.8	87±1.7	92.0±2.5	87±1.9	94±2.8	86±1.7	92±2.6	87±1.2	843
279±25.4	300±28.6	330±18.2	370±20.6	168±1.2	192.0±2.5	215±5.8	260±1.6	185±2.5	194±1.8	220±2.0	234
200±32.2	215±34.2	230±34.8	245±18.0	135±4.6	140±5.4	120±1.2	135±2.8	110±1.8	112±3.2	105±1.6	95±
195±17.6	240±38.4	260±26.4	230±22.6	162±1.5	154±2.6	146±1.8	128±3.6	165±4.5	154±1.8	148±2.5	132
230±16.0	265±14.0	270±15.8	265±14.2	156±4.4	162±1.8	174±3.6	165±4.5	152±1.8	160±2.5	144±3.0	136
190±14.2	215±26.4	235±14.2	250±16.8	145±1.8	160±3.2	165±2.4	172±1.8	165±1.2	172±1.2	170±3.2	165
205±18.6	245±12.4	270±26.4	265±11.4	136±2.8	148±1.6	170±1.5	184±2.0	160±2.0	167±2.4	165±1.8	156
245±12.8	265±10.6	250±14.8	235±10.6	150±1.1	165±4.4	174±1.8	182±3.2	180±1.6	174±3.5	162±2.0	154

Normal; **Group II:-** High-Fat diet; **Group III:-** High-fat diet + Drug (simvastatin); **Group IV:-** High-fat diet + *GSLE* (200 mg/kg); **Group V:-** High-fa
E-I (OPGRs + Blue light); **Group VI:-** High-fat diet + *GSLCE-II* (OPGRs + 5% sucrose); **Group VII:-** High-fat diet + *GSLCE-III* (OPGRs+12 h
d; **Group VIII:-** High-fat diet + *GSLCE-IV* (OPGRs: MS+2,4-D 1.5 mg/l + KN 0.5 mg/l).

ons:

ody weights increased significantly in HFD (Group II) than Control (Group I).
oncentration of blood glucose was higher in HFD fed obese rats (Group II) than in normal rats (Group I).
eas, Leaf and callus extract supplementation normalized the blood glucose level in Table 1.
astatin (Group II), the anti-obesity drug maintained the body weight as well as blood glucose level.

ct leaf and Callus extracts of *G. sylvestre* on Triglycerides, HDL and LDC in normal and in high-fat fed Wister rats

Triglyceride (mg/dl)				HDL-C (mg/dl)				LDL-C (mg/dl)		
10th	20th	30th	40th	10th	20th	30th	40th	10th	20th	30th
65±1.5	68±2.2	70±1.2	67±1.8	54±2.5	57±3.5	60±1.8	55±1.4	20±1.6	23±2.5	28±1.8
120±2.0	125±2.4	132±1.6	145±1.2	27±2.0	25±2.2	22±2.0	20±1.6	115±15.4	123±23.5	128±18.6
62±2.6	64±2.5	72±2.2	76±2.5	60±1.8	64±1.5	56±1.2	58±1.8	70±13.8	65±17.6	50±20.4
84±1.5	90±3.1	86±1.4	81±2.6	37±2.4	42±2.6	43±3.2	45±2.0	74±20.4	68±18.8	54±17.5
90±3.2	86±1.8	81±3.2	78±3.0	40±1.2	46±3.2	44±1.4	48±1.5	86±17.5	75±20.5	61±16.1
87±2.5	90±3.4	92±2.5	86±3.2	31±3.4	35±2.0	38±2.8	41±2.4	95±15.6	88±14.6	76±14.4
102±1.8	98±1.6	94±1.8	87±2.4	36±1.6	39±1.2	38±1.5	42±1.6	85±12.8	80±15.2	78±18.5
96±1.5	93±2.4	86±2.0	80±1.8	28±1.2	31±2.4	36±2.4	38±2.2	87±28.4	79±12.8	72±11.4

rmal; **Group II:-** High-Fat diet; **Group III:-** High-fat diet + Drug (simvastatin); **Group IV:-** High-fat diet + *GSLE* (200 mg/kg); **Group V:-** High-fat d
Rs + Blue light); **Group VI:-** High-fat diet + *GSLCE-II* (OPGRs + 5% sucrose); **Group VII:-** High-fat diet + *GSLCE-III* (OPGRs+12 h Photoperiod; **Gr**
+ *GSLCE-IV* (OPGRs (MS+2,4-D 1.5 mg/l + KN 0.5 mg/l).

total cholesterol, triglycerides and LDL-cholesterol were significantly elevated and HDL-cholesterol level was
sed in the HFD obese rats (Group II), When compared to the normal controls animals (Control I).

ever, HFD obese rats when treated with *G. sylvestre* leaf and callus methanol extracts showed significant imp

leaf and callus extracts-GCE1, GCE4, GCE5 significantly decreased serum cholesterol, LDL, triglyceride than th

Thank you

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Short communication

In vitro callus and *in vivo* leaf extract of *Gymnema sylvestre* stimulate regeneration and anti-diabetic activity in Wistar rats

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ABSTRACT

A methanol extract of *Gymnema sylvestre* leaf and callus showed anti-diabetic a
erating β-cells. Optimum callus was developed under stress conditions of blue lig
and KN (0.5 mg/l), which induced maximum biomass of green compact callus at
by growth curve analysis. Leaf and optimum callus extracts contains gymnemic a
using TLC, HPTLC and HPLC methods. The research reported here deals with leaf
sylvestre, which significantly increase the weight of the whole body, liver, panc
content in alloxan-induced diabetic rats (Wistar rats). The gymnemic acid of leaf
nificantly increases the regeneration of β-cells in treated rats, when compared wi
rats. It could have potential as a pharmaceutical drug for insulin-dependent diab
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Introduction

Type 1 diabetes, or insulin-dependent diabetes mellitus (IDDM), is a common pediatric chronic disease, affecting an increasing number of children every year. IDDM occurs due to autoimmune destruction of insulin-producing β-cells in the pancreas, resulting in low or no production of insulin, a hormone necessary for survival (International Expert Committee 2009).

Gymnema sylvestre (syn. *Periploca sylvestris* Retz) is a traditional medicinal plant, with reported use as a remedy for diabetes mellitus, stomachic and diuretic problems. The plant extracts are also used in folk, ayurvedic and homeopathic systems of medicine (Mitra et al., 1995). The extract of *G. sylvestre* plays a major role in blood glucose homeostasis through increased serum insulin level through regeneration of the endocrine pancreas (Shanmugasundaram et al., 1983; Shanmugasundaram et al., 1990). *G. sylvestre* occurs mainly in the Deccan peninsula of western India, Tropical Africa, Vietnam, Malaysia, Srilanka and is widely available in Japan, Germany and the USA as a health food (Ye et al., 2000). Within the last 10 years, a number of *Gymnema* products, including Gymnema capsules, Gymnema tea, Bioshape®, and Diaxinol® have appeared on the world market.

In the past few decades, secondary metab
plant tissue culture has been identified as a
for new drug development and clinical res
pharmacology and medicine. Plant cell cultu
been used widely in the form of fraction
pounds as potential bioactive molecules (S
In vitro developed callus tends to produce
pounds, including gymnemic acid and gymn
et al., 2006). However, external factors like
ing speeds, pH, and medium play an import
acid production in suspension cultures (Devi
tion, sucrose, inoculum density, auxins, an
a very crucial role in the production of gym
bioreactor-dependent cell growth (Lee et al
studies were undertaken to identify the *G. sy*
extract molecules that contribute to or pron
tion and anti-diabetic effects. In addition, we h
gymnemic acid role in anti-diabetic experime
pancreas weight and glycogen content were
of alloxan-induced diabetic Wistar rats. Furth
is laid on β-cell regeneration and the determ

Materials and methods

Plant material and sterilization

G. sylvestre plants (GS) were collected f
hills, and maintained in the plant science ga
dasan University, Tiruchirappalli, Tamil Nadu

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